Alberta STE Report

Newborn blood spot screening for galactosemia, tyrosinemia type I, homocystinuria, sickle cell anemia, sickle cell/beta-thalassemia, sickle cell/hemoglobin C disease, and severe combined immunodeficiency

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Alberta STE Report: Policy-driven Health Technology Assessment reports that include an analysis of the social and system demographics, technological effectiveness, and economic implications of a health technology. The reports are written under contract with the Alberta Health Technologies Decision Process and contextualized for use in Alberta.

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The views expressed in this report are of the Institute of Health Economics.

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EXECUTIVE SUMMARY

Background

Existing evidence reviews on newborn blood spot screening (for example, health technology assessments, systematic reviews, literature synthesis) are outdated and/or have not assessed the long-term health consequences or health economic impact on the health system. Further, the transferability of the evidence base to the Alberta setting is uncertain, as the value in terms of both health outcomes and costs are ultimately dependent on local epidemiology, clinical practice, system capacity, and costs. Accordingly, there was a need to conduct an updated evidence assessment contextualized to the Alberta setting.

There are seven conditions that are not currently primary targets for screening or secondary conditions identified via screening in Alberta that are widely represented in or being considered for screening programs in many jurisdictions across North America: galactosemia (GALT); tyrosinemia type I (TYRI); homocystinuria (HCY); sickle cell anemia (Hb SS), sickle cell/beta-thalassemia (Hb S/β-thal), and sickle cell/hemoglobin C disease (Hb SC) (hemoglobinopathies collectively referred to here as sickle cell disease [SCD]); and severe combined immunodeficiency (SCID). As such, this evidence assessment focuses on these seven conditions and the associated tests used to identify them, to assess the potential health economic impact of adding any or all of these conditions to the Alberta Newborn Metabolic Screening (NMS) Program.

Alberta STE Evidence Assessment

This evidence assessment was conducted under the auspices of the Alberta Health Technologies Decision Process (*http://www.health.alberta.ca/initiatives/AHTDP.html*). This process involves the use of appropriate evidence and information for decision-making regarding the public provision of health technologies and services. These assessments consider existing evidence and other information relevant to three areas: social and system demographics (**S** section), technology effects and effectiveness (**T** section), and economic analysis (**E** section).

The Alberta NMS Program uses a population-based screening approach to reduce the burden of disease in the community through early detection and treatment of select treatable conditions. As such, this STE review was guided by the generally accepted principles for decision-making for the introduction of population-based screening programs outlined by the Australian Population Health Development Principal Committee's Screening Subcommittee, in its document "Population Based Screening Framework" (Australian Framework). It is important to note that the scope of this review is to assess the impact of adding the seven aforementioned conditions to an already existing population-based screening program. There are criteria outlined in the Australian Framework which are oriented towards the requisite characteristics of a screening program; these were not considered in this review, given that evaluation of the existing screening program is outside the scope of this review.

The criteria outlined in the Australian Framework that were considered in this evidence review are summarized below. According to the framework, for a condition to be included in a population-based screening program:

- The condition must be an important health problem and have a recognizable latent or early symptomatic stage.

- The test for each condition must be highly sensitive and specific, be validated and safe, have a relatively high positive and negative predictive value, and be acceptable to the target population, including important subgroups.
- The treatment for each condition must be effective, available, easily accessible, and acceptable to all patients with the recognized disease or condition.
- There should be clear evidence that screening and treatment leads to better outcomes than finding and treating the disease at a later stage.
- Systems should be in place for evidence-based follow-up assessment of all people with a positive screen, regardless of rurality, ethnicity, socioeconomic status, or disadvantage status.
- Ongoing management referral protocols must be established for individuals who have the condition detected through the screening program.
- The overall benefits of screening outweigh the harm.

Social and System Demographics

Objective

The objective of the S section of this STE report is to provide information describing the patterns of care associated with early and late diagnosis of each of the seven conditions, as well as related health outcomes. The health system capacity in Alberta and general acceptability of screening and condition diagnostic testing will also be described.

Key Findings

Table ES.1: Canadian jurisdictions screening for GALT, TYRI, HCY, SCD, and/or SCID (as of April 2015)

Province	GALT	TYRI	HCY	SCD (Hb SS, Hb S/β-thal, Hb SC)	SCID
British Columbia	+	+	+	+	R
Alberta	Under review	Under review	Under review	Under review	Under review
Saskatchewan	+	+	+		
Manitoba	+	+	+		Under review
Ontario	+	+	+	+	+
Quebec	R	+	R	+*	
New Brunswick	A	A	A	+	A
Nova Scotia	A	A	A	+	A
Prince Edward Island**	R	R	R	R	R
Newfoundland		+	+		
Northwest Territories***					
Yukon	+	+	+	+	R

*Since November 2013, infants born in hospitals and birthing centres in Montreal and Laval regions receive screening for for sickle cell anemia (Hb SS). Screening in other regions of Quebec will be progressively phased in.

**Prince Edward Island will be partnering with IWK Health Centre (used in Nova Scotia and New Brunswick) to increase the number of disorders being screened, including SCD and HCY, within the next two years (*http://www.iwk.nshealth.ca/page/newborn-screening-disorders-facts-questions-families*).

***Newborn screening for infants born in the Northwest Territories is currently conducted in Alberta; as a result, the screening panel is currently the same as in Alberta. Newborn screening for infants born in the Yukon is currently conducted in British Columbia. As a result, the screening panel is currently the same as in British Columbia.

R: recommended, pending funding approval; A: approved but not yet implemented

Table ES.2: Burden of disease, rationale for screening, and system implications

	GALT	TYRI	HCY	SCD (Hb SS, Hb S/β-thal, Hb SC)	SCID
Incidence	1:73,296 (Manitoba screening data) to 1:75,833 (Ontario clinical data)	1:100,000 (Ontario and British Columbia fact sheets; no available Canadian data outside of Quebec*) 1:16:000 to 1:29,000 (Quebec screening data)	0:225,000 (British Columbia screening data) to 1:200,000-300,000 (Ontario fact sheet*) Higher in Irish, Norwegian, and Qatari populations	SCD - 1:17,271 (British Columbia screening data) to 1:5,650 (Ontario clinical data) Hemoglobinopathies - 1:2,737 (Ontario screening data) Higher in African, Arab, and Greek populations	1:58,000 (US screening data) 1:71,429 (Canada clinical data) 1:45,000 (Alberta clinical data)
Potential Sequelae	Cataracts, developmental delay (that is, mental retardation, growth delay, speech problems, and/or motor function issues), liver damage and potential failure, hypoglycemia, seizures, jaundice, bleeding, sepsis and, in females, premature ovarian failure	Progressive liver disease and possible hepatocellular carcinoma, kidney damage, as well as possible neurological crisis, rickets, and cardiomyopathy	Strokes, developmental delays (mental and growth), and eye problems Wide variation in severity of sequelae across patients	Sepsis, acute splenic sequestration, vaso-occlusive crisis, dactylitis, severe anemia, jaundice, acute chest syndrome, abdominal crisis, growth delays, and/or stroke	Severe recurrent infections and failure to thrive
Treatment in Alberta	Lifelong lactose-free diet, formula for infants As needed sequelae management	Lifetime dietary restrictions, NTBC, and dietary supplements As needed sequelae management	Lifelong vitamin B6, betaine, dietary supplements, and possible dietary restrictions As needed sequelae management	Short-term prophylaxis and routine vaccination, and parental education HSCT is offered to patients with sibling match As needed sequelae management	HSCT, with immunoglobulin and prophylaxis therapy a priori

Rationale for Screening	Given the timing of clinical presentation (generally within the first few weeks of life), screening may prevent life-threatening symptoms. Risk of cataracts may also be reduced.	Screening is expected to reduce incidence of liver damage and carcinoma (reducing need for liver transplant), as well as kidney disease. Mortality rates in this group would also be expected to decline significantly.	Screening may not be expected to prevent mortality; however, there is some evidence to suggest reduced risk of thromboembolic manifestations, ocular manifestations, and mental retardation for those who respond to treatment.	Screening may prevent infection and resulting sepsis, a life-threatening condition. Parental education may help reduce disease morbidity from splenic sequestration.	Screening is expected to prevent severe infections that often result in mortality within the first year of life
Clinic Implications**	Metabolic clinic can accommodate***	Metabolic clinic can accommodate*** Dietary supplement cost concerns	Metabolic clinic can accommodate*** Dietary supplement cost concerns	To accommodate the influx of patients, hematology clinics require: • High need for genetic counselling resources, especially if carriers reported • Clinic resources (physician, allied health, clerical, nursing, physical space) • Blood transfusion resources and outpatient space • HSCT resources • Neuropsychologist support	Hematology/ immunology clinic can accommodate*** HSCT unit – at capacity
Lab Implications for recommended screening platforms	a) 1 additional VICTOR^{2}D™, galactose-1-phosphate uridylyl-transferase enzyme measurement screening kits + 0.25 FTE b) 2 GSP®s, kits	Existing MS/MS using succinylacetone screening kit and reagents	Existing MS/MS using methionine screening kit and reagents	2 new HPLC (requested) machines to analyze blood spots	1 additional qPCR QuantStudio™ platform for lab-based test, reagents

Health system Implications**	Reduced ophthalmology due to early treatment No diagnostic odyssey	Reduced liver transplants, hepatocellular carcinoma, and renal treatment due to early treatment No diagnostic odyssey	Reduced ophthalmology due to reduced lens dislocation Reduced hospitalization due to thromboembolic events Reduced social support due to improved mental development No diagnostic odyssey	Reduced hospitalization and treatment of severe infection No diagnostic odyssey	Reduced hospitalization and treatment of severe infections Additional need for HSCT Additional need for isolation space (inpatient and outpatient) Impact on temporary accommodations (e.g. Ronald McDonald House) No diagnostic odyssey

FTE: full-time equivalent; GSP®: Genetic Screening Processor; HPLC: high performance liquid chromatography; HSCT: hematopoietic stem cell transplantation; MS/MS: tandem mass spectrometer; NTBC: 2-(2-nitro-4-triflouromethyl-benzoyl)-cyclohexane-1,3-dione

*The original data source used to generate these estimates is unknown, and no other Canadian data were identified.

**The addition of any (or all) of these conditions will require:

- Additional 0.2 FTE clinic genetic counsellor support would be required to accommodate all seven conditions. Up to additional 2.5 FTE would be required to accommodate SCD variants, secondary targets, and notified carriers.
- It will be necessary to re-configure lab software (Specimen Gate Office), and integrate with Netcare and the NMS Application.
- For implementation and ongoing operation, the following are required: 1.0 FTE Medical Laboratory Technologist I; 2.0 FTE Medical Laboratory Geneticists; 1.0 FTE Geneticist Trainee (CCMG); 3.0 FTE Medical Laboratory Scientists I; 1.0 FTE Information Technology Analyst I; and training of existing staff.
- Possible need to collect an additional blood spot sample.
- Need to also consider resources needed for the maintenance, updating, and/or replacement of equipment.
- Additional lab space may be required to accommodate new screening platforms.

***Existing capacity can accommodate patients that would have otherwise died; and/or the influx of patients that would be identified earlier.

****Expected broader health system requirements only include anticipated increases in system requirements over and above what would be expected given the current method of identifying patients with these conditions (clinical presentation). Therefore, these changes in resource requirements are expected because of: (i) the reduced resource requirements due to reduced morbidity (sequelae) due to the prevention of sequelae based on early treatment because of screening; and (ii) existing capacity constraints within the system. Patients that would have otherwise died from GALT, TYRI, HCY, and SCID may require sequelae management, which would result in added resource utilization at the clinic and health system level. However, each of these conditions is rare, and therefore the overall increase in service utilization would be small.

Technology Effects and Effectiveness

Objective

The objective of the T section of this STE report is to assess the harm, screening test performance, and effectiveness outcomes of newborn screening for the seven conditions.

Key Findings

Table ES.3: Safety, diagnostic accuracy, and effectiveness of screening

Condition	Safety*	Screening sensitivity and specificity	Effectiveness (early vs. late detection)
GALT	No harms as a result of screening were reported	Sn ~99% Sp ~99% Using the galactose-1-phosphate uridylyltransferase enzyme activity test screening detects only classic galactosemia and the milder form known as the Duarte variant.	Effectiveness is uncertain. Limited evidence suggesting that early management may have a favourable impact on mortality, cataract development, and the need for cataract surgery.
TYRI	No harms as a result of screening were reported	Sn ~99% Sp ~99% Succinylacetone is highly specific for TYRI.	Consistent evidence suggests that NTBC is associated with reducing morbidity in terms of reducing the incidence of hepatocellular carcinoma and the need for liver transplantation.
HCY	No harms as a result of screening were reported	Sn ~variable, depending on cut-off Sp ~variable, depending on cut-off Overall, inconclusive diagnostic accuracy of methionine measurement on the dried blood spot.	Scarce, low-level evidence suggests that treatment may reduce morbidity associated with thromboembolic manifestations and developmental delay.
SCID	No harms as a result of screening were reported	Sn ~99% Sp ~99% TRECs measured by real time quantitative PCR are highly sensitive and specific.	Consistent evidence suggests that early identification of infants with SCID and early treatment with HSCT resulted in better survival than late HSCT. Treatment can be curative.
SCD (Hb SS, Hb S/β-thal, Hb SC)	No harms as a result of screening were reported	Sn ~99% Sp ~99% Either HPLC or IEF is highly sensitive and specific.	Consistent evidence suggests that early detection and early initiation of penicillin prophylaxis, parental education, and follow-up/ comprehensive care are associated with reduced early mortality and improved survival.

HPLC: high performance liquid chromatography; HSCT: hematopoietic stem cell transplantation; IEF: isoelectric focusing; MS/MS: tandem mass spectrometer; NPV: negative predictive value; NTBC: 2-(2-nitro-4-triflouromethyl-benzoyl)-cyclohexane-1,3-dione; PCR: polymerase chain reaction; PPV: positive predictive value; Sn: sensitivity; Sp: specificity; TREC: T-cell receptor excision circles

*Although no study was found that specifically examine the psychosocial harms of screening for the seven conditions, it has been well recognized that false positive results of the screening test may cause parents' anxiety and stress while waiting for the results of confirmation diagnosis, or prompt further investigations and unnecessary treatments which may cause family distress and increase use of healthcare services.

Economic Analysis

Objective

The objective of the E section of this STE report is to assess the cost-effectiveness and budget impact of adding one or a combination of the seven conditions to the Alberta NMS Program.

Key Findings

Screening for any of the seven conditions or combination thereof is associated with improvements in health outcomes, but at an additional net cost increase to the health system. Screening did not improve health outcomes at a net cost saving to the health system, which would by definition be considered cost-effective.

Assessing the value for money of the possible combinations or options, therefore, requires an examination of how much health is produced for the dollars invested. When contrasting the additional costs to screen with the quantity of health that is produced (measured in life years gained), adding SCD (that is, Hb SS, Hb S/β-thal, and Hb SC) alone would provide the greatest value among all options, with an estimated incremental cost-effectiveness ratio (ICER) of $2,621 to produce an additional year of life (Table E.S.4).

The option of adding all seven conditions to the screening program would be associated with an estimated cost per additional year of life of $8,155. Hence, decision-makers ought to consider only those combinations that had an estimated cost per additional year of life less than $8,155 (Table ES.4), because those combinations require less money to produce the same additional life year (less money needed for the same unit of health output).

Note that all of these such combinations include SCD. This result is not driven by leveraging an existing platform, given that screening for SCD is not conducted on a MS/MS platform. The cost-effectiveness results are driven by the fact that the amount of health gained for the dollars invested is high for SCD, and SCD therefore has the greatest value among all options. Consequently, the lower technical efficiency (that is, lower health gain for the dollars invested) associated with screening for HCY, TYRI, GALT, or SCID (which each had prohibitively high ICERs, estimated at $25,453 or greater) could be offset by the higher technical efficiency of SCD (that is, higher health gained for the dollars invested). Therefore, when combined, SCD compensates for the other conditions so that their combined result is estimated at $8,155 per additional life year gained.

Table ES.4: Cost-effectiveness rankings

Strategy	ICER
SCD Screening	$2,620.73
HCY + SCD Screening	$2,702.49
TYRI + SCD Screening	$2,989.88
HCY + TYRI + SCD Screening	$3,069.29
GALT + SCD Screening	$4,944.51
HCY + GALT + SCD Screening	$5,016.53
GALT + TYRI + SCD Screening	$5,277.69
GALT + HCY + TYRI + SCD Screening	$5,347.66

SCD + SCID Screening	$5,553.17
HCY + SCD + SCID Screening	$5,623.80
TYRI + SCD + SCID Screening	$5,882.21
HCY + TYRI + SCD + SCID Screening	$5,950.79
GALT + SCD + SCID Screening	$7,800.45
HCY + GALT + SCD + SCID Screening	$7,861.90
GALT + TYRI + SCD + SCID Screening	$8,095.53
Total Screening	**$8,155.21**
HCY Screening	$25,452.50
HCY + TYRI Screening	$30,352.81
TYRI Screening	$31,723.53
HCY + GALT + TYRI Screening	$80,747.11
GALT + TYRI Screening	$86,847.52
HCY + GALT Screening	$107,755.20
GALT Screening	$122,749.04
HCY + GALT + TYRI + SCID Screening	$130,763.89
HCY + TYRI + SCID Screening	$136,984.88
GALT + TYRI + SCID Screening	$139,873.07
TYRI + SCID Screening	$155,353.46
HCY + GALT + SCID Screening	$170,165.83
GALT + SCID Screening	$188,286.63
HCY + SCID Screening	$244,595.49
SCID Screening	$332,360.39

Conclusion

Given the evidence and considerations of the Australian Framework and system readiness in the Alberta context, in principle we conclude that the clinical benefits associated with screening for many of these seven conditions outweigh the potential harms (which can be managed). Expanding a newborn blood spot screening program such as the Alberta NMS Program to include these conditions, however, is ultimately dependent on both the availability of funding and the ability to increase service capacity and provision.

Not adding any of the seven conditions would have the most minimal impact to the existing health system, but would forgo adding conditions for which screening is highly accurate and early intervention is associated with improved clinical outcomes. Adding all seven conditions would be associated with the greatest health system impact and not necessarily the greatest value. With current methods, HCY cannot be screened with high sensitivity and specificity, and the low incidence of HCY results in an increase in unintended health system utilization not outweighed by the benefit gained; screening for HCY is also associated with the lowest cost-effectiveness among the seven conditions reviewed.

Adding a subset of the seven conditions, on the other hand, could balance considerations of clinical benefit and value for the health system. Specifically, this subset of conditions could include GALT, TYRI, SCD (Hb SS, Hb S/β-thal, and Hb SC), and SCID, which were deemed to: be important health burdens; have screening tests with high sensitivity and specificity; have evidence supporting the clinical benefit of early intervention; have established care pathways (from screening to long-term management; and, as a group, not be associated with unreasonable value for money.

Nevertheless, in the Alberta context, in screening for these additional conditions, system readiness and capacity would be a challenge, particularly in the case of SCD. If the Alberta NMS Program were to expand to include all or a subset of the seven conditions, it is imperative that an implementation plan be developed to ensure that the necessary funding, resources, providers, and training be established either prior to their adoption or through a staged implementation strategy; such an implementation plan should also include a strategy for monitoring the impact on these conditions in terms of risks, benefits, and value for money. The incidence rates of these conditions in Alberta remain unknown, and yet they are a strong driver of the value proposition. Moreover, the methodological quality of the evidence examining effectiveness was rated to be poor in general, but is not expected to improve due to the rarity of these conditions. Therefore, the only mechanism of addressing these uncertainties in the Alberta context, as well as identifying both intended and unintended consequences, is to prospectively monitor and evaluate the impact of screening for these conditions.

Abbreviations

All abbreviations that have been used in this report are listed here unless the abbreviation is well known, has been used only once, or has been used only in tables or appendices, in which case the abbreviation is defined in the figure legend or in the notes at the end of the table.

ADA	adenosine deaminase
ADA SCID	adenosine deaminase deficiency
BGL	Biochemical Genetics Lab (North and South)
BIA	budget impact analysis
BMT	bone marrow transplant
CBS	cystathionine beta-synthase or Canadian Blood Services
CEA	cost-effectiveness analysis
CI	confidence interval
CLS	Calgary Laboratory Services
DBS	dried blood spot
EAG	Expert Advisory Group
EBMT	European Group for Blood and Marrow Transplantation
ELISA	enzyme-linked immunosorbent assay
EPHPP	Effective Public Health Practice Project
ESID	European Society for Immunodeficiencies
FAH	fumarylacetoacetate hydrolase (also known as fumarylacetoacetase)
FN	false negative
FP	false positive
FTE	full-time equivalent
GALE	galactose epimerase
GALK	galactokinase
GALT	galactosemia
GSP	Genetic Screening Processor (manufactured by Wallace Oy/PerkinElmer)
GvHD	graft-versus-host disease
Hb	hemoglobin
HCY	homocystinuria
HLA	human leukocyte antigen
HPLC	high performance liquid chromatography

HPPD	hydroxyphenylpyruvate dioxygenase
HPV	human papillomavirus
HSCT	hematopoietic stem cell transplant
HTA	health technology assessment
ICER	incremental cost-effectiveness ratio
IEF	isoelectric focusing
IL-7	interleukin 7
JCAAI	Joint Council of Allergy, Asthma, and Immunology
KREC	Kappa-deleting recombination circle
MAT	methionine adenosyltransferase
MCADD	medium chain acyl-coenzyme A dehydrogenase deficiency
MS/MS	tandem mass spectrometry or spectrometer
NAC	National Advisory Committee on Blood and Blood Products
NBS	newborn screening
NHLBI	National Heart, Lung, and Blood Institute
NHS	National Health Service
NMS	newborn metabolic screening
NPV	negative predictive value
NSO	Newborn Screening Ontario
NTBC	2-(2-nitro-4-triflouromethyl-benzoyl)-cyclohexane-1,3-dione (also known as nitisinone)
PCR	polymerase chain reaction
PID	primary immunodeficiency disorder
PKU	phenylketonuria
PPV	positive predictive value
qPCR	quantitative or real-time polymerase chain reaction
RUSP	United States Department of Health and Human Services' Recommended Uniform Screening Panel
SA	succinylacetone
SCD	sickle cell disease
SCID	severe combined immunodeficiency
TN	true negative
TP	true positive

TREC	T-cell receptor excision circle
TYRI, II, III	tyrosinemia type I, II, or III
UAH	University of Alberta Hospital
XL-SCID	X-linked severe combined immunodeficiency

Glossary

The glossary terms listed below were obtained and adapted from the following sources:

Dictionaries/glossaries

- A dictionary of epidemiology (6th edition, Oxford University Press)
- Concise dictionary of modern medicine (2002, McGraw-Hill)
- HTA glossary.net (*http://htaglossary.net/HomePage*)
- Merriam-Webster medical dictionary (*http://www.merriam-webster.com*)
- Mosby's medical dictionary (9th edition, Elsevier)
- National Human Genome Research Institute glossary (*https://www.genome.gov/glossary/*)
- Newborn Screening Ontario, NSO's Diagnostic Definitions Key
- Oxford Dictionaries (*http://www.oxforddictionaries.com*)

Health organizations, studies, and other documents

- Alberta Health Technologies Decision Process, Newborn blood spot screening - Project charter
- Health Canada (*http://www.hc-sc.gc.ca*)
- Indiana Hemophilia & Thrombosis Center (*http://www.ihtc.org/patient/blood-disorders*)
- National Center for Biotechnology Information, Genes and diseases (*http://www.ncbi.nlm.nih.gov/books/NBK22183/*)
- National Heart, Lung, and Blood Institute (*http://www.nhlbi.nih.gov/*)
- UCSF Children's Hospital, Intensive care nursery house staff manual (*http://www.ucsfbenioffchildrens.org/pdf/manuals/53_Metabolism.pdf*)
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Adverse event – Any noxious, pathological, or unintended change in a physical or metabolic function, revealed by signs or symptoms or a change in the results of laboratory tests in any phase of a clinical study, whether or not the change is considered treatment-related. May involve the exacerbation of a pre-existing condition, intercurrent diseases, an accident, a drug interaction, or a significant worsening of the disease.

Allele – Alternative forms of a gene occupying the same locus on a chromosome. An individual inherits two alleles for each gene, one from each parent. If the two alleles are the same, the individual is homozygous for that gene. If the alleles are different, the individual is heterozygous. Though the term allele was originally used to describe variation among genes, it now also refers to variation among non-coding DNA sequences.

Amino acids – A set of 20 different molecules used to build proteins. Proteins consist of one or more chains of amino acids called polypeptides. The sequence of the amino acid chain causes the polypeptide to fold into a shape that is biologically active. The amino acid sequences of proteins are encoded in the genes.

Antibody – A protein molecule produced in response to exposure to a foreign or extraneous substance (e.g. invading microorganisms responsible for infection) or active immunization.

Biosynthesis – A multi-step, enzyme-catalyzed process where substrates are converted into more complex products in living organisms.

Bone marrow transplant (BMT) – Also called a stem cell transplant, is a procedure that infuses healthy cells (stem cells) into a patient's body to replace damaged or diseased bone marrow.

Carrier – An individual who carries and is capable of passing on a genetic mutation associated with a disease and may or may not display disease symptoms. Carriers are associated with diseases inherited as recessive traits. In order to have the disease, an individual must have inherited mutated alleles from both parents. An individual having one normal allele and one mutated allele does not have the disease. Two carriers may produce children with the disease.

Carrier screening – A type of genetic testing performed on people who display no symptoms for a genetic disorder but may be at risk for passing it on to their children. A carrier for a genetic disorder has inherited one normal and one abnormal allele for a gene associated with the disorder. A child must inherit two abnormal alleles in order for symptoms to appear. Prospective parents with a family history of a genetic disorder are candidates for carrier screening.

Cascade testing – The identification of close relatives of an individual with a disorder to determine whether the relatives are also affected or are carriers of the same disorder. It is intended as a form of medical screening.

Chromosome – An organized package of DNA found in the nucleus of the cell. Humans have 23 pairs of chromosomes: 22 pairs of numbered chromosomes, called autosomes, and one pair of sex chromosomes, X and Y. Each parent contributes one chromosome to each pair so that offspring get half of their chromosomes from their mother and half from their father.

Confidence interval – A range of values below and above the point estimate that has a given probability of including the true value of a given parameter, such as a treatment effect.

Confirmatory test – The test required to verify the analysis.

Congenital – Present or recognized at (or before) birth and developed during intrauterine life.

Deoxyribonucleic acid (DNA) – The chemical name for the molecule that carries genetic instructions in all living things. The DNA molecule consists of two strands that wind around one another to form a shape known as a double helix. Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases: adenine, cytosine, guanine, and thymine. The two strands are held together by bonds between the bases;

adenine bonds with thymine, and cytosine bonds with guanine. The sequence of the bases along the backbones serves as instructions for assembling protein and RNA molecules.

Diagnosis – The art or act of identifying a disease from its symptoms.

Diagnostic odyssey – The process of diagnosis by sequentially ruling out differential diagnoses.

Dried blood spot (DBS) – A form of biosampling where blood samples obtained by a finger prick (or heel prick, in the case of newborns) lancet are blotted and dried on filter paper. The DBS platform is especially advantageous in studies of infants and small children since it is minimally invasive and small volumes often are available.

Duarte variant or syndrome – A rare and less severe type of galactosemia that is often, but not always, detected during newborn screening. Infants with this variant may need less treatment or no treatment at all.

Electrophoresis – A laboratory technique used to separate DNA, RNA, or protein molecules based on their size and electrical charge. An electric current is used to move molecules to be separated through a gel. Pores in the gel work like a sieve, allowing smaller molecules to move faster than larger molecules. The conditions used during electrophoresis can be adjusted to separate molecules in a desired size range.

Enzyme – A biological catalyst that is almost always a protein (though it could be RNA, a nucleic acid). It speeds up the rate of a specific chemical reaction in the cell. The enzyme is not destroyed during the reaction, and is used over and over. A cell contains thousands of different types of enzyme molecules, each specific to a particular chemical reaction.

Galactosemia (GALT) – A metabolic disorder inherited as an autosomal recessive trait in which galactose accumulates in the blood due to deficiency of an enzyme (galactose-1-phosphate uridylyltransferase) catalyzing its conversion to glucose.

GALE deficiency (galactose epimerase deficiency, galactosemia type III, UDP-galactose-4'-epimerase deficiency) – A rare, autosomal recessive form of galactosemia associated with a deficiency of the enzyme galactose epimerase.

GALK deficiency (galactokinase deficiency, galactosemia type II) – A rare, autosomal recessive form of galactosemia associated with a deficiency of the enzyme galactokinase.

Gene – A sequence of DNA that codes for a particular protein product or that regulates other genes.

Genetic counselling – The professional interaction between a healthcare provider with specialized knowledge of genetics and an individual or family. Genetic counsellors determine whether a condition in a family may be genetic, and estimate the chances that another relative may be affected. Genetic counsellors also offer and interpret genetic tests that may help to estimate risk of disease, convey information in an effort to address patient concerns, and provide psychological counselling to help families adapt to their condition or risk.

Genetic screening – The use of genetic, clinical, and epidemiological knowledge, reasoning, and techniques to detect genetic variants that have been demonstrated to place an individual at increased risk of a specific disease.

Genetic testing – The use of a laboratory test to look for genetic variations associated with a disease. The results of a genetic test can be used to confirm or rule out a suspected genetic disease, or to determine the likelihood of a person passing on a mutation to their offspring. Genetic testing may be performed prenatally or after birth. Ideally, a person who undergoes a genetic test will discuss the meaning of the test and its results with a genetic counsellor.

Genotype – The genetic constitution inherited by an organism or a person, as distinct from the physical characteristics and appearance that emerge with development.

Graft-versus-host disease (GvHD) – A condition that might occur after an allogeneic transplant. The donated bone marrow or peripheral blood stem cells view the recipient's body as foreign, and thus attack the body.

Hematopoietic stem cell transplantation (HSCT) – The transplantation of multipotent hematopoietic stem cells, usually derived from bone marrow, peripheral blood, or umbilical cord blood.

Hemoglobinopathy – A group of inherited disorders in which there is abnormal production or structure of the hemoglobin molecule. Such disorders include hemoglobin (Hb) C disease, Hb SC disease, sickle cell anemia, and various types of thalassemia.

Heterozygous – A genetic condition where an individual has inherited different alleles of a particular gene from each parent.

Homocystinuria (HCY) – An inherited disorder that affects the metabolism of the amino acid methionine.

Homozygous – A genetic condition where an individual has inherited the same alleles of a particular gene from both parents.

Inborn errors of metabolism – A group of disorders in which a single gene defect causes a clinically significant block in a metabolic pathway, resulting either in an accumulation of substrate behind the block or a deficiency in the pathway's product.

Incidence – The number of newly diagnosed cases during a specific time period.

Incidental – Not a specifically targeted disease (or variant thereof), but may be ascertained by newborn screening. It may be a consequence of multiplex testing (i.e. the technology used generates information beyond only the markers for the targeted disease), or because the additional diseases share the same biomarkers as the targeted disease.

Inherited – Genetically determined, passed from parent to offspring according to the rules of Mendelian genetics. Most traits are not strictly determined by genes, but rather are influenced by both genes and environment.

Kappa-deleting recombination excision circles (KRECs) – During the B-cell maturation process, KRECs are produced by recombination events that detemine the allelic and isotypic exclusion of the kappa chain. The KREC measurement can be used to identify certain types of B-cell deficiencies such as X-linked agammaglobulinemia. The combined TREC/KREC assay can be used to identify certain types of SCID and other primary immunodeficiencies.

Low birth weight – A birth weight of a liveborn infant of less than 2,500 grams, regardless of gestational age.

Lymphocyte – A type of white blood cell that is part of the immune system. There are two main types of lymphocytes: B-cells produce antibodies that are used to attack invading bacteria, viruses, and toxins; and T-cells destroy the body's own cells that have themselves been taken over by viruses or become cancerous.

Mass-to-charge ratio – A number defining how a particle will respond to an electric or magnetic field that can be calculated by dividing the mass of a particle by its charge.

Methionine adenosyltransferase (MAT) I/III deficiency – Caused by mutations in the *MAT1A* gene, is characterized by persistent hypermethioninemia without elevated homocysteine or tyrosine.

Mutation – A change in a DNA sequence. Mutations can result from DNA-copying mistakes made during cell division, exposure to ionizing radiation, exposure to chemicals called mutagens, or infection by viruses. Germ line mutations occur in the eggs and sperm and can be passed on to offspring, while somatic mutations occur in body cells and are not passed on.

Negative predictive value (NPV) – The probability that a person with a negative test result is a true negative (i.e. does not have a disease).

Newborn screening (NBS) – Testing performed on newborns to detect a wide variety of disorders. Typically, testing is performed on a blood sample obtained from a heel prick when the infant is two or three days old. Newborn metabolic screening (NMS) is specific to metabolic disorders.

Pathognomonic – A sign or symptom specifically characteristic or indicative of a particular disease or condition.

Peptide – One or more amino acids linked by chemical bonds. The term also refers to the type of chemical bond that joins the amino acids together. A series of linked amino acids is a polypeptide.

Phenotype – An individual's observable traits, such as height, eye colour, and blood type. The genetic contribution to the phenotype is called the genotype. Some traits are largely determined by the genotype, while other traits are largely determined by environmental factors.

Positive predictive value (PPV) – The probability that a person with a positive test result is a true positive (i.e. does have a disease).

Pre-term – Infants born alive before 37 weeks of pregnancy are completed.

Prevalence – The number of people in a population with a specific disease or condition at a given time, usually expressed as a proportion of the number of affected people to the total population.

Primary target – A condition deemed to meet the criteria for screening set out by Alberta.

Proteins – A class of molecules found in all living cells. A protein is composed of one or more long chains of amino acids, the sequence of which corresponds to the DNA sequence of the gene that encodes it. Proteins play a variety of roles in the cell, including structural (cytoskeleton), mechanical (muscle), biochemical (enzymes), and cell signaling (hormones). Proteins are also an essential part of diet.

Recessive – A quality found in the relationship between two versions of a gene. Individuals receive one version of a gene (an allele) from each parent. If the alleles are different, the dominant allele will be expressed, while the effect of the other allele, called recessive, is masked. In the case of a

recessive genetic disorder, an individual must inherit two copies of the mutated allele in order for the disease to be present.

Screening – The presumptive identification of unrecognized disease or defects by means of tests, examinations, or other procedures that can be applied rapidly. Screening is intended for all people in an identified target population who do not have symptoms of the disease or condition being screened for.

Secondary target - classic – A condition that may be identified either through screening for a primary target or in the diagnostic evaluation following a screen positive result for a primary target. They are generally genetically or pathophysiologically related to the primary targets, and are also often amenable to treatment. Screening systems are generally not optimized to try and ascertain infants with these diseases.

Secondary target - variant – A variant of the targeted condition. Often this is a milder version of the target condition, or where the clinical severity of the disease is indeterminate.

Sensitivity (Sn) – The effectiveness of a test in detecting disease in those that have the disease, expressed as the proportion of those with a condition who have a positive test result (inversely related to the proportion of false negative results).

Sequelae – The consequences of a particular condition or therapeutic intervention.

Serious adverse event – Any untoward medical occurrence that at any dose: results in death, is life-threatening (i.e. an event in which the patient was at risk of death at the time of the event), requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.

Severe combined immunodeficiency (SCID) – A group of rare, sometimes fatal, congenital disorders characterized by little or no immune response.

Sickle cell – Contains abnormal hemoglobin called sickle hemoglobin or hemoglobin S. Sickle hemoglobin causes the cells to develop into a sickle, or crescent, shape.

Sickle cell anemia – People who have sickle cell disease (SCD) inherit two abnormal hemoglobin genes, one from each parent. In all forms of SCD, at least one of the two abnormal genes causes a person's body to make hemoglobin S. When a person has two hemoglobin S genes, hemoglobin SS, the disease is called sickle cell anemia. This is the most common and often most severe kind of SCD.

Sickle cell disease (SCD) – A hereditary disease seen most often among people of African ancestry, caused by mutations in one of the genes that encode the hemoglobin protein, the disease is inherited as an autosomal recessive trait. The mutation causes the red blood cells to take on an unusual sickle shape. Individuals affected by SCD are chronically anemic and experience significant damage to their heart, lungs, and kidneys.

Specificity (true negative rate) (Sp) – The effectiveness of a test in giving negative results for those that do not have the disease.

Splenic sequestration – An acute condition of intrasplenic pooling of large amounts of blood. Children with sickle cell disease between ages 5 months and 2 years represent most of the cases of splenic sequestration. During severe sequestration crisis, the blood-filled spleen may enlarge to the

point of filling the entire abdomen. The child's hemoglobin may drop acutely (to as low as 1-3g/dl), resulting in hypovolemic shock and death within hours of initial onset.

Tandem mass spectrometry (MS/MS) – A two-step technique used to analyze a sample for a predetermined set of substances, either by using a separate mass spectroscope for each step or by using the same spectroscope to perform the steps sequentially. In the first stage, a predetermined set of ions is selected for fragmentation. In the second, mass spectra are produced for the fragments. This technique is used in screening newborns for multiple metabolic disorders from a single blood sample.

T-cell receptor excision circles (TRECs) – Small circles of DNA created in T-cells during their passage through the thymus as they rearrange their T-cell receptor genes; their presence indicates maturation of T-cells. TRECs are reduced in severe combined immunodeficiency disease.

Trait – Denotes the heterozygous state of a recessive disorder, such as sickle cell anemia.

Transient hypertyrosinemia – A rise in plasma tyrosine during the first few weeks of life, which later returns to normal.

Tyrosinemia – A rare inherited disorder of tyrosine metabolism caused by the shortage (deficiency) of one of the enzymes required for the multistep process that breaks down tyrosine. Tyrosinemia is characterized by abnormally high concentrations of tyrosine in the blood and urine, with associated abnormalities especially of the liver and kidneys. There are three types of tyrosinemia:

- **Tyrosinemia type I (TYRI) –** The most severe form of this disorder, is caused by a shortage of the enzyme fumarylacetoacetate hydrolase.
- **Tyrosinemia type II (TYRII) –** Caused by a deficiency of the enzyme tyrosine aminotransferase.
- **Tyrosinemia type III (TYRIII) –** Caused by a deficiency of the enzyme 4-hydroxyphenylpyruvate dioxygenase.

Variant – A form of the targeted condition. Often this is a milder version of the target condition, or where the clinical severity of the disease is indeterminate.

Table of Contents

SECTION ONE: BACKGROUND AND CONTEXT

1.1 Background

Existing evidence reviews on newborn blood spot screening (for example health technology assessments, systematic reviews, literature synthesis) are outdated and/or have not assessed the long-term health consequences or the health economic impact on the health system. Further, the transferability of the evidence base to the Alberta setting is uncertain, as the value in terms of both health outcomes and costs are ultimately dependent on local epidemiology, clinical practice, system capacity, and costs. Accordingly, there is a need to conduct an updated evidence assessment contextualized to the Alberta setting.

The Alberta Newborn Metabolic Screening (NMS) Program currently screens for 17 core conditions that are considered primary targets, including 14 metabolic conditions, two endocrine conditions, and cystic fibrosis. There are seven conditions that are not currently primary targets for screening or secondary conditions[i] identified via screening in Alberta that are widely represented in or being considered for screening programs in many jurisdictions across North America: galactosemia (GALT), tyrosinemia type I (TYRI), homocystinuria (HCY), sickle cell anemia (Hb SS), sickle cell/beta-thalassemia (Hb S/β-thal), sickle cell/hemoglobin C disease (Hb SC), and severe combined immunodeficiency (SCID). As such, this evidence assessment focuses on these seven conditions and the associated tests used to identify them, to assess the potential health economic impact of adding any or all of these conditions to the Alberta NMS Program. Note that Hb SS, Hb S/β-thal, and Hb SC are all hemoglobinopathies that fall under the broad classification of sickle cell disease (SCD); they are therefore grouped and referred to collectively in this report as SCD.

1.2 Alberta STE Evidence Assessment

Alberta Health's mission is to set policy and direction to improve health outcomes for all Albertans, support the well-being and independence of Albertans, and achieve a high quality, appropriate, accountable, and sustainable health system. The 2015-2020 Alberta Health Business Plan describes the desired outcomes, priority initiatives, and performance measures and indicators for the Ministry. One of the priority initiatives identified in the Business Plan is to increase Alberta's health system capacity for evidence-informed practice through data, clinical information systems, research, innovation, health technology assessment, and other structures as appropriate.

Accordingly, this evidence assessment is being conducted under the auspices of the Alberta Health Technology Decision Process (*http://www.health.alberta.ca/initiatives/AHTDP.html*). This process involves the use of appropriate evidence and information for decision-making regarding the public provision of health technologies and services. These assessments consider existing evidence and other information relevant to three areas:

- The *Social and System Demographics* assessment (**S** section) describes the profiles of illness and patterns of care, and identifies potential inequities in health status or care across population groups. Sources of evidence include:

[i] Secondary conditions can become evident through screening for primary targets, or are identified as part of the differential diagnosis of a primary target.

  - a comprehensive search of bibliographical databases and grey literature sources; and
  - consultation with targeted clinical and operational experts to provide information on screening program's characteristics, disease profiles, system capacity, and clinical care pathways for screening, diagnosis, treatment, and management.

- The *Technology Effects and Effectiveness* assessment (**T** section) provides an examination of the safety, risks, screening test performance, and benefits/harms of screening and early detection. Sources of evidence include a systematic and comprehensive search of bibliographical databases and grey literature sources including manual hand-searching of reference lists for relevant studies. A two-step research methodology was used:
  - First, a comprehensive database search was performed to identify the most recently published systematic reviews and HTA reports that evaluated screening test performance, treatment effectiveness, or effectiveness of newborn screening programs for the seven conditions.
  - Second, a comprehensive systematic review of primary studies was performed to update and complement the information retrieved from the first step.

  Quality assessment and data extraction was conducted using standard health technology assessment methodology.
- The *Economic Analysis* assessment (**E** section) evaluates the cost-effectiveness and budget impact of adding one or a combination of the seven conditions to the Alberta NMS Program, using the following approach:
  - An economic model was developed based on the care pathways and operational constraints found in the S section.
  - The model was populated with input data (for example, epidemiological, clinical, financial, and operational) obtained from the S and T sections, as well as other sources in the published literature.
  - All costs are reported in 2015 Canadian dollars.

The Alberta NMS Program uses a population-based screening approach to reduce the burden of disease in the community through early detection and treatment of select treatable conditions, with a goal to minimize the morbidity and mortality of Alberta infants.[ii] As such, this STE review will be guided by the generally accepted principles for decision-making for the introduction of population-based screening programs outlined by the Australian Population Health Development Principal Committee's Screening Subcommittee, in its document "Population Based Screening Framework" (Australian Framework).[iii] It is important to note that the scope of this review is to assess the impact of adding the seven aforementioned conditions to an already existing population-based screening program. There are criteria outlined in the Australian Framework which are oriented towards the

[ii] Alberta Health and Wellness, Community and Population Health Division. *Alberta newborn metabolic screening program policy document*. Edmonton (AB): Government of Alberta; 2010. Available from: *http://www.health.alberta.ca/documents/Newborn-Metabolic-Screening-Policy-2010.pdf*.

[iii] Australian Population Health Development Principal Committee, Screening Subcommittee. Population based screening framework. Australian Health Ministers' Advisory Council; 2008. Available from: *http://www.cancerscreening.gov.au/internet/screening/publishing.nsf/Content/population-based-screening-framework*.

requisite characteristics of a screening program; these were not considered in this review, given that evaluation of the existing screening program is outside the scope of this review.

The criteria outlined in the Australian Framework that were considered in this evidence review are summarized below. According to the framework, for a condition to be included in a population-based screening program:

- The condition must be an important health problem and have a recognizable latent or early symptomatic stage.
- The test for each condition must be highly sensitive and specific, be validated and safe, have a relatively high positive and negative predictive value, and be acceptable to the target population, including important subgroups.
- The treatment for each condition must be effective, available, easily accessible, and acceptable to all patients with the recognized disease or condition.
- There should be clear evidence that screening and treatment leads to better outcomes than finding and treating the disease at a later stage.
- Systems should be in place for evidence-based follow-up assessment of all people with a positive screen, regardless of rurality, ethnicity, socioeconomic status, or disadvantage status.
- Ongoing management referral protocols must be established for individuals who have the condition detected through the screening program.
- The overall benefits of screening outweigh the harm.

In the final section of this report (section 5), informed by the evidence assessments of the S, T, and E sections, we discuss if/how these criteria are met by adding the seven conditions to the Alberta NMS Program. We also examine the readiness of the health system to add some or all of the conditions to the program.

SECTION TWO: SOCIAL AND SYSTEM DEMOGRAPHICS

Arianna Waye, PhD

2.1 Introduction

Objective

The objective of the *Social and Systems Demographics* (**S**) section of this STE report is to provide information describing the patterns of care associated with early and late diagnosis of each of the seven conditions under review, as well as related health outcomes. The health system capacity in Alberta and general acceptability of screening and condition diagnostic testing will also be described.

Research Questions

The S section aims to address the following research questions from the project charter:

1. What is the nature of the condition?
    i. Is the condition well-defined?
    ii. Will variants of the condition be identified through screening?
        a) Are the variants clinically significant?
        b) Will the variants be clearly distinguished from the target condition at screening, or as part of the differential diagnosis?
    iii. Is the natural history and outcome of the disease known?
    iv. What are the characteristics of the latent or early symptomatic stage?
2. What is the prevalence and incidence of the condition?
    i. Is prevalence known to vary across populations?
    ii. If applicable, has there been an increase in observed prevalence in jurisdictions with newborn blood spot screening for the condition?
3. What is the burden of disease associated with the condition?
    i. Is the condition associated with significant morbidity and mortality if not treated, or not treated early before onset of symptoms?
    ii. Is there a period before onset of clinical disease during which medical intervention improves the burden of illness on the patient?
    iii. What is known about improvements in outcomes if the condition is diagnosed in the latent or early symptomatic stage?
    iv. If applicable, how has the spectrum of severity of the condition changed in jurisdictions with newborn blood spot screening?
4. Are there special considerations (i.e. serial screening) that would need to be understood for pre-term, low birth weight, and otherwise sick infants when screening for this condition?
5. Is the technology licensed by Health Canada and for what indications?
6. Is the test based on a platform that offers high throughput capability?

7. What ancillary information (e.g. about other conditions, carrier status) is generated by the screening test, if any?
8. What kind of expertise, resources, and capacity in the workforce/medical facilities are required to assess patients with a positive screening test?
9. Is there capacity in Alberta's current healthcare system to deliver the services including the screening tests as well as the follow-up assessments for all patients with a positive screen?
10. What is the current model of care related to the condition?
    i. What are the current diagnostic options/standard diagnostic procedures and monitoring approaches for the condition?
    ii. Are there clinical practice guidelines (Alberta, Canada, or international) for management of this condition?
11. Is there reason to be concerned about the acceptability of diagnostic investigations among families of screen-positive infants?
    i. Are there any issues related to disclosure of incidental findings (e.g. carrier status, secondary conditions)?
12. Is treatment available for the condition?
    i. What are the associated health outcomes with treatment?
    ii. Is the treatment available and accessible to all Albertans?
    iii. Is the effective treatment acceptable to the patient group?
    iv. Is treatment lifelong?
13. Is there evidence to support the acceptability of screening for this condition in newborns/children, among families of screened children, the public, and/or health professionals?
14. What additional resources (for screening, treatment, genetic counselling, education, etc.) are likely to be needed to support screening for this condition?
15. What is the treatment and ongoing management protocol?
16. How does treatment and ongoing management differ in cases of early diagnosis (before onset of symptoms) and later diagnosis?
17. How do outcomes vary between cases being managed after early diagnosis and later diagnosis?

Method

Sources of Information

Search strategy

The IHE information specialist conducted a comprehensive search of relevant bibliographic databases and grey literature sources to identify the best available evidence. Reference lists of the retrieved articles were checked for additional publications. Primary and review studies were searched for GALT, TYRI, HCY, and SCID. However, due to the volume of information on sickle cell

disease (SCD), only review articles were searched for the forms of SCD that are relevant to this report (that is, Hb SS, Hb S/β-thal, and Hb SC).

- **Bibliographic databases:** Medline (Ovid)
- **Grey literature resources:** Health Technology Assessment (HTA) agency websites, clinical trial registries, clinical practice guidelines, position statements, Health Canada coverage status, and Google
- **Reference lists:** from retrieved articles for relevant studies
- **Search terms:** keywords (including synonyms) and subject headings for newborns, for metabolic, endocrine, and hemoglobin disorders, and for the seven core conditions, along with a filter for social and system demographics information (see Appendix S.A).

Expert Advisory Group consultation

Consultation with targeted clinical and operational experts (collectively referred to as our Expert Advisory Group [EAG])[iv] occurred to provide information on screening program characteristics, disease profiles, system capacity, and clinical care pathways for screening, diagnosis, treatment, and management.

Study Selection

The initial study selection was conducted by one reviewer (AW), who selected studies using predefined criteria, based on titles or abstracts only. The final selection was based on the review of full-text articles. Abstracts that clearly did not meet the inclusion criteria were excluded. Copies of the full text of potentially relevant papers were retrieved and assessed for eligibility.

Published articles were included if they reported on etiology, epidemiology, pathology, prognosis, screening, diagnosis, treatment, and/or prevention of sequelae (that is, associated consequences of a condition and/or treatment for a condition) for patients with GALT, HCY, TYRI, SCD, or SCID.

Clinical practice guidelines were included if they provided recommendations for the treatment or management of any of the seven conditions. Clinical practice guidelines that focused on the diagnosis of the seven conditions were not included. Only evidence-based published guidelines were considered. Clinical practice guidelines that were not evidence-based, such as consensus statements containing recommendations based only on expert opinion, were included only if they were developed in Canada or provided relevant information regarding the current practice in Canada or Alberta.

Background

Currently, Alberta does not screen for GALT, TYRI, HCY, SCD, or SCID. As a result, patients with one of these conditions are identified after becoming symptomatic and presenting clinically. Due to the rarity and non-specific nature of the symptoms of these conditions, the underlying cause of symptoms is often not immediately evident. Therefore, there will be a long sequence of clinical diagnostic investigations and referrals prior to the eventual diagnosis of the condition (herein referred to as the 'diagnostic odyssey').

[iv] For a full list of EAG members and others on the project team, please see Appendix A, at the end of this report.

Patients with these conditions are eventually diagnosed using existing confirmatory tests, which are already well-established in Alberta. Given that patients are generally not identified prior to the onset of symptoms, they may experience clinical manifestations of the condition, also referred to as sequelae. Sequelae must then be managed as they are often irreversible, even with treatment.

Treatment and follow-up for each of the conditions under review is presently available in Alberta. Specifically, there are specialty clinics that specialize in each of the conditions under review: the metabolic clinics (including GALT, TYRI, and HCY), the hematology clinic (SCD), and the hematology/immunology clinic (SCID).

Children identified early with these conditions may experience reduced morbidity and mortality. This report will assess the evidence around the impact of identifying these patients early through screening, as compared to late through clinical presentation.

The remainder of the S section is organized in response to the charter questions posed above, which have been based upon the Australian Framework (see section 1.2). Specifically,

a) section 2.2 provides context for screening for these conditions, based upon programs in other jurisdictions, and highlights the existing newborn screening program and infrastructure available in Alberta;

b) section 2.3 describes what is known about each condition in terms of burden of illness, patterns of care associated with early and late diagnosis, as well as the associated health outcomes; and

c) section 2.4 describes the health system capacity in Alberta and how this system can be leveraged to accommodate additional conditions on the screening panel; and

d) section 2.5 discusses the general acceptability of diagnostic testing, treatment, and expanding the existing screening program.

2.2 Newborn Screening Programs

Newborn Screening Programs in Canada

There is variation in the inclusion of GALT, HCY, TYRI, SCD, and SCID across the provincial and territorial newborn screening programs in Canada (Table S.1). British Columbia screens for all seven conditions currently under review in Alberta except SCID, and SCID has been recommended for screening in the province. Manitoba screens for the three metabolic diseases (that is, GALT, TYRI, and HCY),[1] and has been examining the utility of screening for SCID.[2] Saskatchewan screens for GALT, TYRI, and HCY. Ontario screens for all seven conditions. New Brunswick, Nova Scotia, and Prince Edward Island currently screen only for SCD, though GALT, TYRI, HCY, and SCID have also been approved or recommended; these provinces all use a common laboratory service, the IWK Centre for Health, which has standardized screening. Newfoundland screens for TYRI and HCY. Quebec screens for TYRI, and is progressively phasing in SCD screening; the addition of HCY and GALT have also been recommended.[3]

Table S.1: Canadian jurisdictions screening for GALT, TYRI, HCY, SCD, and/or SCID (as of April 2015)

Province	GALT	TYRI	HCY	SCD (Hb SS, Hb S/β-thal, Hb SC)	SCID
British Columbia[4]	+	+	+	+	R*
Alberta	Under review	Under review	Under review	Under review	Under review
Saskatchewan	+	+	+		
Manitoba[1,2]	+	+	+		Under review*****
Ontario[5]	+	+	+	+	+
Quebec[6]	R	+	R	+**	
New Brunswick	A	A	A	+	A
Nova Scotia	A	A	A	+	A
Prince Edward Island* **	R	R	R	R	R
Newfoundland		+	+		
Northwest Territories** **					
Yukon** **	+	+	+	+	R

*Perinatal Services BC, personal communication, 2015

**Since November 2013, infants born in hospitals and birthing centres in Montreal and Laval regions receive screening for sickle cell anemia (Hb SS). Screening in other regions of Quebec will be progressively phased in.

***Prince Edward Island will be partnering with IWK Health Centre (used in Nova Scotia and New Brunswick) to increase the number of disorders being screened, including SCD and HCY, within the next two years (*http://www.iwk.nshealth.ca/page/newborn-screening-disorders-facts-questions-families*).

****Newborn screening for infants born in the Northwest Territories is currently conducted in Alberta, as a result, the screening panel is currently the same as Alberta. Newborn screening for infants born in the Yukon is currently conducted in British Columbia. As a result, the screening panel is currently the same as British Columbia.

*****According to the annual report for 2012-2013.[2]

R: recommended, pending funding approval; A: approved but not yet implemented

Source (unless otherwise noted): Alberta Health, personal communication, 2015

Newborn Screening Programs in the United States

All states in the United States require that newborns be screened; however, like in Canada, each state runs its own newborn screening program. As a result, newborn screening panels vary from state to state.

The United States Department of Health and Human Services' Recommended Uniform Screening Panel (RUSP) includes all seven conditions under review in Alberta, though not all states screen for all seven conditions. Specifically, 48 of the 50 states and the District of Columbia screen for TYRI (representing 95% of live births); thirty-two states currently screen for SCID;[v] and all 50 states and the District of Columbia screen for GALT, SCD, and HCY.[7]

[v] US states and territories screening all newborns for SCID as of July 2015 are: Arkansas, California, Colorado, Connecticut, Delaware, Florida, Hawaii, Illinois, Iowa, Maine, Massachusetts, Michigan,

Reduction in International Newborn Screening Programs

An international scan of jurisdictional screening programs was beyond the scope of this project. However, data were collected from studies that reported international jurisdictions had removed conditions from their screening panels. It was found that, Norway and Poland have elected to remove GALT from their screening panel due to the rarity of disease, and the reality that infants can clinically present before screening results can be reported.[8-13] In addition, given current methods of treatment, long-term complications still occur for the majority of cases, irrespective of whether the patient was screened.[8-11, 13, 14]

As of 1998, a number of programs discontinued screening for HCY, including: Austria (incidence 1 in 500,000), Belgium (no cases identified), Italy (incidence 1 in 55,000), and Scotland (incidence 1 in 1,000,000), as well as Switzerland, Australia, and New Zealand (incidence not reported).[15]

Current Newborn Screening Program in Alberta

The Alberta Newborn Metabolic Screening (NMS) Program was initiated in 1967 as a population-based screening program. At the time of initiation, screening was only conducted for phenylketonuria (PKU). Congenital hypothyroidism was added to the panel in 1977, and biotinidase deficiency in 1990.[16, 17] In 2007, the program expanded to screen for the current 17 conditions.[16] There were 53,770 registered live births in Alberta in 2013-2014, an increase of 6.35% since 2010-2011. In 2013-2014, 99.49% of registered infants in Alberta were screened for the 17 conditions on the current panel. Of the 53,575 infants screened in 2013-2014, 59 were found to have abnormal diagnostic results.[18]

According to a 2010 policy document by Alberta Health,[16] there are three main goals of the Alberta NMS Program:

1. Morbidity and mortality of Alberta infants with the screened disorders is minimized through timely and effective screening to allow the early diagnosis and treatment of affected infants.
2. All infants born in Alberta have timely access to newborn metabolic screening as an integral component of their health care. The program goal is that all infants have an initial screen reported on or before the tenth day of age.
3. Parents, health professionals, and the public are informed about the NMS Program.

Current Blood Spot Collection

In Alberta, screening is conducted between 24 to 72 hours of age (following informed verbal consent), using a blood spot sample collected through a heel prick. The blood spot is sent via routine lab courier to the Alberta NMS Laboratory and delivered within 72 hours of collection.

NMS Laboratory – Analysis of Blood Spot

The Alberta NMS Lab is located in Edmonton within the Department of Laboratory Medicine and Pathology at the University of Alberta Hospital. The NMS Lab performs tests for newborn screening and is equipped with instrumentation for the current blood spot screening program,

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Minnesota, Mississippi, Nebraska, New Hampshire, New Jersey, New Mexico, New York, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, Texas, Utah, Virginia, Washington, West Virginia, Wisconsin, Wyoming, District of Columbia, and the Navajo Nation.

including: two tandem mass spectrometers; two AutoDELFIA® automatic immunoassay systems; and one VICTOR²D™ fluorometer.

- Tandem mass spectrometer (MS/MS): Used to screen 13 of the 17 disorders currently on the panel (not cystic fibrosis, congenital adrenal hyperplasia, congenital hypothyroidism, or biotinidase deficiency). This platform qualitatively and quantitatively analyzes blood spot samples by separating them according to their molecular mass (size) and mass-to-charge ratio. MS/MS is automated, can analyze between 500 and 1,000 samples in 24 hours, and can screen more than 30 disorders at once.

  Specific kits are required for the analysis of target metabolites on the provincial screening panel.
- AutoDELFIA® automatic immunoassay system: Currently performs fluoroimmunoassay for cystic fibrosis, congenital adrenal hyperplasia, and congenital hypothyroidism.
- VICTOR²D™ fluorometer: Currently used to screen for biotinidase deficiency, and is not fully automated.

Abnormal Screen Results

Assuming an adequate screening sample is received, screen results will be reported as normal or abnormal. Inadequate samples (that is, any issue with sample or requisition that prevent accurate analysis) require collection of a repeat screen (Alberta Health, personal communication, 2015).

There are two categories of abnormal results that may be reported on a screen, critically abnormal and borderline abnormal (Alberta Health, personal communication, 2015). Infants with critically abnormal results are immediately referred to specialty clinics for assessment and confirmatory testing, and infants with borderline abnormal results will have a repeat screen arranged by zone public health nursing (Alberta Health, personal communication, 2015). The majority of infants with borderline abnormal results screen normal on the repeat screen; infants with persistent borderline abnormal screening results are referred to specialty clinics for assessment and confirmatory testing.[vi]

Confirmatory Testing

Genetic counsellors who work in the NMS Lab (lab genetic counsellors) are responsible for the notification of positive screen results to ordering physicians and specialty clinics. They may also notify families of positive screen results, if requested by the ordering physician.

Clinical assessment of infants with positive screen results is generally performed by clinicians at specialty clinics. Genetic counsellors may also work in specialty clinics (clinic genetic counsellors) and counsel families of patients during the process of confirmatory testing and potential diagnosis of heritable conditions. In the Calgary metabolic specialty clinic, this function is performed by trained nursing staff (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015). The physicians at the specialty clinics will arrange confirmatory testing, either before clinical assessment (as is often the case for SCD and SCID), or after clinical assessment (as is the case for metabolic conditions) (EAG members, personal communication, 2015).

[vi] The remainder of this section will refer to a positive screen as one that is either critically abnormal or persistent borderline abnormal.

Confirmatory testing is performed on all samples that screen positive. Details regarding confirmatory testing for conditions within the scope of this project can be found in section 2.3.

A genetic test may also be ordered by a physician simultaneously or sequentially to study a single gene mutation or, in some instances, to fully sequence a gene. Genetic testing for common mutations may be conducted in the province by the north and south Molecular Diagnostics Labs, located in Edmonton and Calgary, respectively. Rare mutation analyses are referred out of province, and require application from the referring physician and special approval by the province.

Treatment and Management at Specialty Clinics

Possible diagnostic outcomes of a positive screen include that the infant is a false positive, or that the infant has one of the screened conditions, a variant of the condition, or a secondary condition. Infants with positive confirmatory diagnoses will be treated and followed by the specialist physician and their allied healthcare team. Clinic details for conditions within the scope of this review can be found in section 2.4.

2.3 Conditions Under Review

Galactosemia (GALT)

Nature of Disease

Galactosemia (GALT) is a rare inherited condition that prevents or limits the digestion of galactose, due to a deficiency in the enzyme galactose-1-phosphate uridylyltransferase.[vii] Galactose is an element that makes up the sugar lactose, which is found in all milk products, including breast milk. Infants most often begin to experience the effects of GALT within the first few days of life because of feeding on breast milk or formulas containing milk.

Infants will typically present within the first two weeks of age with feeding problems, failure to gain weight, lethargy, and irritability. Infants who survive the first few weeks of life are likely to develop sequelae such as: cataracts, developmental delay (that is, mental retardation, growth delay, speech problems, and/or motor function issues), liver damage and potential failure, hypoglycemia, seizures, jaundice, bleeding, sepsis and, in females, premature ovarian failure.

Depending on the timing of clinical presentation, screening for GALT may prevent life-threatening symptoms as well as cataracts; the timing of result notification is critical, as infants may present within the first weeks of life. For more details, see the *Outcomes of Early Versus Late Treatment* section below, as well as the T section (specifically, section 3.2).

Epidemiology in Canada and Abroad

Incidence in Canada

There are no known Canada-wide estimates of incidence of GALT; however, estimates were located for samples drawn from provinces other than Alberta (see Table S.2). The Manitoba Perinatal Screening Program identified one infant with GALT out of 70,336 between 1983 and 1986, and

[vii] Galactose-1-phosphate uridylyltransferase is often abbreviated as 'GALT', and thus classic galactosemia is often referred to as 'GALT deficiency'. However, for clarity, the abbreviation is used in this report only to refer to the condition, and when used in the name for the gene that encodes the enzyme (GALT gene).

between 1966 and 1989, the reported incidence in Manitoba was 1 in 73,296.[19] A retrospective review of lab testing conducted in Ontario between 1977 and 1998 suggests an incidence rate of 1 in 75,833; this estimate is based upon extrapolating data from 18 cases over 21 years, given 65,000 births per year.[11]

Known differences in incidence across ethnic populations

The United States screening programs voluntarily report annual GALT case data to the National Newborn Screening and Genetic Resource Center (NNSGRC). The 2006 NNSGRC report suggests that GALT incidence in the United States is similar to Canadian estimates, as 62 cases were identified out of 4,171,306 screened, or 1 in 67,279.[20] As seen in Table S.2, based upon screening data, Austria, Hamburg, Ireland, Southern Iran, and Greece have found slightly higher incidence than either the United States or Manitoba, Canada.

All ethnic backgrounds are affected by GALT; however, as seen in Table S.2, based upon screening program data, the disease is more common in the Irish, Iranian, and Greek populations. Meanwhile, the condition appears to be much less common among the Japanese and Chinese/Taiwanese populations.

Table S.2: Galactosemia reported estimated incidence rates

Incidence, Canada (per number of births)	Incidence, USA (per number of births)	Incidence, international (per number of births)
Based on screening program *Manitoba* 1:73,296[19] Based on clinical presentation *Ontario* 1:75,833[11]	Based on screening program 1:30,000 to 1:67,279[20, 21]	Based on screening program *Hamburg* 1:39,000[22] *Ireland* 1:23,000[23] *Austria* 1:30,000[22] *Southern Iran* 1:6,000[24] *Greece* 1:22,182[25] *Japan* 1:788,969[26] *China/Taiwan* 1:419,286[26] Based on clinical presentation *Norway* 1:96,000[27] *Saudi Arabia* 1:8,333[28] Based on study sample *Brazil* 1:19,984[29] *New Zealand* 1:44,600[30]

Alberta Care Pathway (Diagnosis, Treatment, and Management)

The care pathway for GALT diagnosis (including diagnostic odyssey and confirmatory testing), treatment, sequelae, and follow-up in the context of screening and no screening can be found in Appendix S.B (Figure S.B.1).

Current Alberta care pathway – without screening

Diagnosis: Clinical presentation, diagnostic odyssey, and confirmatory testing

GALT patients will necessarily present clinically (unless an affected sibling has been identified) and cycle through a diagnostic odyssey prior to being diagnosed. The diagnostic odyssey begins when infants with GALT first develop symptoms and present clinically, which generally occurs very early in life. A retrospective study from Toronto, Canada found that, of the 18 GALT patients identified

(through lab results between 1977 and 1998), 17 were symptomatic at diagnosis, all presenting within 28 days of age and half within 14 days.[11] These infants clinically presented with jaundice (82%), cataracts (59%), hepatomegaly (41%), failure to thrive (41%), liver failure (29%), sepsis (24%), and hypoglycemia (17%). A number of differential diagnoses may be considered before an infant with GALT is identified; this process can take days to weeks in Alberta (EAG members, personal communication, 2015). Our EAG suggests that infants who present earlier are more likely to be diagnosed quickly in the diagnostic odyssey (EAG members, personal communication, 2015).

Confirmatory testing in Alberta is conducted at the Biochemical Genetics Lab (BGL) in Calgary (not Edmonton, as of yet), and includes a blood test to measure the amount of total galactose and galactose-1-phosphate (EAG members, personal communication, 2015). Genetic testing may also be conducted to determine if there are mutations to the GALT gene (which encodes galactose-1-phosphate uridylyltransferase); samples are sent to Seattle Children's Hospital for mutation analysis (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015). The costs of confirmatory testing for GALT are paid for by the government.

Treatment

Diagnosed GALT patients in Alberta are treated with a lifelong lactose-free diet, and infant formula is purchased by the family over the counter.

National or international best practice guidelines have yet to be established for GALT. A 2012 study suggests that, internationally, there is variation in what constitutes best practice,[9] and there is a need for more comparative studies to better understand the benefits and drawbacks of different approaches. Broadly speaking, providers seem to agree that a diet restricting lactose intake is necessary. A formula is generally recommended for infants, which is relatively inexpensive and can be purchased by the family over the counter. Older children and adults are asked to adhere to a lactose- and galactose-free diet, which means the following foods should be avoided: milk and dairy products; pre-packaged foods containing lactose; tomato sauces; some candies; and fruits and veggies that contain galactose.[31] No medications are prescribed for GALT, though some may be prescribed for comorbid conditions.

Disagreement can be found in the strictness of dietary recommendations related to the intake of certain types of foods containing galactose and dairy, as well as the type of formula to prescribe (soy or elemental). For example, some European providers will suggest that dietary restrictions extend beyond lactose and include other sources of galactose, including fruits and vegetables. Providers in the United Kingdom, the United States, Germany, and Mexico recommended less stringent restrictions on galactose intake. In support of a more liberal approach, a task force consisting of a metabolic physician, dieticians, and food scientists conducted a literature review and international survey of 138 dieticians, and recommend allowing fruits, vegetables, soy products that are not fermented, and some aged cheese.[32]

Management

Infants with GALT are monitored every three months at the metabolic clinics in Alberta, while children are followed up every six months and adults annually (EAG members, personal communication, 2015). Standard monitoring of these patients requires monitoring of galactose-1-phosphate in their blood. The purpose of these tests is to determine whether galactose is building up in the blood and is under control. If sequelae develop, management of sequelae will be required.

Potential Alberta care pathway – with screening

Compared to no screening, the major changes in the care pathway of infants identified by screening for GALT include avoidance of the diagnostic odyssey and early initiation of treatment (with dietary restrictions and infant formula) before the onset of symptoms, which may prevent cataract development and death, as is described in the next section.

Outcomes of Early Versus Late Treatment

Screening for GALT has the potential to prevent life-threatening symptoms that generally present within the first few weeks of life in affected individuals. As such, standard lifelong treatment with a lactose-restricted diet is expected to prevent life-threatening complications as a result of an anticipated reduction in the risk of death due to sepsis.[33]

Only three studies were identified that quantify mortality resulting from GALT. The most cited reference regarding the difference in GALT mortality as a result of screening is a meeting document from Washington, which suggests that, over a 10-year period, it can be expected that 4.76 unscreened children will die, compared to 0.3 children screened; no information is provided as to how these numbers were arrived at, and the total sample size is not indicated.[34] A retrospective study by Schweitzer-Krantz discusses 148 German GALT patients born between 1955 and 1989.[10] Universal screening for GALT was introduced in 1977. The study examines 49 GALT patients who were not screened and were identified clinically (late), and 99 who were screened before the onset of symptoms. Of the patients identified late, 19 of 49 died (39%), and of those identified through screening, only 1 out of 99 died (1%). Of the 19 who were identified late and died: four infants died between 7 and 14 days of age; five died between 15 and 21 days of age; nine died between 4 and 9 weeks of age; and one child died at 3 years of age. The last study located, Honeyman et al., reports the death of one infant at the age of 4 days, out of 60 GALT patients (identified through clinical presentation) in the UK between 1988 and 1990.[8] It is not clear if this infant had been screened or not, but, either way, the results of the screen would not have been available within four days after birth.

The evidence related to the effectiveness of treatment for GALT will be appraised in more detail in the T section (specifically, section 3.2). Briefly, studies examining long-term outcomes across screened and unscreened populations have found that fewer patients die in the screened group.[8, 10] However, the logistics of screening and the associated timing of reporting the results are critical to preventing life-threatening complications.[11] For example, studies suggest that between 47 and 86% of GALT patients are symptomatic before 14 days of age.[8, 14] As a result, screening programs must report results quickly to prevent life-threatening complications. Alberta NMS Program data between 1 April 2014 and 31 March 2015 show that 93% of infant results were reported within 7 days of age (Alberta Health, personal communication, 2015).

Despite the reduction in mortality given early treatment, there is evidence to suggest that, of the sequelae caused by GALT, early identification may be effective in reducing the incidence of cataracts only.[23, 33] There is little difference in the incidence of other sequelae across screened and unscreened GALT patients. Specifically, patients experience mental retardation, motor function, growth, verbal dyspraxia, and ovarian failure despite screening and early treatment.[8-10, 12, 22, 35-45] Health-related quality of life is negatively affected for people with GALT.[46-48] Quality of life is lower than for other conditions such as PKU because of cognitive and speech impairment, not from dietary restrictions with the burden of chronic disease.

The causal pathways of these long-term impacts are not well understood. Some theorize that the tissue damage is done in utero,[49] while others suggest that there may be some endogenous galactose production.[50]

Ancillary Information from Expanded Screening (Variants, Secondary Targets, and Carriers)

Classic GALT is the primary screening target; however, other variants of GALT may be detected. Specifically, Duarte syndrome, resulting from a benign mutation of the GALT gene, may be identified through screening, depending upon the cut-off values set when measuring the galactose-1-phosphate uridylyltransferase enzyme activity, and requires differential diagnosis from classic GALT.[43] The Duarte variant is estimated to be close to ten times as prevalent as classic galactosemia, and patients with Duarte syndrome may need little or no treatment.[51] Patients identified with Duarte syndrome in Alberta often receive an initial assessment and then are monitored for one to two years for complications (EAG members, personal communication, 2015).

A pilot study involving the biochemical and neurodevelopmental assessment of 28 Duarte patients examined the impact of lactose-restricted diet on long-term outcomes, including developmental, physical, and ophthalmological outcomes.[51] The results suggest that there is no difference for those with or without restricted diets across clinical or developmental outcomes. However, in a study on children screened with Duarte in Atlanta between 1988 and 2001, it was shown that 15% (5 out of 33) of 8-year-olds with Duarte had received special education, some of which was for speech problems, compared to 5.9% of children without Duarte.[52]

Other variants of GALT, galactose epimerase (GALE) deficiency and galactokinase (GALK) deficiency, will not be detected through screening if only the activity of the galactose-1-phosphate uridylyltransferase enzyme is measured because these conditions are identified by measuring total galactose. The lab has proposed measuring galactose-1-phosphate uridylyltransferase enzyme activity to screen for GALT, therefore GALK and GALE will not be detected.

Carriers of GALT may be identified through screening, depending upon the cut-off values set for enzyme activity using a VICTOR^{2}D™ fluorometer. Specifically, carriers will possess one healthy and one mutated gene, and as a result may exhibit slightly lower than normal GALT activity, despite enzyme activity of one normal gene.[53] As a result, these carriers may be identified, though not consistently.

The impact on prevalence rates following the introduction of a GALT screening program is not known. Evidence of GALT prevalence changes in other jurisdictions after the implementation of a GALT screening program was not located in this review. Given the rarity of disease and that patients will eventually present clinically, it is unlikely that the prevalence of GALT would change substantially following the initiation of a universal screening program. However, due to incidental findings from the program, the number of Duarte syndrome patients seen in the clinic may increase.

Tyrosinemia Type I (TYRI)

Nature of Disease

Tyrosinemia type I (also known as hepatorenal tyrosinemia) is a disorder that results in the inability to break down the amino acid tyrosine, due to a deficiency in the enzyme fumarylacetoacetate hydrolase (FAH), also known as fumarylacetoacetase. The buildup of upstream metabolites in the blood leads to liver damage including hepatocellular carcinoma and kidney damage, as well as

possible neurological crisis, rickets, and cardiomyopathy. The risk of hepatocellular carcinoma is an estimated 18 to 37% in untreated patients.[54]

Two clinical groups of TYRI have been characterized, based upon the severity of disease and timing of initial clinical presentation and subsequent diagnosis. Acute TYRI accounts for upwards of 75% of cases; these infants will generally present with early signs of liver failure in the first week of life.[55] Chronic TYRI is usually identified by the age of 6 months, based upon clinical presentation of liver and kidney dysfunction and poor weight gain.[55-57] A small number of infants (generally, pre-term) may experience transient hypertyrosinemia. It is speculated that this condition, which involves a rise in the level of plasma tyrosine during the first few weeks of life, occurs because of immature 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) in the liver,[58, 59] which over time matures and the condition spontaneously resolves. Most infants with transient hypertyrosinemia are asymptomatic, though some do temporarily experience lethargy and poor feeding.[58, 60] Currently in Alberta, patients identified with transient hypertyrosinemia cannot be differentiated from those with TYRI. Therefore, transient cases are treated and followed the same as those with true TYRI until the case is recognized as transient.

Untreated infants with TYRI who survive the first few months of life are likely to experience sequelae including: liver and/or kidney damage, hepatocellular carcinoma, and the need for liver treatment. Based upon data from Quebec, before population-wide screening, the mortality rate was upwards of 90% before the age of 2 years, mostly as a result of acute liver failure.[59] Of those receiving treatment, the 2-year survival rate is 38% if infants present with symptoms before the age of 2 months, 74% if infants present with symptoms between the ages of 2 and 6 months, and 96% if they first present with symptoms after the age of 6 months.[58] This evidence suggests that, the earlier symptoms develop, the poorer the prognosis. However, the 10-year survival rate declines for each of these groups to 30% for the 2 to 6 month group, and to 60% for those diagnosed after 6 months.[55, 60]

Screening for TYRI is expected to reduce the incidence of liver damage and hepatocellular carcinoma and the need for liver transplantation, as well as kidney damage. Mortality rates in this group would also be expected to decline significantly as a result of screening. For more details, see the *Outcomes of Early Versus Late Treatment* section below, as well as the T section (specifically, section 3.2).

Epidemiology in Canada and Abroad

Incidence in Canada

The incidence of TYRI in Alberta is not known. The incidence in Canada based on other provinces' newborn screening factsheets is 1 in 100,000 (see Table S.3).[5, 61] No Canadian primary studies estimating TYRI were located outside of Quebec.

Incidence rates are known to be higher in Quebec. Based upon screening program data between 1974 and 1982, 58 cases of TYRI were identified in Quebec, or 1 in 16,000, and between 1983 and 1988, 20 cases were identified, or 1 in 29,000.[62] Of these, there was a disproportionate amount from the Saguenay–Lac-Saint-Jean region, with an incidence of 1 in 1,042 between 1967 and 1986, and of 1 in 1,846 between 1982 and 1992 (see Table S.3).

Known differences in incidence across ethnic populations

The incidence worldwide is an estimated 1 in 100,000 to 1 in 120,000.[63] These worldwide estimates agree with results from a retrospective study of a regional screening program in the United Kingdom between 1985 and 1994 within the Birmingham and Midland health region, where the incidence rate is an estimated 1 in 105,000 (see Table S.3).[64] Incidence rates in the United States have not been well studied; the 2000 NNSGRC report suggests that, of 393,491 infants screened in 2000 in Georgia, Maryland, Massachusetts, and North Carolina, no cases were identified.[65] No other primary studies from the United States were located.

Most of the reported cases are in Quebec and Norway.[59, 66] Based upon patients who have clinically presented in Norway, the reported incidence is 1 in 74,800,[66] where 14 tests were found positive out of 1,181,636 TYRI lab tests conducted (at the only lab offering selective metabolic screening). A point estimate of the undiagnosed patients was made based upon the age of patients and the time of diagnosis, calculating the estimated number of undiagnosed cases between 1991 and 2010 to be 1.8.

Table S.3: Tyrosinemia type I reported estimated incidence rates

Incidence, Canada (per number of births)	Incidence, USA (per number of births)	Incidence, international (per number of births)
Based on fact sheets *Ontario & British Columbia* 1:100,000[5, 61] Based on screening program *Saguenay–Lac-Saint-Jean, Quebec* 1:1,846[62] *Quebec* 1:16,000-29,000[62]	Based on screening program *Georgia, Maryland, Massachusetts, and North Carolina* 0:393,491[65]	Based on screening program *Birmingham and Midland, UK* 1:105,000[64] Based on clinical presentation *Norway* 1:74,800[66]

Alberta Care Pathway (Diagnosis, Treatment, and Management)

The care pathway for TYRI diagnosis (including diagnostic odyssey and confirmatory testing), treatment, sequelae, and follow-up in the context of screening and no screening can be found in Appendix S.B, Figure S.B.2.

Current Alberta care pathway – without screening

Diagnosis: Clinical presentation, diagnostic odyssey, and confirmatory testing

In order for infants to be identified, they will necessarily present clinically and cycle through the diagnostic odyssey prior to being diagnosed.[viii] It can take days to weeks in Alberta to diagnose infants with TYRI (EAG members, personal communication, 2015).

Seventy-seven percent of affected infants suffer an acute form of TYRI and are expected to present clinically with TYRI within weeks of being born; the remainder suffer from chronic TYRI, and tend to present later.[67] Most acute TYRI patients present with liver failure, renal tube dysfunction, or kidney disease before the age of 6 months. In a study of 168 TYRI patients from 21 centres (Europe, Israel, and Turkey), 118 were not screened and were diagnosed because of clinical presentation.[68] Forty of the 118 patients had only one symptom at presentation, being: acute liver

[viii] Unless a sibling has been affected by TYRI, then it may be possible to identify the child prior to clinical presentation without screening.

failure (42%), liver dysfunction (18%), hepatomegaly (5%), cirrhosis (12.5%), nephromegaly (2.5%), renal dysfunction (7.5%), and rickets (2.5%). Seventy-four patients presented with multiple sequelae, mostly liver dysfunction along with renal dysfunction or rickets. A number of differential diagnoses may be considered before a child clinically presenting with TYRI is identified, resulting in a delay of diagnosis. The average age of diagnosis in the above study was 15.5 months.

Confirmatory testing in Alberta is conducted at the BGL in Calgary, and consists of measuring tyrosine in the blood, as well as an enzyme test measuring FAH. Genetic testing may also be conducted to determine if there are mutations to the FAH gene (which encodes the FAH enzyme); samples are sent to Seattle Children's Hospital for mutation analysis (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015).

Though Alberta does not presently screen for TYRI in blood spots, infants with elevated tyrosine levels may be identified through the current screening program, specifically through screening for phenylketonuria (PKU), which measures phenylalanine and tyrosine levels in blood spots. If an infant is found to have elevated tyrosine based on the PKU screen, a second blood spot will be collected four to six weeks later. Infants with persistently elevated tyrosine levels will be referred for confirmatory diagnostic testing. Not all cases of TYRI will be detected through measurement of tyrosine as a result of low specificity in testing for tyrosine, as is described in the T section (specifically, section 3.2); in addition, given these methods, it is not possible to differentiate between transient hypertyrosinemia and TYRI through the measurement of tyrosine alone.

The costs of confirmatory testing for TYRI in Alberta are paid for by the government.

Treatment

In Alberta, patients diagnosed with TYRI are treated for life with medication (2-(2-nitro-4-triflouromethyl-benzoyl)-cyclohexane-1,3-dione or NTBC, also known as nitisinone), as well as with dietary modifications and supplements.

NTBC (brand name Orfadin®) prevents the formation of maleylacetoacetic acid and fumarylacetoacetic acid, both of which may be converted to succinylacetone, which is toxic to the liver and kidneys. Patients have access to NTBC with the treatment cost covered through the metabolic clinic or the Health Canada Special Access Program (EAG members, personal communication, 2015).

Dietary supplements provided to TYRI patients contain all amino acids except tyrosine, phenylalanine, and methionine (EAG members, personal communication, 2015).[56, 58] Dietary modifications include a diet low in tyrosine, phenylalanine, and methionine. The degree to which diets are restricted has been shown to vary in the literature, as does the age at which restrictions are relaxed.[68] The costs of TYRI dietary formula in Alberta varies depending upon age and weight of the patient (EAG members, personal communication, 2015). These costs are also paid for out of the metabolic clinic operational budget (Metabolic Clinic, Alberta Health Services, personal communication, 2015).

If patients are diagnosed after the onset of liver and kidney damage, transplantation may be necessary.

While clinical guidelines have not yet been developed for the management and treatment of TYRI, two review articles have been published describing treatment options for TYRI patients.[56, 58]

Management

TYRI patients in Alberta are most commonly followed up every three to six months until the age of 18 years, and annually through adulthood (EAG members, personal communication, 2015). Blood and urine tests are collected to monitor amino acid levels, NTBC levels, liver and kidney function, and succinylacetone levels.[31] If sequelae develop, management of sequelae will be required.

Potential Alberta care pathway – with screening

Compared to no screening, the major changes in the care pathway of infants identified by screening for TYRI include avoidance of the diagnostic odyssey and early initiation of effective treatment (with NTBC and dietary restrictions/supplements) before the onset of liver and kidney complications, which may prevent severe liver/kidney damage and liver carcinoma and associated death, as is described in the next section.

Outcomes of Early Versus Late Treatment

NTBC, the first-line medication to treat TYRI, has been on the market for nearly 25 years. However, there are few studies to monitor the long-term effectiveness of treatment. Three studies have shown that, based upon nine years or less of follow-up, the treatment of TYRI with NTBC has reduced mortality and largely mitigated acute liver and kidney complications associated with TYRI for patients who responded to NTBC treatment.[54, 68, 69] In addition, it is found that those who are responders who were treated late (after 2 years of age) experience a range of sequelae, and NTBC is said to help alleviate some of the symptoms.[70] However, those who are treated late are at higher risk of sequelae, including liver failure, liver carcinoma, hepatomegaly, rickets, renal dysfunction, and risk of death.[68] Please refer to the T section for a detailed appraisal of this evidence (specifically, section 3.2).

Ancillary Information from Expanded Screening (Variants, Secondary Targets, and Carriers)

There are two variants of TYRI, tyrosinemia type II (TYRII), also called oculocutaneous tyrosinemia or Richner-Hanhart Syndrome, and tyrosinemia type III.[71, 72] Neither of these variants, nor transient hypertyrosinemia, will be identified through screening for TYRI through the measurement of succinylacetone. More information about variants of TYRI can be found in the T section (specifically, section 3.2).

Carriers of TYRI are not identified through screening.

The impact on prevalence rates following the introduction of a TYRI screening program is not known. Evidence of TYRI prevalence changes in other jurisdictions after the implementation of a TYRI screening program was not located in this review. However, TYRI is a very rare condition and patients will eventually present clinically, and, therefore, very little change in the prevalence of the disease would be expected as a result of screening.

Homocystinuria (HCY)

Nature of Disease

HCY is a disorder that results in the inability to break down the amino acid homocysteine, most often due to a deficiency in the enzyme cystathionine beta-synthase (CBS), resulting from a mutation of the CBS gene (which encodes the CBS enzyme). Homocysteine is produced from the

biosynthesis of another amino acid, methionine, found in foods such as breast milk and formulas. The result of the inability to break down homocysteine is a buildup of homocysteine and methionine to toxic levels in the body, which is associated with serious permanent health problems including strokes, developmental delays (mental and growth), and eye problems.[73, 74] There is variation in the severity of clinical symptoms experienced by people with HCY.

The natural history of HCY has been described by Mudd et al., using the results from an international questionnaire of 629 HCY patients to describe the effect of untreated HCY on ocular, skeletal, neurological, and vascular systems.[74] In particular, eye problems related to nearsightedness often occur after 12 months, which for 55 to 82% of patients is expected to result in lens dislocation by age 10 years, and in some cases glaucoma. Some may also experience seizures, osteoporosis (36 to 64% by age 15 years), psychiatric problems, and dystonia. Twenty-eight percent of HCY patients without treatment would be expected to have a thrombolytic event by age 15 years.[73, 74] Vascular events are the major cause of mortality in patients with HCY, and, without treatment, an estimated 4 to 23% will not survive to the age of 30 years.[74, 75] Mudd et al. suggest that the nature of disease is more severe for untreated patients that are non-responders to treatment, as indicated by the upper limit of each of the risk estimates listed above.[74]

Screening for HCY may not be expected to prevent mortality; however, it is likely that the incidence of select sequelae, including thromboembolic manifestations, ocular manifestations, and mental retardation, will be reduced for those who respond to treatment. For more details, see the *Outcomes of Early Versus Late Treatment* section below, as well as the T section (specifically, section 3.2).

Epidemiology in Canada and Abroad

Incidence in Canada

The incidence of HCY in Canada is not known. HCY is very rare, and is said to affect approximately 1 in 200,000 to 300,000 infants in Ontario (see Table S.4),[5] though the data from which these estimates were drawn could not be identified. No other Canadian estimates were located in this review. Based upon personal communication with the Newborn Screening Program in British Columbia, no cases have been found over the last five years, given 225,000 infants screened (BC Children's Hospital, personal communication, 2015).

Known differences in incidence across ethnic populations

International HCY incidence rates indicate that different ethnicities are differentially impacted (see Table S.4), based upon data from screening programs in the United Kingdom, Ireland, and Qatar. In the United Kingdom between 1970 and 1977, the screening program at The Hospital for Sick Children in London identified three infants with HCY out of 670,634 screened using Guthrie cards, including one infant with a transient form; the incidence is therefore 1 in 223,588.[76] Ireland has screened for HCY since 1971, and, since 1998, 1.58 million newborns have been screened with 25 cases identified from 19 families, resulting in an estimated incidence of 1 in 65,000.[77] In Qatar, between 2006 and 2009, 46,406 infants were screened using MS/MS, and, in this time, 14 infants were diagnosed with HCY; based on these data, the incidence is 1 in 3,000.[78] This study suggests that native Qatari's may have an even higher incidence of 1 in 1,800.

Mudd et al. estimate the worldwide rate to be 1 in 344,000.[79] This cumulative rate of detection is based upon rates of detection from 13 countries, each of which screened at least 200,000 infants for HCY. Across these 13 countries, 145 cases were detected out of 49,886,727 newborns screened.

Other studies have estimated the incidence of HCY by genotyping blood samples and identifying CBS gene mutations. The incidence of HCY is then calculated on the basis that infants with two mutated alleles will have HCY. Using these methods of estimation, a study of 5,000 blood samples taken from the Rikshosptalet University Hospital in Oslo suggest an incidence of 1 in 20,500.[80] Similarly, a study of 600 blood samples in central Europe suggest an incidence of 1 in 40,000.[81]

Table S.4: Homocystinuria reported estimated incidence rates

Incidence, Canada (per number of births)	Incidence, USA (per number of births)	Incidence, international (per number of births)
Based on fact sheets *Ontario* 1:200,000-300,000[5] Based on screening programs *British Columbia* 0:225,000 (BC Children's Hospital, personal communication, 2015)	Based on screening programs 1:291,000[79]	Based on screening programs *Thames Regional Health Authority, London UK* 1:223,588[76] *Ireland* 1:65,000[77] *Qatar* 1:3,000[78] Based upon study sample *Central Europe (Czech)* 1:40,000[81] *Norway* 1:20,500[80]

Alberta Care Pathway (Diagnosis, Treatment, and Management)

The care pathway for HCY diagnosis (including diagnostic odyssey and confirmatory testing), treatment, sequelae, and follow-up in the context of screening and no screening can be found in Appendix S.B, Figure S.B.3.

Current Alberta care pathway – without screening

Diagnosis: Clinical presentation, diagnostic odyssey, and confirmatory testing

In order for infants to be identified, they would necessarily present clinically and cycle through the diagnostic odyssey prior to being diagnosed.[ix] The duration of the diagnostic odyssey for HCY patients tends to vary considerably, which may be partially due to the variation in symptom severity with which individuals with HCY typically present.[82] In a study of 34 consecutive patients with HCY in the Netherlands, 88% presented with ectopia lentis, glaucoma, scoliosis, marfanoid features, mental retardation, epilepsy, and/or psychosis.[82] First signs of clinical presentation for HCY has been found to be around age 13 years on average (ranging from 1 to 40 years of age), and the average age of diagnosis is age 24 years (ranging from 1 to 61 years of age).[82] Therefore, the average delay between the onset of clinical symptoms and timing of diagnosis is approximately 11 years (ranging from 0 to 43 years of age).

Confirmatory testing in Alberta is conducted at the BGL in Calgary, and consists of measuring total plasma homocysteine, as well as enzyme testing, measuring CBS (see the T section for more details) (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015). Genetic testing may be conducted as requested by the physician, and is conducted at the Mayo clinic (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015). The costs of these tests are paid for by the government.

[ix] Unless a sibling has been affected by HCY, then it may be possible to identify the child prior to clinical presentation without screening.

Treatment

Clinical guidelines have not yet been developed for the management and treatment of HCY.

In Alberta, all patients are treated lifelong with vitamin B6 and betaine (Metabolic Clinic, Alberta Health Services, personal communication, 2015). Lifelong treatment with vitamin B6, also known as pyridoxine, helps to reduce homocysteine in the blood, and betaine achieves biochemical control for HCY patients. Recommended supplements to be taken in conjunction with vitamin B6 include folic acid, vitamin B12, and vitamin C. Vitamin B6, betaine, and the recommended supplements are paid for by the family/patient, and are available through pharmacies.

Depending upon the patient's response to vitamin B6, patients in Alberta may also be advised to make dietary modifications and take dietary supplements. An estimated 10 to 50% of HCY patients do not respond to treatment.[82, 83] Lifelong treatment for vitamin B6-nonresponders include dietary restrictions, betaine, and dietary supplements.[84] Specifically, patients may be asked to adhere to a diet low in methionine, which may consist of restricting cow's milk, regular formula, meat, dairy, regular flour, and nuts. A cysteine dietary supplement is also provided to compensate for the deficiencies that could be caused from the dietary restrictions. The cost of the formula supplement varies, depending upon age and weight (EAG members, personal communication, 2015). These costs are paid for out of the metabolic clinic operating budget (Metabolic Clinic, Alberta Health Services, personal communication, 2015).

Management

Infants with HCY who are vitamin B6-responsive are monitored every three months at the metabolic clinics in Alberta, older children are followed up every three to six months, and adults annually (EAG members, personal communication, 2015). Patients who are not responsive to vitamin B6 will be seen more frequently. Standard monitoring of these patients requires monitoring levels of homocysteine to determine whether it is building up in the blood, or is under control.

Potential Alberta care pathway – with screening

Compared to no screening, the major changes in the care pathway of infants identified by screening for HCY include avoidance of the diagnostic odyssey and early initiation of treatment, which may reduce morbidity associated with thromboembolic manifestations, ocular manifestations, and mental retardation, as is described in the next section. Management of chronic complications will be the same across those screened and not screened. If sequelae develop, management of sequelae will be required.

Outcomes of Early Versus Late Treatment

Evidence of screening for HCY has not shown the potential to prevent mortality. Based on relatively few studies, there is little evidence to substantiate speculative differences in outcomes across those who are treated early as compared to late. There is some evidence to suggest that those treated early experience fewer ocular issues, in terms of lens dislocation and vascular events.[85, 86] In addition, vitamin B6-responsive patients had a significantly higher IQ than those who were non-responsive, late-detected, or not treated.[87] Please refer to the T section (specifically, section 3.2) for a detailed appraisal of this evidence.

Ancillary Information from Expanded Screening (Variants, Secondary Targets, and Carriers)

Two phenotypic clinical variants are identified through screening, vitamin B6-responsive HCY, and vitamin B6-non-responsive HCY.

Other conditions that may be identified based on screening and later differentially diagnosed from HCY include methionine adenosyltransferase (MAT) I/III deficiency (persistent hypermethioninemia without elevated homocysteine or tyrosine, caused by mutations in the *MAT1A* gene), cystanthioninemia, and glycine n-methyltransferase deficiency; the course of treatment for these disorders is uncertain, as they are very rare.[88, 89] British Columbia has been screening for HCY for five years and no cases of HCY have been identified; however, patients with the aforementioned variant conditions have been identified (BC Children's Hospital, personal communication, 2015). More information can be found in the T section (specifically, section 3.2).

Carriers of HCY are not identified through screening.

The impact on prevalence rates following the introduction of a HCY screening program is not known. Evidence of HCY prevalence changes in other jurisdictions after the implementation of a HCY screening program was not located in this review. However, HCY is a rare condition, and therefore very little change in the prevalence of the disease would be expected as a result of screening.

Sickle Cell Disease (SCD) (Hb SS, Hb S/β-thal, and Hb SC)

Nature of Disease

The hemoglobinopathies are a clinically heterogeneous group of inherited disorders associated with mutations in both the α-globin and β-globin genes, and are broadly classified as sickle cell disorders (SCD) and thalassemias.[90] Hemoglobin (Hb) variants are caused by changes that alter the structure of the affected globin chain(s), whereas mutations that lead to reduced or absent synthesis of the affected globin chains cause thalassemias.[91, 92] Sometimes these characteristics are not mutually exclusive, whereby some globin chain variants are also synthesized at reduced levels.[92] This review focuses on SCD.

SCD is an autosomal recessive disease, which means that gene mutations must be inherited from both parents; the inheritance of the gene for hemoglobin S in a homozygous state (Hb SS), or with the gene for another abnormal hemoglobin (for example, Hb SC), results in the production of abnormal sickle-shaped red blood cells.[93] The underlying cause of clinical complications is a sickling of hemoglobin in response to conditions where oxygen concentration is reduced.[94] Red blood cells will "sickle", or change from a normal shape to a crescent shape. Re-oxygenating can help these blood cells to recover; however, after a few episodes, they will not regain their shape.[94] These affected blood cells do not flow through the body as easily as normal red blood cells; sickled red blood cells can block blood vessels (sickling crisis), as well as cause tissue and organ damage, strokes, and alterations to the immune system.[95]

The two key features of SCD are chronic hemolytic anemia and vaso-occlusion.[96] One of the most detrimental effects of sickling is vaso-occlusion within the spleen, which results in functional asplenia in 94% of sickle cell anemia patients by age 5 years.[97] With functional asplenia, the patient can no longer filter waste product such as damaged sickle cells or bacteria from the blood.[97] The spleen is especially important in the removal of encapsulated organisms in children under the age of

2 years who are unable to develop antibodies to encapsulated organisms (for example, *Streptococcus pneumoniae*).[97] As a result, children with SCD are also more susceptible to infections, particularly respiratory infections, which are often caused by pneumococcal bacteria.[94] *Streptococcus pneumoniae* infections often progress quickly, with death in less than 24 hours from onset.[97] Infection occurs in patients with SCD from infancy, with the highest risk before the age of 3 years, and significantly lower risk in older children and adults.[98]

Infants with SCD appear normal at birth; life-threatening symptoms may develop within the first few months of life, or more gradually with chronic sequelae later in adolescence. In early childhood, symptoms of the disease are unlikely before the age of 4 months, and only approximately 6% of affected infants will be symptomatic by the age of 6 months. About 32% of Hb SS patients become symptomatic by age 1 year, 61% by age 2 years, 78% by age 3 years, and 90% by age 5 years.[93] The first symptoms often appear between 3 and 9 months of life, and thus early implementation of medical care can prevent complications.[99] The peak incidence of death occurs in the first three years.[93]

About 40 years ago, the median life expectancy of American patients with sickle cell anemia was only 14.3 years, primarily because of high early mortality.[100] More recently, the natural history of disease has been best described for Hb SS in two studies, finding that the median life expectancy for male patients is between 42 and 53 years of age, and for female patients between 46 to 58.5 years.[94] The mortality rate curve has been shown to be U-shaped, with higher rates under age 10 years and over age 20 years.[94] This can be attributed to the fact that the causes of death are different for children as opposed to adults.[101] School-aged children will more often die of infection, acute chest syndrome, cerebrovascular complications, and pulmonary artery hypertension.[101] Meanwhile, adults will more often die from chronic end organ dysfunction, thrombotic disease, and treatment-related complications.[101]

Evidence suggests that screening for SCD (Hb SS, Hb SC, Hb S/β-thal) can prevent infection and resulting sepsis, a life-threatening condition, and allow for the education of parents on disease management sooner.[102] Parental education to recognize signs of the disease may help reduce disease morbidity from infection and splenic sequestration, as children who are not screened typically present with these preventable sequelae. Early identification will reduce mortality. For more details, see the *Outcomes of Early Versus Late Treatment* section below, as well as the T section (specifically, section 3.3).

Types of SCD

The most common and severe form of SCD, sickle cell anemia or Hb SS, is the homozygous state for Hb S, a variant of Hb A (normal adult Hb) caused by a Glu6Val mutation on the β-globin gene to become β^S (that is, the sixth amino acid in the gene, glutamic acid, is replaced by valine).[103] Hb SS accounts for approximately 60 to 65% of SCD cases.[104] Heterozygous forms of SCD result from coinheritance of β^S with one of several other abnormal β-globin genes. The most common interacting variants include Hb C (Hb SC), accounting for 25 to 30% of all SCD cases, and β-plus thalassemia (Hb S/β^+-thal) and β-zero thalassemia (Hb S/β^0-thal), accounting for 5 to 10% of all SCD cases.[104] Other less common β-globin mutations that lead to SCD when coinherited with Hb S include Hb O-Arab, Hb D-Punjab, and Hb E.[104]

In general, Hb SS and Hb S/β^0-thal are the most severe forms of SCD, and they may be clinically indistinguishable. In comparison, Hb SC and Hb S/β^+-thal are usually less severe.[96]

It is important to differentiate the underlying mutations causing SCD, because different forms of the disease have different sequelae and clinical significance (see Table S.5.). An estimated 700 to 1,000 hemoglobin variants can be identified, depending upon the screening platform.[105] Only 25 to 30 variants are considered clinically significant; these include the four main genotypes of SCD: Hb SS, Hb SC, Hb S/β^+-thal, and Hb S/β^0-thal.[106] However, it should be noted that SCD has been shown to exhibit significant phenotypic variability even within consistent genotypes, as patients with the same mutation can have vastly different clinical sequelae.

Table S.5: Primary targets and variants of SCD, including severity of disease and proportion of all cases or rarity

Name	Severity of SCD	Proportion of cases or rarity*
Primary target		
Hb SS	Severe[107]	60-70% of SCD cases[104]
Hb SC	Moderate[107]	25-30% of SCD cases[104]
Hb S/β^0-thal	Severe[107]	5-10% of SCD cases[104]
Hb S/β^+-thal	Moderate[107]	
Variants of SCD		
Hb S/Hb variant**	Variable[107]	Rare[104]
Hb S/Hb E disease	Mild[107]	Southeast Asia[104]
Secondary targets		
α thalassemia	Severe (Hb Bart); moderate to severe (Hb H disease),[108] all others variable	Variable
β-thalassemia major and intermedia	Severe (β-thal major); moderate (β-thal intermedia)[109, 110]	Variable
Hb EE	Asymptomatic, mild[111-113]	Asia
Hb CC	Mild to moderate[114]	Rare
Hb S-HPFH	Asymptomatic, mild	Rare

Note: The proportions in this table assume African descent. Different ethnicities are expected to have different incidence rates and distribution of primary targets and variants.

*Rare denotes incidence rates of less than 1 in 50,000; Very rare denotes incidence rates of less than 1 in 100,000

**For example, Hb O Arab, Hb D, Hb C Harlem, Hb Antilles, Hb Oman, Hb Quebec-CHORI

Epidemiology in Canada and Abroad

Incidence in Canada

The incidence of SCD in Alberta is unknown; however, estimates exist for other parts of Canada (see Table S.6).[x] Based on the Ontario Provincial Hemoglobinopathy Reference Laboratory, between 1978 and 2004, it has been estimated that 23 SCD cases clinically present and are identified

[x] Currently, one clinic in Alberta has 68 Hb SS patients, 10 Hb SC patients, and five Hb S/β-thal. However, it is not possible to infer incidence based upon these data.

per year, totaling 1 in 5,650 live births.[115] British Columbia, on the other hand, has screened 224,526 infants for SCD since 2010, and identified 11 cases of Hb SS (1 in 20,411), one case of Hb SC (1 in 224,526), and one case of Hb S/β-thal (1 in 224,526), for an overall SCD incidence of 1 in 17,241 (BC Children's Hospital, personal communication, 2015).

A Quebec HTA estimates an incidence in Quebec of 1 in 1,851.[116]

A more recent poster presentation[117] based on data from Newborn Screening Ontario finds that, between 2006 and 2010, 174 infants out of 476,382 (1 in 2,737) were affected by hemoglobinopathies; however, based upon these data, it is not possible to estimate the incidence of SCD separately.

Known differences in incidence across ethnic populations

The variation in incidence identified across provinces is likely due to known variation in the genetic disorder across ethnicities. In particular, sickle cell anemia is prevalent where malaria was endemic (that is, in sub-Saharan Africa, India, and the Arabian Peninsula),[94] as heterozygous carriers are protected against malaria.[116]

Migration from these regions has resulted in hemoglobinopathies including SCD becoming much more common in Europe and North America (see Table S.6). Estimated hemoglobinopathy incidence in the United States (based upon screening program data) is 1 in 6,579[118] and, as expected, there is wide variation across ethnicities within the United States, with SCD incidence highest among the African-American population. SCD is said to be the most common hereditary disease in France and England.[94, 119, 120]

Table S.6: Hemoglobinopathy reported estimated incidence rates

Incidence, Canada (per number of births)	Incidence, USA (per number of births)	Incidence, international (per number of births)
Based on screening program *Ontario (hemoglobinopathy)* 1:2,737[117] *British Columbia (SCD)* Hb SS 1:20,411; Hb SC 1:224,526; Hb S/β-thal 1:224,526; Hb EE 1:56,131 (BC Children's Hospital, personal communication, 2015) Based on clinical presentation *Ontario (SCD)* 1:5,650[115] Based on study sample *Quebec (SCD)* 1:1,852[116]	Based on screening program *California (hemoglobinopathy)* 1:6,579[118] Based on study sample *African-American population (hemoglobinopathy)* 1:365 to 1:500[101, 121]	Based on screening program *England (SCD)* 1:2,000[119] Based on targeted screening program *France (SCD)* 1:674[120] Based on clinical presentation *Africa (SCD)* 1:57,[122] 1:54[123] Based on study sample *Europe (hemoglobinopathy)* from 1:156 (Cyprus) to 1:10,000 (Luxembourg)[102] *Greece* 1:139[102] *Middle East (hemoglobinopathy)* 1:1,190[124]

Assessing the transferability or generalizability of incidence rates requires consideration of the underlying populations at risk, given the known variation across ethnic backgrounds. Table S.7 outlines select visible minorities across select provinces in which SCD incidence data exists in terms of the distribution of these visible minorities most "at risk". Quebec and Ontario have high incidence of SCD relative to British Columbia, which is thought to be related to the number of visible minorities, in particular black (6- to 16- fold difference) and Arabic (12- to 13- fold

difference) populations. The incidence rate in Alberta is unknown. However, given that the Alberta population more closely resembles the population in British Columbia relative to Ontario or Quebec, it is likely that the incidence rate of SCD more closely resembles that of British Columbia.

Table S.7: Visible minority population, by province (2011 data)

	Alberta	British Columbia	Quebec	Ontario	Canada
Black	74,435	33,260	243,624	539,205	**945,665**
Arab	34,920	14,090	166,260	151,645	**380,620**
Multiple visible minority	18,840	31,160	17,425	96,735	**171,935**

Source: Statistics Canada, 2011 National Household Survey[125]

Alberta Care Pathway (Diagnosis, Treatment, and Management)

The care pathway for SCD diagnosis (including diagnostic odyssey and confirmatory testing), treatment, sequelae, and follow-up in the context of screening and no screening can be found in Appendix S.B, Figure S.B.4.

Current Alberta care pathway – without screening

Diagnosis: Clinical presentation, diagnostic odyssey, and confirmatory testing

In order for infants to be identified without screening, they would necessarily present clinically and cycle through the diagnostic odyssey prior to being diagnosed. The diagnostic odyssey is quite variable, as it depends on when SCD patients first present and are subsequently diagnosed. A number of differential diagnosis may be considered before a child with SCD is identified, as these children typically present with the following:[115]

- vaso-occlusive crisis (37%) – this is likely to occur as the first clinical presentation, but also across the life course;
- dactylitis (28%) – painful inflammation of the hands and feet;
- severe anemia (14%);
- jaundice (11.5%);
- acute chest syndrome (5.5%) – often caused by lung infection that is exacerbated by sickling of red blood cells, symptoms include: fever, cough, pain, low oxygen levels;
- acute splenic sequestration (5%) – results from intrasplenic sickling that prevents blood from leaving the spleen, happens mostly in childhood;
- abdominal crisis (8.2%);
- sepsis (3.3%); and/or
- stroke – usually only seen in Hb SS (0.55%).

The duration of the diagnostic odyssey in Alberta can be days to weeks (EAG members, personal communication, 2015). Most in Alberta will present and be diagnosed at school age; some are diagnosed as late as in their teens (EAG members, personal communication, 2015). Data from the Hospital for Sick Children in Toronto indicate that, before screening was introduced, the average age of presentation was 18 to 24 months (EAG members, personal communication, 2015).

Confirmatory testing of SCD in Alberta is currently conducted using an HPLC analyzer on a liquid blood sample at select labs, including the University of Alberta Hospital (UAH) and Calgary Lab Services (CLS). Additional genetic testing of the β-globin gene, conducted at McMaster University, may also be requested by the diagnosing physician (EAG members, personal communication, 2015). The costs of these tests are paid for by the government.

Treatment

SCD patients identified in Alberta are treated with penicillin prophylaxis, as well as vaccination against encapsulated bacteria (with the pneumococcal conjugate and polysaccharide, meningococcal and *Haemophilus influenzae* type b vaccines) (EAG members, personal communication, 2015). Parental education and comprehensive follow-up and management are also provided, and the costs of vaccinations are covered by the government. The clinical guidelines for treatment are described below.

In addition, all SCD patients in Alberta are offered hematopoietic stem cell transplantation (HSCT), if a sibling match is available. Alberta offers a reduced intensity conditioning regimen, which has fewer side effects than traditional HSCT (EAG members, personal communication, 2015). In addition, Alberta is one of the top ten jurisdictions for the number of SCD-related HSCTs performed. HSCTs are performed at the Alberta Children's Hospital in Calgary, and therefore are paid for by the government.

Management

Clinical follow-up for SCD is primarily to monitor the presence and progression of SCD-related chronic sequelae (that is, vascular occlusion and subsequent chronic pain or injury of bones/marrow, spleen, liver, lungs, brain, kidneys, or joints, as well as hemolysis). In Alberta, children with SCD are followed up every three to six months for the first few years of life, and then annually with blood, kidney, heart, and liver functioning tests. Diagnostic imaging is used as needed. The American Academy of Pediatrics (AAP) and the National Heart, Lung, and Blood Institute (NHLBI) suggest that patients are followed up every two to four months from birth to age 1 year, and every six to twelve months from ages 1 to 21 years.[126]

Patients are generally prescribed amoxicillin prophylaxis, folate, and hydroxyurea for disease management (EAG members, personal communication, 2015). The medication for SCD management must be purchased by the family, and costs approximately $500 per year.

Clinical Guidelines

A number of guidelines[xi] for specific aspects of SCD management have been published, including: pain management,[128-130] stroke prevention,[131] vaccination,[132, 133] hydroxyurea treatment,[134] and inpatient care.[135] Others have published recommendations on screening,[136, 137] including carrier screening.[138] One Quebec report was also located in this review, summarizing evidence from the NICE 2012 and Haute Autorité de Santé 2010 guidelines.[116, 130]

In 2014, the NHLBI developed a comprehensive set of evidence-based guidelines for the management of SCD based upon evidence from a systematic review of literature, including

[xi] The Canadian Haemoglobinopathy Association (CanHaem) has also produced a comprehensive set of draft guidelines.[127] However, they have not yet been finalized and published, and are therefore not reviewed here.

recommendations made in many of the aforementioned published guidelines; these are discussed below.[139] Depending upon the clinical sequelae under consideration, recommendations from existing SCD and non-SCD (for example, chronic pain therapy) guidelines were considered in the NHLBI guidelines.

Prophylaxis, vaccination, and prevention of invasive pneumococcal infection

Patients with Hb SS are recommended to receive oral prophylactic penicillin daily up to the age of 5 years, unless they have had a splenectomy or invasive pneumococcal infection. Children with Hb SC and Hb S/β-thal are not recommended to receive penicillin prophylaxis, unless they have had a splenectomy (though the evidence for this recommendation is weak).

Conjugate pneumococcal vaccination series should also be completed before the age of 5 years. According to the Canadian National Advisory Committee on Immunization[133] and the NHLBI, children should receive doses of the 13-valent conjugate pneumococcal vaccine (PCV13) at the ages of 2, 4, and 12 months, and of the 23-valent pneumococcal polysaccharide vaccine (PPV23) at the ages of 2 and 5 years. Adults should also receive PCV13, followed by one dose of PPV23.[133]

Parental education is also highly recommended by the NHLBI guidelines, to encourage parents to immediately seek medical attention if their child develops a fever.

Chronic sequelae management

Management of other SCD sequelae is the same, irrespective of timing of diagnosis. In other words, periodic episodes cannot be prevented, even with early treatment. Specifically, vascular occlusion, which leads to tissue ischemia (and subsequent chronic pain or injury of bones/marrow, spleen, liver, lungs, brain, kidneys, or joints), as well as hemolysis (chronic anemia and vascular issues affecting leg ulcers, pulmonary artery hypertension, and priapism leading to organ damage) cannot be prevented, but rather must be managed.[101] The severity of these sequelae vary across people, even with the SCD caused by the same genotype.[101] The NHLBI guidelines outline recommendations for management of each of these sequelae associated with SCD.

Opioids for chronic pain management

It is recommended that opioid treatment be initiated in both children and adults with vaso-occlusive crisis and are in severe pain. The NHLBI guidelines outline the routes of administration, as well as timing and dosage. Many patients require hospitalization for pain management (EAG members, personal communication, 2015).

Hydroxyurea and parental education for minimizing complications

Hydroxyurea therapy is recommended with strong evidence for adults with vaso-occlusive crisis, SCD pain, chronic anemia, or repeated or severe acute chest syndrome. The NHLBI guidelines also suggest hydroxyurea for infants older than 9 months with Hb SS, irrespective of clinical severity, to reduce SCD-related sequelae such as pain, dactylitis, acute chest syndrome, and anemia. Almost all patients with SCD will experience a vaso-occlusive crisis in their lifetime, which consists of severe acute pain. In children with Hb SC or Hb S/β-thal with recurrent pain, it is suggested that a sickle cell expert be consulted regarding hydroxyurea treatment.

Informing parents of early indications of clinical events provides them with the knowledge and skills needed to closely monitor their children, particularly when that information includes scenarios such as fever, pain episodes, monitoring the spleen function, and how to assess and address an

emergency. It is suggested that parental education continue for the duration of a child's early years, as the risks of other events become more likely (for example, stroke and splenic sequestration).[126] Parental education will not prevent events from occurring, but can help to minimize the morbidity associated with each event.

Blood transfusion and prevention of stroke

Hb SS patients are at higher risk of stroke. As a result, long-term transfusion therapy is recommended by NHLBI for children with Hb SS that have abnormal transcranial Doppler velocity. Monitoring of iron load and chelation therapy are also strongly recommended with chronic transfusion therapy for patients with transfusion-acquired iron overload.

Hematopoietic stem cell transplantation

While HSCT shows considerable promise, there is limited data available for long-term impacts, and therefore they are not an explicitly recommended therapy in the NHLBI guidelines.

Guidelines for screening for sequelae

Hb SS patients are followed up with annual transcranial Doppler examinations for those aged 2 to 16 years to reduce the risk of stroke. The American Heart Association/American Stroke Association (AHA/ASA) and the NHLBI suggest that children with SCD receive a transcranial Doppler ultrasound beginning at age 2 years and continuing until age 16 years, though recommended screening intervals have not yet been established.

Patients should receive abdominal ultrasound and retinal examinations as needed after the age of 5 years. The NHLBI suggests retinal exams every one to two years. Renal disease screening should begin by age 10 years, and continue annually. Hypertension screening was recommended for adults and children. No recommendations were made for pulmonary hypertension screening, or for electrocardiogram screening.

Patients (children and adults) with signs or symptoms of respiratory disease should be tested with pulmonary function tests, depending upon medical history and physical exam. Asymptomatic SCD patients should not receive pulmonary function testing.

Potential Alberta care pathway – with screening

Compared to no screening, the major changes in the care pathway of infants identified by screening for SCD include avoidance of the diagnostic odyssey and early initiation of effective treatment (with prophylactic treatment, vaccination, and parental education) before the onset of infection or splenic sequestration, which may prevent severe infections, splenic sequestration, and death, as is described in the next section.

Outcomes of Early Versus Late Treatment

The risk of most SCD-related sequelae cannot be modified through early identification and treatment, except for risk of infection and splenic sequestration.

The risk of infection has been shown to be reduced as a result of early identification and treatment with antibiotic prophylaxis and pneumococcal vaccinations. One randomized clinical trial provides evidence that the use of antibiotic prophylaxis before the age of 3 years reduces the incidence of invasive pneumococcal infections in patients with SCD, and that the administration of prophylactic penicillin in children with SCD reduces the risk of childhood mortality from pneumonia and

resulting sepsis.[95] The life expectancy for patients with SCD is still, on average, 30 years less than the general population.[140]

The risk of splenic sequestration may be reduced as a result of parental education. A longitudinal study indicates lower incidence and reduced severity of splenic sequestration following the implementation of a parental education program.[116]

A more detailed appraisal of the effectiveness of prophylactic treatment, vaccination, and parental education can be found in the T section (specifically, section 3.3).

Ancillary Information from Expanded Screening (Variants, Secondary Targets, and Carriers)

The number of variants of SCD is more than 475.[141] Some of these variants can be identified at the screening stage using HPLC on a blood spot.[39, 118] SCD screening using HPLC has been shown to identify SCD variants as well as other hemoglobinopathies such as thalassemia and rare homozygote genotypes: β-thalassemia, E/β-thalassemia, EE disease, CC disease, and C/β-thalassemia.[118, 142] Clinical manifestations of these SCD variants, as well as other hemoglobinopathies, may be mild to severe (Table S.5). However, it is not clear from the literature whether all possible variants of each of these conditions will be identified through HPLC, as many of these variants are very rare.

Using the HPLC platform for screening will identify carriers,[xii] including: Hb S, Hb E, Hb C, and Hb D carriers; and also hemoglobin variants (except α-/β-globin gene variants) including γ-globin variant, HPFH, α-globin variants, β-globin variants, β- and α-globin variants, and other combination of globin variants. A few variants will not, however, be identified, such as β-thalassemia intermedia and β-thalassemia trait (EAG members, personal communication, 2015).

Carriers of the sickle cell trait are generally healthy carriers, with the exception of Hb AS, which may experience symptoms such as hematuria or splenic sequestration in rare cases.[94] Serious complications including death have been recorded in extreme conditions, such as in United States military exercises.[94] With the exception of β-thalassemia intermedia, thalassemia carriers are asymptomatic; therefore, no clinical monitoring is required. Patients with β-thalassemia intermedia generally present with symptoms of anemia after 6 months of age, and outcomes for β-thalassemia patients would not be affected by newborn screening (EAG members, personal communication, 2015).

Severe Combined Immunodeficiency (SCID)

Nature of Disease

Primary immunodeficiency (PID) refers to a heterogeneous group of over 130 disorders that result from defects in immune system development or function.[143] PIDs are generally classified as disorders of adaptive immunity (that is, T-cell, B-cell, or combined immunodeficiencies) or disorders of innate immunity (for example, phagocyte and complement disorders).[143] Over 150 genes have been shown to be associated with different kinds of PID.[144]

Severe combined immunodeficiency (SCID) is the most profound and severe form of PID.[143] SCID is inherited as an X-linked or autosomal recessive defect. Although SCID is genetically

[xii] The annual average numbers of carriers identified in British Columbia based upon newborn screening between 2010 and 2015 are: 84 Hb SS; 15 Hb SC; 43 Hb D; 75 Hb SE; and 15 Hb rare variants.

heterogeneous with over 18 different gene defects identified, all patients with SCID share a common characteristic, being the complete absence or an extremely low level of functional T-cells due to an abnormality of T-cell development in the thymus, which results in a severe defect of both cellular and humoral immunity.[144, 145] The combined defects of T- and B-cells, with absent natural killer cells in some forms of SCID, severely compromise an infant's ability to resist infections, as these cells are all lymphocytes, a subgroup of white blood cells that are essential to the immune system.[146, 147] infants with SCID are highly susceptible to developing serious recurrent and life-threatening infections.[5]

SCID arises from a variety of molecular defects that have profound effects on lymphocyte development and function, including defects in the lymphocyte-specific signaling molecules (common γ-chain, JAK-3, and IL-7 receptor α) and in molecules that control rearrangement of the T-cell receptor and immunoglobulin genes (recombination-activating-gene [RAG]-1/2, Artemis, DNA ligase IV, DNA-dependent protein kinase catalytic subunit [DNA-PKcs], Cernunnos/XLF) or signaling through the pre-T-cell receptor (CD3δ, CD3ε, CD3ξ, and CD45).[148]

Infants with SCID are healthy at birth, but all infants with SCID develop infections from both common and opportunistic pathogens by age 4 to 7 months, as protection from maternal antibodies wanes during the first months of life.[146, 149] Three of the most common clinical presentations include: interstitial pneumonia (48%); failure to thrive (43%); and persistent bronchiolitic illness (40%).[150]

The natural history of disease suggests that, if not identified and treated by the age of 1 year, mortality is nearly uniform due to severe infection, and the condition is fatal in the first two years of life.[144] A Canadian study of 40 cases reported by Canadian pediatricians between 2004 and 2010 suggests 30% of infants identified late and subsequently treated died (58% of these died of infection before they could receive HSCT).[150] Program data from Alberta indicates a 73% mortality rate for those infants identified with SCID (EAG members, personal communication, 2015).

Screening for SCID is expected to prevent severe infections that often result in mortality within the first year of life. HSCT has been found to be curative (immune system reconstitution),[151] and thus, if identified and treated early, infants may be spared from developing serious and recurrent infections. Identifying infants late may result in severe life-threatening infections. The evidence suggests that optimal outcomes are achieved when infants receive transplants before the age of 3.5 months, from a matched donor, and do not have an infection prior to transplant.[149] For more details, see the *Outcomes of Early Versus Late Treatment* section below, as well as the T section (specifically, section 3.3).

Types of SCID

Forms of SCID are classified according to immunological phenotype: 1) T- B+ SCID is characterized by the absence of T-cells; and 2) T- B- is characterized by the absence of both T- and B-cells. "Typical SCID" cases have with fewer than 300/μL autologous T-cells, and are defined by no or very low function of T-cells (response to phytohemagglutinin <10% or evidence of engraftment of maternal T-cells).[152] Typical SCID consists of two sub-classifications: X-linked SCID (XL-SCID), the most common form of SCID, linked to the X chromosome and affects only males[153] and adenosine deaminase (ADA) deficiency (ADA deficiency, or ADA SCID).[154] So-called "leaky SCID" cases are due to incomplete mutation(s) in a typical SCID gene, with 300-1,500/μL T-cells. Leaky SCID is defined by a partial loss of function of T-cells, and may have a later age of onset of clinical symptoms, with an increased risk of severe life-threatening infections.[154]

There are some other specific sub-types such as reticular dysgenesis and Omenn syndrome in the definition of SCID, because they are characterized by an absence of T-cells.[149] In Canada, some special forms of SCID have been known to be present in the province of Manitoba, such as inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β) deficiency, where the infants have T-cells but the T-cell function is abnormal (T+ SCID).[155]

There are a number of secondary targets related to SCID, which are further discussed in the *Ancillary Information from Expanded Screening* section below.

Table S.8: Classification of SCID – primary and secondary targets

Classification	Description
Primary target	
Typical SCID	Fewer than 300 autologous T-cells/μL
Leaky SCID	Between 300-1,500 T-cells/μL; few naïve T-cells
Omenn syndrome	Oligoclonal T-cells
Secondary target	
Syndrome with low T-cell numbers (DiGeorge syndrome, trisomy 21, ataxia telangiectasia, trisomy 18, CHARGE, Jacobsen, CLOVES, ECC, Fryns, Nijmegen breakage, Noonan, Rac2 defect, Renpenning, TAR)	Spectrum of clinical findings
Secondary T-cell lymphopenia	Congenital malformation or disease process without an intrinsic defect in production of circulating T-cells (cardiac anomalies, multiple congenital anomalies, neonatal leukaemia)
Pre-term birth alone	Pre-term birth and low birth weight, with low T-cell numbers early in life that normalize over time
Idiopathic T-cell lymphopenia, also called variant SCID	Low T-cell numbers without recognized cause; six programs used 300-1,500 autologous T cells/μL plus evidence of functional immune cell impairment, while other programs included infants with higher T-cell numbers

Source: [156]

Epidemiology in Canada and Abroad

Incidence in Canada

An estimated 1 in 71,429 live births in Canada will have SCID (see Table S.9).[150] This estimate is based upon 40 confirmed cases of SCID reported by Canadian pediatricians between 2004 and 2010.[150]

The frequency of SCID cases in First Nations, Metis, and Inuit (FNMI) infants is higher than in the general Canadian pediatric population, with one expected case for every 22,727 live births.[150] In Manitoba, an overall incidence of SCID was 3-fold higher than the national average, with two distinct ethnic populations (Mennonites and First Nations of Northern Cree ancestries) known to have a higher incidence.[155] The Mexican Mennonite population is known to carry both the CD3-delta deficiency and the zeta-chain-associated protein kinase 70 (ZAP70) deficiency forms of SCID; however, the size of the Mexican Mennonite population in Alberta, as well as their carrier rate, is

unknown (EAG members, personal communication, 2015). As is mentioned in the Jilkina et al. (2014) study,[155] a number of forms of SCID, including those that affect Northern Cree and Mennonite populations in Manitoba, are not detected using the TREC assay, though it may be possible to detect through lab-based tests (see section 2.4 for more details).

The estimated incidence in Alberta, based upon 11 cases out of 498,758 live births identified and treated at the Alberta Children's Hospital hematology/immunology clinic between 2005 and 2014,[157] is approximately 1 in 45,000 (EAG members, personal communication, 2015).

Known differences in incidence across ethnic populations

Estimates of SCID have changed over time. It was previously suggested, based upon retrospective clinical diagnoses, that the incidence of SCID in the United States was 1 in 50,000 to 1 in 150,000 live births;[158-160] it is now suggested, based upon screening data from 11 states,[xiii] that the incidence is 1 in 58,000 across states that screen for SCID (see Table S.9).[156] The incidence is not statistically different across states, but is statistically different for the Najavo Nation (1 in 3,500).[156]

It has also been shown that SCID may differ across ethnicity, as the incidence of SCID is an estimated 1 in 33,000 overall in California, compared to 1 in 22,000 live births within Hispanic populations.[158]

Internationally, one study suggests that the incidence in France is an estimated 1 in 150,000, based upon a retrospective study of 117 cases of SCID that clinically presented between 1970 and 1992.[160]

Table S.9: Severe combined immunodeficiency reported estimated incidence rates

Incidence, Canada (per number of births)	Incidence, USA (per number of births)	Incidence, international (per number of births)
Based on clinical presentation *Canada* 1:71,429[155] *Alberta* 1:45,000 (EAG members, personal communication, 2015) *First Nations, Metis, and Inuit populations* 1:22,727[155] *Manitoba* 1:15,000-20,000[160]	Based on screening program *USA* 1:58,000[161] *California* 1:33,000[163] *Hispanic population*1:22,000[163]	Based on clinical presentation *France* 1:100,000-1:150,000[165]

Alberta Care Pathway (Diagnosis, Treatment, and Management)

The care pathway for SCID diagnosis (including diagnostic odyssey and confirmatory testing), treatment, sequelae, and follow-up in the context of screening and no screening can be found in Appendix S.B (Figure S.B.5).

[xiii] There were 52 cases identified out of 3,030,083 screened in the United States between January 2008 and July 2013.

Current Alberta care pathway – without screening

Diagnosis: Clinical presentation, diagnostic odyssey, and confirmatory testing

In order for infants to be identified, they would necessarily present clinically and cycle through the diagnostic odyssey prior to being diagnosed.[xiv] A number of differential diagnoses may be considered before an infant with SCID is identified, as these infants generally present with symptoms that are typical of many diseases or conditions within the first few months of life: failure to thrive, severe infections (pneumonia, gastrointestinal infections, sepsis), recurrent or persistent thrush, chronic diarrhea, and/or absent lymph nodes. Diagnosis of SCID is additionally difficult because less than 10% of the infants born with SCID have a positive family history.[144]

In Canada, the average age of diagnosis is 4.2 months,[150] though the time ranges from one day to 583 days. An American study suggests the mean time to diagnosis without screening is 138.5 days.[161] Our EAG suggests that the average age of diagnosis in Alberta is 7 months. Of those with family history, these infants are usually diagnosed and treated at a much younger age before severe infection.

Confirmatory testing for suspected SCID in Alberta is currently conducted using flow cytometry on a blood sample at select major labs, including the UAH and CLS. Genetic testing may also be conducted (requiring approval), at certified clinical genetics labs outside of Alberta. The costs of these tests are paid for by the government.

Treatment

Immunity restoration (reconstitution) include allogeneic HSCT, enzyme replacement (for example, for ADA SCID), and gene therapy. The standard treatment for SCID is HSCT, a life-saving treatment, preferably from a human leukocyte antigen (HLA)-matched relative.

Clinical monitoring of SCID patients in Alberta prior to HSCT occurs at the Alberta Children's Hospital in Calgary. Currently, following diagnosis, SCID patients are kept isolated until a donor can be located and the HSCT is performed. Often siblings are first tested. Only about 15% have a family match, which takes up to one month to identify and transplant; otherwise it takes two to three months to identify a donor and organize transplantation (EAG members, personal communication, 2015). Many patients require hospitalization during this time due to complications of their SCID such as infection, or because they live far from the transplant centre and there are currently no other alternative housing arrangements that offer appropriate isolation close to the hospital. For families in Calgary, patients may be monitored as outpatients if appropriate isolation precautions can be followed in the infants' home and the infant does not require hospitalization (EAG members, personal communication, 2015).

Enzyme replacement therapy and gene transfer are potential options for certain genetic subtypes.[162] Treatment of infections alone (in the absence of HSCT) do not cure this condition. In Alberta, enzyme replacement therapy may be used in rare cases for infants with ADA SCID.

[xiv] Unless a sibling has been affected by SCID, then it may be possible to identify the child prior to clinical presentation without screening.

Clinical guidelines

Immunoglobulin therapy and the process of immunity restoration through HSCT are described in the clinical guidelines by: the Joint Council of Allergy, Asthma, and Immunology (JCAAI);[163] Canadian Blood Services (CBS)/National Advisory Committee on Blood and Blood Products (NAC);[164] and the European Group for Blood and Marrow Transplantation (EBMT)/European Society for Immunodeficiencies (ESID)[165] (see Table S.10).

- The JCAAI 2005 guidelines suggest patients with XL-SCID and ADA SCID should be treated immediately with antimicrobial prophylaxis and immunoglobulin therapy, and receive HSCT or gene therapy as soon as possible to restore T-cells to normal function (though gene therapy is considered investigational at this time).[xv] Patients with ADA SCID additionally receive enzyme replacement therapy (polyethylene glycol-modified bovine adenosine deaminase [PEG-ADA]).[167] These guidelines are the most recent available, however, a task force is currently in place to review.[168]
- The CBS/NAC 2010 guidelines outline the warning signs of primary immunodeficiency and timing by which patients with this condition should receive immunoglobulin therapy for the purpose of decreasing frequency of infection and associated hospitalization, subsequently reducing the risk of developing various chronic illnesses, as well as improving survival and quality of life. Guidance on dosage, routes of administration, and management of reactions are provided, and the different immunoglobulin therapy formulations are described.
- The EBMT/ESID 2011 guidelines for HSCT suggest that preference be given to genotypically and phenotypically identical donors. According to these guidelines, patients should not receive conditioning treatment, T-cell-depletion serotreatment, or graft-versus-host disease (GvHD) prophylaxis. These guidelines also provide recommendations on conditioning, serotreatment, and GvHD prophylaxis for HLA-non-identical family donors, whose course of treatment largely depends on whether the donor is phenotypically identical or not. If genotypically and phenotypically identical donors cannot be located, alternative therapies may be considered, including: HLA-matched unrelated or HLA-non-identical family HSCT donors; or gene therapy.

[xv] Gene therapy is not an alternative currently employed in Alberta. Gene therapy is an experimental method being investigated, and consists of isolating stem cells from the patient's bone marrow, modifying these cells, and infusing them back into the patient. This has only been offered to patients that do not have a sibling-matched donor. Long-term impacts of gene therapy are not well understood, as there have been few cases of leukaemia in children that received gene therapy.[163] Currently, in the United States, gene therapy for XL-SCID is restricted to patients who have failed HSCT.[166]

Table S.10: Clinical guidelines for SCID

Guideline	Description
JCAAI, *Practice parameter for the diagnosis and management of primary immunodeficiency*[163]	These guidelines describe the following for SCID: - *Clinical presentation* associated with SCID for the purpose of identification - *Physical exams* show that lymphoid tissue is not present and the thymus is not detectable using radiography - *Abnormal lab results* should indicate severe age-adjusted lymphopenia and panhypogammaglobulinemia, one or more reduced or absent or profoundly reduced T-cell proliferation to mitogens and antigens - *Genetic mutation analysis* determines type of SCID - *Treatment with HSCT or gene therapy* and the success of these treatments for different subpopulations o SCID IL-2RG, ADA receive IVIG, HSCT, and gene therapy o SCID JAK2, IL-2RA, IL7RA, RAG-1, RAG-2, CD45, MHC I/II, CD3, ZAP-70, Artemis, NP (unknown) receive IVIG, HSCT, but not gene therapy - *Treatment with prophylaxis* for *Pneumocytis carinii* pneumonia
CBS/NAC, *The use of immunoglobulin therapy for patients with primary immune deficiency*[164]	These guidelines are organized according to a number of clinical questions focused on standardizing and optimizing the care of adult and pediatric patients with primary immunodeficiency that receive immunoglobulin therapy. The specific objectives are: "(i) to examine the evidence for the use of immunoglobulin therapy in patients who have primary immune deficiency; (ii) to provide guidance for health care professionals in Canada (and potentially elsewhere) involved in the care of patients with primary immune deficiency on the optimal utilization of immunoglobulin therapy in patients with primary immune deficiency; and (iii) to provide assurance for funders of the Canadian blood system that the effectiveness of immunoglobulin therapy is being assessed."
EBMT/ESID, *Guidelines for haematopoietic stem cell transplantation for primary immunodeficiencies*[165]	Some of the key recommendations for patients with SCID include guidance related to conditioning, provision of T-cell depletion serotherapy, and GvHD prophylaxis treatment depending upon whether donors are genotypically identical, unmatched, or HLA-non-identical matched.

GvHD: graft versus host disease; HLA: human leukocyte antigen; HSCT: hematopoietic stem cell transplant

Management

Clinical monitoring following HSCT is as an inpatient until blood counts recover and the patient's medical care can be managed as an outpatient (generally one to three months), then twice weekly immediately following discharge, then every six months, and then annually (EAG members, personal communication, 2015). Typically, SCID patients who receive HSCT fully recover within one year.

Patients in Alberta generally only require anti-rejection drugs for one year post-transplant; through this time, they are seen annually by an immunologist. After one year of follow-up, they do not require additional medication, and are only followed by the transplant team and immunologist, as HSCT is typically highly effective and the patients' T-cells generally return to normal functioning.

Potential Alberta care pathway – with screening

Compared to no screening, the major changes in the care pathway of infants identified by screening for SCID include avoidance of the diagnostic odyssey and early initiation of effective treatment

(with HSCT) before the onset of infections, which will prevent severe infection/sepsis and associated early mortality, as is described in the next section.

Outcomes of Early Versus Late Treatment

Screening for SCID is expected to prevent severe infections that often result in mortality within the first year of life.

Identifying infants early reduces the likelihood of infection, and allows early treatment with HSCT. HSCT has been found to be curative (immune system reconstitution),[151] and, if identified and treated early, these infants may be spared from developing serious and recurrent infections. Since the first HSCT was conducted in 1968, the standard treatment for all forms of SCID has been HSCT.[169] Because of the absence of functional T-cells in patients with typical SCID, graft rejection is rare, even when immunoablative conditioning is omitted.[162] HSCT has been under continuing evolution, with many innovations in HSCT introduced between 2000 and 2005.[170]

Identifying infants late may result in severe life-threatening infections. The evidence suggests that optimal outcomes are achieved when infants receive transplants before the age of 3.5 months, from a matched donor, and do not have an infection prior to transplant.[149] Of the infants identified (late) in Canada, 30% died following diagnosis, with 58% of these before receiving life-saving treatment.[150] However, studies have shown that infants who are identified early and receive HSCT before 28 days of age have better survival rates than those who receive later treatment.[151] Survival rates may vary by donor type (HLA sibling- or phenotypically-matched), conditioning status, and infection status at the time of transplant.[158, 161] The impact of early treatment is significant, as those who receive a sibling-matched HSCT have over a 90% survival rate, and those who receive a non-matched transplant have around a 50 to 90% survival rate.[160, 161, 171-173] A more detailed analysis of these studies can be found in the T section (specifically, section 3.4).

Ancillary Information from Expanded Screening (Variants, Secondary Targets, and Carriers)

Screening for SCID may identify a number of conditions upon differential diagnosis, each of which requires treatment,[156] the incidence of which may exceed that of SCID (see section 5 for estimated cases). Infants with T-cell lymphopenia will be identified; however, this disorder would not generally present until later in life, and the management of the disorder is not well-established.[158] Secondary targets of screening for SCID, including variants, can be found in the T section (specifically, section 3.4).

Carriers of SCID will not be identified from screening.

The impact on prevalence rates following the introduction of a SCID screening program is not known. Evidence of SCID prevalence changes after screening comparing the population prevalence before and after the initiation of screening programs was not located in this review. As mentioned above, based upon results from screening programs in the United States, the estimated incidence of SCID is 1 in 58,000, as opposed to previous estimates of 1 in 100,000.[156] However, this gives very little indication of the population prevalence of SCID.

Given that SCID is a rare condition and patients will eventually present clinically, very little change in the prevalence of the disease would be expected as a result of screening.

2.4 System Implications of Expanded Alberta NMS Program

Additional resources will be required for the implementation and operation of an expanded screening program.

Existing capacity, constraints, and suggested condition-specific resource requirements are listed below for each condition under review, in terms of required operating resources and resources needed for implementation. Additional lab resources required for implementation and operation of expanded screening, for the addition of any/all conditions are also described separately below.

Additional System Operating Resources Required

The existing Alberta NMS Program may be leveraged to accommodate screening for additional conditions. While there are known capacity constraints within the system (as will be described below), the infrastructure for newborn screening already exists, specifically:

- the screening protocol and logistics around the collection of blood spots and their transportation to the NMS Lab are well-established;
- confirmatory testing is already being done for these conditions, and these processes and protocols would likely not change given the expansion of screening; and
- specialty clinics already exist and are treating patients with these conditions that have been identified as a result of clinical presentation.

The main system implications concerning the addition of the seven conditions center around the operating resources required to obtain or modify the necessary newborn screening platforms, and as well as addressing potential resource and capacity constraints that may arise in the treatment and follow-up of patients being identified early.

The additional operating and implementation resources required, as well as the system implications, will be considered for each condition below (Table S.11).

Table S.11: Existing capacity, additional system level operating and implementation resources required, and system implications for expansion of screening

	GALT	TYRI	HCY	SCD (Hb SS, Hb S/β-thal, Hb SC)	SCID
Alternative screening platforms and associated requirements					
Screening platform alternatives	a) VICTOR^{2}D™ b) GSP®	MS/MS	MS/MS	a) HPLC (requested) b) IEF analyzers	qPCR lab-based test**
Lab resource requirements for screening (see Appendix S.C)	a) 1 additional VICTOR^{2}D™, galactose-1-phosphate uridylyltransferase enzyme measurement screening kits +0.25 FTE b) 2 GSP®s, kits	Succinylacetone screening kit and reagents	Methionine screening kit and reagents	2 new HPLC machines (requested) to analyze blood spots Reagents	1 additional qPCR QuantStudio™ platform, additional items, reagents
Confirmatory testing implications					
Existing confirmatory testing platform	Existing galactose-1-phosphate uridylyltransferase enzyme levels in the blood ±Genetic testing	Existing tyrosine levels in the blood ±Genetic testing	Existing plasma total homocysteine in the blood ±Genetic testing	Existing confirmatory HPLC on whole blood ±Genetic testing	Existing flow cytometry test on blood ±Genetic testing
Resource requirements for confirmatory testing	Existing capacity	Existing capacity	Existing capacity	Existing capacity	Existing capacity
Treatment and follow-up implications					
Existing speciality clinic capacity and capacity constraints	Metabolic clinic can accommodate***	Metabolic clinic can accommodate*** Dietary supplement cost concerns	Metabolic clinic can accommodate *** Dietary supplement cost concerns	To accommodate influx of patients, hematology clinics require: • genetic counselling resources (high need), especially if carriers reported • Clinic resources (physician, allied health, clerical, nursing, physical space) • Blood transfusion resources and outpatient space • HSCT resources • Neuropsychologist support	Hematology/ immunology clinic can accommodate*** HSCT unit – at capacity

	GALT	TYRI	HCY	SCD (Hb SS, Hb S/β-thal, Hb SC)	SCID
Expected broader health system resource implications resulting from screening*	Reduced ophthalmology due to early treatment No diagnostic odyssey	Reduced liver transplants, hepatocellular carcinoma, and renal treatment due to early treatment No diagnostic odyssey	Reduced ophthalmology due to reduced lens dislocation Reduced hospitalization due to thromboembolic events Reduced social support due to improved mental development No diagnostic odyssey	Reduced hospitalization and treatment of severe infections and splenic sequestrations No diagnostic odyssey	Reduced hospitalization and treatment of severe infections Additional need for HSCT Additional need for isolation space (inpatient and outpatient) Impact on temporary accommodations (e.g. Ronald McDonald House) No diagnostic odyssey

*Expected broader health system requirements only include anticipated increases in system requirements over and above what would be expected given the current method of identifying patients with these conditions (clinical presentation). Therefore, these changes in resource requirements are expected because of: (i) effectiveness of early treatment resulting in increased resource requirements to meet the needs of children who survive, or the reduced resource requirements due to reduced morbidity (sequelae) due to the prevention of sequelae based on early treatment because of screening; and (ii) existing capacity constraints within the system. Survivors of GALT, TYRI, HCY, and SCID may require sequelae management, which would result in added resource utilization at the clinic and health system level. However, each of these conditions is rare, and therefore the overall increase in service utilization would be small.

**GSP® and VICTOR™ EnLite™ have also been listed as an alternative screening method for SCID. However, the GSP TREC kits are not approved by Health Canada, and therefore are not commercially available. As a result, this alternative is not currently a feasible screening option. VICTOR™ EnLite™ is commercially available; however, laboratory services have suggested that it would employ the single-wash, in-situ qPCR method, due to quality assurance issues that have been identified with VICTOR™ EnLite™ (personal communication, September 2015).

***Existing capacity can accommodate patients that would have otherwise died; and/or the influx of patients that would be identified earlier.

GSP®: Genetic Screening Processor; HPLC: high performance liquid chromatography; HSCT: hematopoietic stem cell transplant; IEF: isoelectric focusing; MS/MS: tandem mass spectrometer; PCR: polymerase chain reaction; qPCR: quantitative (or real-time) polymerase chain reaction

Screening Platform Requirements

GALT: The VICTOR^{2}D™ fluorometer (manufactured by PerkinElmer) can be used to screen for GALT. The NMS Lab has a VICTOR^{2}D™, which is currently being used to screen for biotinidase deficiency. Screening kits for GALT would be required to add GALT to the panel.

Samples are manually loaded into the VICTOR^{2}D™. Adding more conditions to be screened using this method is difficult, and an additional 0.25 FTE would be required if the VICTOR^{2}D™ is the selected screening method, because the platform is not automated (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015).

Our EAG has recommended acquiring a Genetic Screening Processor (GSP®) instead, which is a new automated and integrated screening plate processor (manufactured by Wallac Oy, a subsidiary of PerkinElmer). The GSP® would reduce the number of semi-manual assays, and therefore minimize potential errors and improve workflow in the NMS Lab (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015). GSP® Neonatal GALT analyte kits would also be required. See Appendix S.C for more details.

There is only one VICTOR^{2}D™ in the NMS Lab, and a second would be needed for redundancy, if screening for GALT is initiated and the VICTOR^{2}D™ is the selected screening method instead of a GSP® (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015).

TYRI and HCY: TYRI and HCY screening require a MS/MS. The NMS Lab has two MS/MSs (manufactured by PerkinElmer), which are currently used to screen for 13 of the 17 primary target conditions on the existing panel. Screening kits would be required to add TYRI and/or HCY to the panel. It has been recommended that succinylacetone be used to screen for TYRI and methionine for HCY. See Appendix S.C for more details.

SCD: Most of the United States currently screen using isoelectric focusing (IEF) on dried blood spots, with a few programs using high performance liquid chromatography (HPLC) or MS/MS to screen.[101] More information about each alternative platform can be found in the T section (specifically, section 3.3). HPLC is currently used in Ontario, and is the screening and confirmatory method of choice, according to our EAG. Alberta has HPLC machines to analyze whole blood samples; however, they are not equipped to analyze blood spots. As a result, new HPLC systems will be required. Two analyzers would be needed to ensure screening back-up when machine maintenance is required. See Appendix S.C for more details. HPLC results would be reviewed by technologists and existing senior staff in the NMS Lab.

SCID: Laboratory services have suggested that it would employ real-time/quantitative polymerase chain reaction (qPCR) using a lab-based test (see Appendix S.C for more details on the lab-based test) to screen for SCID. The VICTOR™ EnLite™ fluorometer is commercially available for SCID screening; however, this method is not considered a screening alternative by laboratory services due to quality assurance issues that have been identified with this platform (personal communication, September 2015).

a) A single-wash, in-situ qPCR is a lab-based test method developed by the Centers for Disease Control and Prevention (CDC) in the United States, and is currently being used in Ontario to screen for SCID (Genetic Laboratory Services, Alberta Health Services, personal communication, July 2015). The lab has an existing qPCR platform (QuantStudio™) to perform these lab-based tests for SCID. Redundant equipment is necessary, and the EAG

has recommended obtaining another QuantStudio™ for SCID screening. Additional equipment-related items will also be required (see Appendix S.C).

- This equipment is multi-functional and can accommodate more than just the TREC assay (that is, the kappa-deleting recombination excision circle [KREC] assay could be added to improve the detection of immunodeficiency disorders). Additionally, this instrument can be used on an entirely different assay that would enable the identification of mutations that may be missed by the TREC assay (that is, SCID in the Northern Cree population as reported by Manitoba). The lab-based testing method for SCID can accommodate up to 16 mutations, which can be analyzed alongside the lab-based testing method developed by the CDC.

Confirmatory Testing Implications

The methods for confirmatory testing, described above for each of the seven conditions (section 2.3), are expected to remain the same. There are no known capacity constraints within the system in terms of resource constraints for confirmatory testing of these seven conditions. It should be noted, however, that there may be an increase in additional tests as a result of false positives, as well as secondary conditions, and an associated increase in specialty clinic utilization.

Treatment and Follow-Up Implications

Existing specialty clinic capacity and capacity constraints

As mentioned above, there are three types of clinics that are involved in the identification, treatment, and management of patients who clinically present with the seven conditions under review. Following consultation with each of the clinics, we have identified the following key areas in terms of existing capacity, as well as capacity constraints.

Metabolic clinics (to treat and manage GALT, TYRI, and HCY)

Metabolic clinics are located in Edmonton and Calgary to treat metabolic disorders including GALT, TYRI, and HCY. Given the rarity of the disorder, with the exception of the genetic counsellors, the metabolic clinical teams in Alberta have confirmed existing capacity (in both Edmonton and Calgary) to accommodate both patients who would have otherwise died as well as the influx of additional patients as a result of screening for GALT, TYRI, and HCY (additional clinic genetic counsellor FTE requirements are considered below).

The cost of dietary supplements is currently paid for out of the metabolic clinics' operational budgets for TYRI and HCY. These costs would be higher in the context of screening, as treatment would be started earlier. In addition, treatment is shown to reduce TYRI mortality, and these survivors would then also need dietary treatment.

Few differences were mentioned by our EAG regarding variation in terms of newborn screening protocols or treatment and management practices between the Edmonton and Calgary clinics. The only noted difference between the regions was some variation across the metabolic clinics' genetic counselling staff. Specifically, the Calgary clinic utilizes a nurse to consult with families, instead of a clinic genetic counsellor.

Hematology clinics (to treat and manage SCD)

There are currently two pediatric hematology clinics, one in Calgary and one in Edmonton. The Calgary clinic has four hematologists, while the Edmonton clinic has two, and are currently

recruiting a third. At age 18, patients in both Edmonton and Calgary are transferred to an adult hematology clinic (also known as the Blood Disorder Program).

Newborn screening will not immediately impact the adult clinic, but will immediately affect the pediatric clinics. Specifically, the addition of SCD to the screening panel should increase resource utilization at the pediatric clinics. The clinics have indicated that they are already at capacity, and need additional physician, allied health, and nursing support, as well as clinic space. The expected surge of patients resulting from increased screening would be difficult for the current clinics to accommodate. There is currently a one- to two-month wait to get in to the hematology clinics, and the added number of patients would amplify these wait times. At the time of writing, there was an open vacancy for a hematologist in Edmonton. Given the current case load, additional hematologists as well as additional clinic staff would be required.

In addition, outpatient space for blood transfusions (for thalassemia patients identified as a secondary target of screening) is at capacity; additional space would be needed to accommodate new patients. An increase in neuropsychologist capacity is said to also be needed.

Currently, Alberta is within the top ten jurisdictions for the most children who receive HSCT for SCD in North America (EAG members, personal communication, 2015). The EAG indicated that, with more patients being identified with SCD, additional HSCT resources would be required.

Given the relatively high incidence of the disease, the expected impact on hematologists and genetic counsellor resources would be significant, especially initially, as the number of children identified (SCD cases, variants, and carriers) both with screening (a proportion of which would otherwise have presented at some point in the future) and without screening would require support simultaneously (additional clinic genetic counsellor FTE requirements are considered below).

Hematology/immunology clinic (to treat and manage SCID)

One of the hematologists at the Calgary hematology clinic is also an immunologist, and is involved in the treatment of hematologic and immunologic disorders, including SCD and SCID. A second immunologist has been hired in Calgary. Edmonton does not have an immunologist (but is in the process of recruiting one); as a result, all SCID patients in Alberta are currently referred to the clinic in Calgary for treatment until an immunologist is hired. The addition of SCID to the screening panel may increase resource utilization at the hematology/immunology clinic. However, with the exception of the clinic genetic counsellors, the clinic team can accommodate both patients who would have otherwise died before being diagnosed as well as the influx of additional patients with existing resources (additional clinic genetic counsellor FTE requirements are considered below).

Expected broader health system implications resulting from screening

GALT: A decrease in the utilization of specialists for select comorbid conditions such as cataracts would be expected. Evidence regarding outcomes of early treatment suggests that more children will survive; however, most children are still expected to experience sequelae as a result of GALT, though a lower incidence of cataracts among those treated early as compared to late has been reported (see section 2.3, as well as the T section, specifically section 3.2).

Acute symptom hospitalization is likely in the context of no screening throughout the diagnostic odyssey, and would therefore be reduced in the context of screening.

TYRI: A decrease in resource utilization resulting from clinical presentation of sequelae would be expected, as incidence of liver damage (and resulting hepatocellular cancer and/or liver transplants)

and kidney damage should be reduced with current standard treatment. Acute symptom hospitalization is likely in the context of no screening throughout the diagnostic odyssey, and would therefore be reduced in the context of screening.

HCY: Screening and treating HCY early may result in a decrease in resource utilization resulting from certain sequelae, as the risk of lens dislocation, thrombosis, and mental retardation can be reduced or mitigated with current standard treatment. Therefore, a reduction in ophthalmology resources, hospitalizations for thrombotic events, and social supports for mental retardation would be expected in the context of screening. Acute symptom hospitalization is likely in the context of no screening throughout the diagnostic odyssey, and would therefore be reduced in the context of screening.

Other jurisdictions have experienced an influx of secondary conditions identified as a result of screening (BC Children's Hospital, personal communication, 2015). For example, over the five years of screening for HCY in British Columbia, no cases have been identified. However, five cases of MAT I/III have been identified (1 in 45,000 live births), each of which require clinical assessment and follow-up.

SCD: Evidence suggests that early treatment may reduce the incidence of severe infection and splenic sequestration in children under the age of 5 years, and therefore a reduction in hospitalization is expected in the context of screening. In addition, acute symptom hospitalization is likely in the context of no screening throughout the diagnostic odyssey, and would therefore be reduced in the context of screening.

SCID: Acute symptom hospitalization for severe infection is likely in the context of no screening throughout the diagnostic odyssey, and would be reduced in the context of screening.

The need for HSCT may increase as a result of SCID cases being identified and treated early. The HSCT unit in Alberta is currently at capacity. A consideration related to HSCT capacity in Alberta is that Saskatchewan and eastern British Columbia do not perform HSCT, and thus, should either province screen for SCID, infants requiring this treatment would be sent to Alberta.

Isolation space in hospital is limited, and more space is required (EAG members, personal communication, 2015). Care protocol is to isolate patients at home or as outpatients if identified early (without infection). There is a need for outpatient isolation space for patients who do not reside in Calgary.

In the context of SCID screening, resource requirements of temporary accommodations for housing patient families awaiting transplants, such as the Ronald McDonald House, may also be impacted.

Additional clinic genetic counsellor capacity needs

Clinic genetic counsellors already care for patients who present clinically with the seven conditions under review. However, their maximum workloads have already been reached. Any addition to the screening panel would result in significant resource challenges, as each new urgent patient requires five to eight hours of work-time, and non-urgent positive cases require approximately four hours (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015). An increase of 0.2 FTE would be required to accommodate all seven conditions. Should SCD carriers and variants be reported, up to an additional 2.5 FTE clinic genetic counsellors would be needed.

Protocol for pre-term, low birth weight, and transfused babies

Pre-term and/or low birth weight infants may not have an accurate screen for the conditions under review (for more details, see the T section, sections 3.2-4). Ontario recommends a repeat screen at 3 weeks of age;[5, 174 xvi] Alberta's existing protocol[18] for low birth weight babies similarly recommends a repeat screen between 21 and 28 days of age.

Infants that require a blood transfusion should have a specimen collected prior to transfusion, if at all possible, as the blood transfusion interferes with newborn screening for GALT, SCD, and SCID (Newborn Screening Laboratory Services, Alberta Health Services, personal communication, 2015). The lab protocol for re-testing transfused infants is currently under review in Alberta.

Additional Considerations for Lab Implementation and Operation

Additional Resources Required

The resources required for the initial implementation and operation of the expanded screening programs were discussed with the NMS Lab management staff. Resource requirements were noted with respect to required changes to blood spot sample collection, changes to software, and additional training for lab staff (see Appendix S.C for more details).

- **Additional physical space:** There may be a need for additional physical space, depending upon the platforms and equipment to be acquired should screening be expanded (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015).
- **Changes to blood spot sample collection:** A change to the newborn screen requisition form will be necessary. A fifth blood spot may be required should all seven conditions be included on the panel (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015). One-time education would then be required for those collecting blood spot samples.
- **Software requirements:** For the addition of any condition, it will be necessary to re-configure lab software (Specimen Gate Office) and integrate with Netcare and the NMS Application for the expanded screening panel.
- **Equipment maintenance:** The lifecycles of screening platforms are approximately five to seven years long (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015). Consideration should be given to equipment replacement that will be required over the long-term, due to equipment age and service obsolescence.
- **Additional capacity and training for lab staff:** Additional staff would also be required for the expansion of screening (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015), as noted in Table S.12. Additional training would also be required for existing lab staff on new methodologies.

[xvi] See also: *http://newbornscreening.on.ca/bins/content_page.asp?cid=7-294-342&lang=1*.

Table S.12: Additional staff required for expansion of screening

Condition	Labour	Implementation Phase and/or Ongoing Operational Needs
GALT	0.3 FTE Laboratory Technologist I 0.1 FTE Genetic Counsellor 0.2 FTE Information Technology Analyst I 0.2 FTE Geneticist 0.2 FTE Geneticist Trainee (CCMG) 0.5 FTE Laboratory Scientist I	Implementation phase and ongoing
TYRI	0.3 FTE Laboratory Technologist I 0.1 FTE Genetic Counsellor I 0.3 FTE Information Technology Analyst I 0.4 FTE Geneticist 0.4 FTE Geneticist Trainee (CCMG) 0.7 FTE Laboratory Scientist I	Implementation phase and ongoing
HCY	No additional labour required	NA
SCD	0.4 FTE Laboratory Technologist I 0.2 FTE Genetic Counsellor I 0.3 FTE Information Technology Analyst I 0.4 FTE Geneticist 0.4 FTE Geneticist Trainee (CCMG) 0.8 FTE Laboratory Scientist I	Implementation phase and ongoing
SCID	1.0 FTE Laboratory Scientist I 0.1 FTE Genetic Counsellor I 0.2 FTE Information Techology Analyst I 1.0 FTE Geneticist	Implementation phase and ongoing

Health Canada Approval

Health Canada approval was searched for all alternatives under consideration in terms of screening platforms and analyte kits.[xvii]

For GALT screening, Health Canada issued a licence:

- in May 2002, for the Wallac 1420 VICTOR^{2}D™ Multilabel Counter (manufactured by PerkinElmer) as a class 3 device;
- in August 2006, for the Neonatal GALT Kit (manufactured by Wallac Oy, a subsidiary of PerkinElmer) as a class 2 device;
- in August 2006, for the GSP® Neonatal GALT Kit (manufactured by Wallac Oy, a subsidiary of PerkinElmer) as a class 3 device; and
- in June 2011, for the GSP® (manufactured by Wallac Oy, a subsidiary of PerkinElmer) as a class 3 device.

[xvii] Health Canada [Internet]. Medical devices active licence listing [modified 10 July 2012]. Available from: *http://webprod5.hc-sc.gc.ca/mdll-limh/prepareSearch-preparerRecherche.do?type=active&lang=eng*.

For TYRI and HCY screening, Health Canada issued a licence in May 2006, for the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit (manufactured by Wallac Oy, a subsidiary of PerkinElmer) as a class 3 device.

For SCD screening, Health Canada issued a licence:

- in August 2006, for the VARIANT™nbs Sickle Cell Program (manufactured by Bio-Rad Laboratories, Inc.) as a class 3 device, which can be used for the HPLC test; and
- in July 2004, for the RESOLVE® Hemoglobin Kit (manufactured by Wallac Oy, a subsidiary of PerkinElmer) as a class 3 device, which can be used for the IEF test.

For SCID screening, the QuantStudio™ qPCR platform (manufactured by Thermo Fisher Scientific, Inc.) has not been approved by Health Canada. However, the Edmonton site molecular labs already have a QuantStudio™ in place, and it is used routinely for other lab-developed diagnostics (EAG members, personal communication, 2015).[xviii]

Platform Throughput

Table S.13 describes the platform throughput, or maximum number of samples that each alternative platform can accommodate per day (Genetic Laboratory Services, Alberta Health Services, personal communication, 2015).

Table S.13: Platform throughput estimates

Condition	Platform	Throughput, theoretical maximum (n samples per day)	Throughput, after accounting for other testing (n samples per day)
GALT	VICTOR^{2}D™ GSP®	384 2,496	384 499
TYRI	MS/MS	600	600
HCY	MS/MS	600	600
SCD	HPLC	1,500 (with a 2-minute per sample run time)	1,500
SCID	QuantStudio™	936	468

2.5 Acceptability of Expanding the Alberta NMS Program

The acceptability of diagnostic testing, treatment, and overall screening program were assessed by our EAG on behalf of their patient population, as described below. Qualitative studies were not conducted to assess the acceptability of testing and treatment from the perspective of parents or Albertans. However, our EAG has first-hand experience diagnosing and treating patients with these conditions, and are therefore familiar with patient and family responses to screening, testing, and treatment of genetic conditions.

[xviii] The GSP® platform has also been suggested by the lab to screen for SCID. As mentioned above, the GSP® platform was approved by Health Canada in 2011. However, the TREC kit has not been approved by Health Canada, and, therefore, the kits cannot yet be purchased, so it is not possible to screen for SCID using GSP® at this time.

Acceptability of Confirmatory Testing

Based upon the opinion of physicians in our EAG, confirmatory testing is thought to be acceptable to physicians, as well as to their patients and the patients' caregivers, for each proposed condition (EAG members, personal communication, 2015).

Acceptability of Treatment

Our EAG also considers treatment for the conditions under review to be acceptable to physicians, as well as their patients and the patients' caregivers (EAG members, personal communication, 2015). HSCT for the hemoglobinopathies is broadly accepted in Alberta. However, there are known social stigmas associated with SCD across certain ethnic populations, such as people of African descent.[175, 176] One Canadian qualitative study of mothers of African and Caribbean descent whose children were diagnosed with SCD states that all except one experienced stigma related to SCD in their communities.[177] These women suggest that the stigma stems from notions about SCD being contagious, a religious curse, or that they willingly conceived an ill child. In addition, these mothers reported being stigmatized by employment insurance, as SCD medications are not often covered and have large additional fees. Our EAG has noted that Ontario has experienced some resistance towards HSCT for SCD, although this has not been the Alberta experience (EAG members, personal communication, 2015). In Alberta, out-of-pocket medication costs for the management of the condition are an estimated $500 annually for the patient's family (EAG members, personal communication, 2015).

Acceptability of Expanded Provincial Newborn Screening

Based upon conversations with our EAG, expanded provincial screening appears to be generally accepted by physicians, parents/guardians, and the general public.

2.6 Conclusions

This *Social and Systems Demographics* review has summarized the evidence from the scientific literature and information from our EAG to address questions about seven conditions (GALT, TYRI, HCY, SCID, Hb SS, Hb SC, and Hb S/β-thal) given the Alberta context, related to: burden of illness, nature of condition, current patterns of care, long-term outcomes if diagnosed and treated early as compared to late, system capacity for expanded screening, and acceptability of screening to Albertans and affected families.

Key Findings

Burden of Illness and Nature of Condition

- GALT, TYRI, HCY, and SCID are rare conditions, with incidences in Canada of approximately 1 in 70,000, 1 in 100,000, 1 in 200,000 to 300,0000, and 1 in 58,000 to 71,000, respectively.
- Different ethnic backgrounds have been found to be differentially impacted by GALT, TYRI, HCY, and SCD. In particular, GALT is found to be more common in the Irish, Iranian, and Greek populations; TYRI is more common in Quebec and Norway; HCY is more prevalent in Irish and Norwegian populations; and SCD more often affects people of African, Mediterranean, or Middle Eastern descent. Ontario has an estimated incidence rate of SCD in the range of 1 in 5,600, while British Columbia has observed an incidence rate of 1 in 17,300.

Rationale for Screening – Outcomes from Early Versus Late Treatment

- Compared to no screening, the major changes in the care pathway of infants identified by screening for GALT include avoidance of the diagnostic odyssey and early initiation of treatment before the onset of symptoms, which may prevent cataract development and death. Norway and Poland have elected to remove GALT from their screening panel due to the rarity of disease and the reality within those jurisdictions that patients often clinically present before screening results can be reported. In addition, given current methods of treatment, long-term complications still occur for the majority of cases, irrespective of whether the patient was screened.
- Compared to no screening, the major changes in the care pathway of infants identified by screening for TYRI include avoidance of the diagnostic odyssey and early initiation of treatment with NTBC. NTBC treatment has been shown to reduce morbidity and mortality associated with hepatocellular carcinoma and the need for liver transplantation, as well as kidney damage.
- Compared to no screening, the major changes in the care pathway of infants identified by screening for HCY include avoidance of the diagnostic odyssey and early initiation of treatment, which may reduce morbidity associated with thromboembolic manifestations, ocular manifestations, and mental retardation. Select jurisdictions have opted to discontinue HCY screening due to low incidence, including Austria, Belgium, Italy, Scotland, Switzerland, Australia, and New Zealand.
- Compared to no screening, the major changes in the care pathway of infants identified by screening for SCD include avoidance of the diagnostic odyssey and early initiation of effective treatment (with prophylactic penicillin and parental education) before the onset of infection or splenic sequestration, which may prevent severe infections, splenic sequestration, and associated mortality. The risk of the other SCD-related chronic sequelae may not be modified through early identification and treatment, and must be managed.
- Compared to without screening, the major changes in the care pathway of children identified by screening for SCID include avoidance of the diagnostic odyssey and early initiation of effective treatment (with HSCT) before the onset of infections, which will prevent severe infection/sepsis and associated early mortality

System Implications

- Existing system capacity is largely sufficient to accommodate treatment and follow-up for the influx of newly identified patients with GALT, TYRI, HCY, and SCID. Specialty clinics have indicated that they can accommodate the additional infants identified because each of these conditions is relatively rare. Genetic counsellors, however, are currently at full capacity and require additional resources should any of the conditions be added to the panel.
- There is not existing system capacity to accommodate treatment and follow-up for the influx of newly identified patients with SCD. Expanding newborn screening to include SCD may require investment in terms of equipment, genetic labs and clinic counsellors, and hematology and immunology clinic physicians, nurses, allied health staff, and physical space. Additional FTE requirements for genetic counsellors for SCD depend upon whether carrier status is reported. Blood transfusion and HSCT resources will also be required.
- Based upon our EAG's recommendations for methods of testing for each condition, existing screening platforms can be modified to accommodate TYRI and HCY. New platforms are

required for SCD. GALT could be screened for using the existing VICTOR^{2}D™ fluorometer (with additional staff) or through acquiring a new GSP®. A new qPCR platform would be acquired to accommodate SCID. The need for redundant equipment within the lab needs to be considered as well.

- Additional lab resources will also be required in terms of additional lab staff and training of staff; and configuration of lab software. Changes to blood spot collection may also be required in terms of collecting an additional blood spot.

Acceptability of Screening and Treatment

- There is general agreement, based upon EAG interviews, that screening for the seven conditions, in terms of the diagnostic interventions and available treatments, is considered to be clinically acceptable in Alberta.

Appendix S.A: Literature Search Summary

The grey literature search was conducted by the IHE Research Librarian from 29 October 2014 to 11 June 2015.

Table S.A.1: Grey literature search summary

Source	Edition or date searched	Search Terms
Systematic reviews and HTAs		
CADTH *http://www.cadth.ca/*	29 October 2014 2 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
INESS *http://www.inesss.qc.ca/en/home.html*	29 October 2014 6 results	Newborn screening or neonatal screening or infant screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
CHSPR *http://www.chspr.ubc.ca/*	29 October 2014 0 results	Newborn screening or neonatal screening or infant screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
ICES *http://www.ices.on.ca/*	29 October 2014 0 results	Newborn screening or neonatal screening or infant screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
OHTAC *http://www.hqontario.ca/evidence/publications-and-ohtac-recommendations/ontario-health-technology-assessment-series*	29 October 2014 0 results	Browsed list
NLCAHR: Newfoundland and Labrador Centre for Applied Health Research (CHRSP) *http://www.nlcahr.mun.ca/CHRSP/*	29 October 2014 0 results	Browsed list
THETA *http://theta.utoronto.ca/*	29 October 2014 0 results	Browsed list
Adelaide Health Technology Assessment *http://www.adelaide.edu.au/ahta/pubs/*	29 October 2014 0 results	Browsed list

MSAC *http://www.msac.gov.au/internet/msac/publishing.nsf/Content/completed-assessments*	29 October 2014 0 results	Browsed list
Euroscan *http://www.eurosca n.org.uk/*	29 October 2014 2 results	Newborn screening or neonatal screening or infant screening or immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
UK National Health Technology Assessment Programme *http://www.nets.nihr.ac.uk/projects?collection=netscc&meta_P_sand=Project*	29 October 2014 9 results	Newborn screening or neonatal screening or infant screening or immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
NICE *http://www.nice.org.uk/*	29 October 2014 0 results	Browsed list
AHRQ *http://ahrqpubs.ahrq.gov/OA_HTML/ibeCZzpHome.jsp*	30 October 2014 6 results	Newborn screening or neonatal screening or infant screening or immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Aetna *http://www.aetna.com/health-care-professionals/clinical-policy-bulletins/medical-clinical-policy-bulletins.html*	30 October 2014 0 results	Browsed list
BlueCross and Blue Shield (BCBS) Association *http://www.bcbs.com/blueresources/tec/vols/*	30 October 2014 0 results	Browsed list
Google *http://www.google.com*	30 October 32014 19 results	immunodeficiencies OR "immune deficiency" OR SCID OR galactosemia OR thalassemia OR "sickle cell" OR tyrosinemia OR homocystinuria OR homocysteinemia OR homocysteinurea "health technology assessment" filetype:pdf
Guidelines		
Guidelines Clearinghouse *http://www.guideline.gov/*	2 February 2015 12 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea

CMA Infobase	13 February 2015 2 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Clinical Trials		
Clinical trials.gov	9 March 2015 478 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Websites		
Google	2 April 2015	severe combined immunodeficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea severe combined immunodeficiency OR SCID therapy OR treatment OR screening OR diagnosis OR testing -pubmed filetype:pdf galactosemia therapy OR treatment OR screening OR diagnosis OR testing -pubmed filetype:pdf thalassemia OR sickle cell OR ss disease OR sc disease therapy OR treatment OR screening OR diagnosis OR testing -pubmed filetype:pdf homocystinuria OR homocysteinemia OR homocysteinurea therapy OR treatment OR screening OR diagnosis OR testing -pubmed filetype:pdf Newborn screening or neonatal screening
Government of BC *http://www2.gov.bc.ca/*	5 April 2015 2 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Alberta Health *http://www.health.alberta.ca/*	5 April 2015 4 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of Saskatchewan *http://www.saskatchewan.ca/*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of Manitoba *https://www.gov.mb.ca/index.html*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of Ontario Ministry of Health *http://www.health.gov.on.ca/en/*	15 April 2015 3 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of New Brunswick *http://www2.gnb.ca/*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of Nova Scotia *http://novascotia.ca/*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or

		homocystinuria or homocysteinemia or homocysteinurea
Government of PEI *http://www.gov.pe.ca/*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Newfoundland Ministry of Health *http://www.gov.nl.ca/*	15 April 2015 0 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Yukon Government *http://www.gov.yk.ca/*	15 April 2015 See BC	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
NWT Government *http://www.gov.nt.ca/*	15 April 2015 See Alberta	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Nunavut Government *http://www.gov.nu.ca/*	15 April 2015 0 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Regulatory Information		
Health Canada Medical Devices Active Licence Listing (MDALL) *http://www.hc-sc.gc.ca/dhp-mps/md-im/licen/mdlic-eng.php*	11 June 2015 7 results	Hb variants OR HPLC VARIANT OR Bio-Rad Laboratories OR TRECs OR Qiagen OR IL-7 OR ELISA OR R&D system OR Tandem mass spectrometry OR Fluorescence spectrometry OR fluorescence spectroscopy OR spectrofluorometry OR Victor fluorometer OR PerkinElmer

The main literature search was conducted by the IHE Research Librarian on 4 May 2015. The search for sickle cell disease and related conditions was limited to reviews.

Table S.A.2: Main literature search summary

Database	Edition or date searched	Search Terms††	
MEDLINE (includes in process and other non-indexed citation) OVID Licensed Resource	4 May 2015 213 results	1	Homocystinuria/
		2	(homocystinuria or homocysteinemia or homocysteinurea).ti.
		3	Tyrosinemias/
		4	Tyrosinemia*.ti.
		5	exp Anemia, Sickle Cell/
		6	sickle cell*.ti.
		7	ss disease.ti.
		8	beta-Thalassemia/
		9	beta thalassemia.ti.
		10	exp Hemoglobin SC Disease/

		11	sc disease.ti.
		12	Galactosemias/
		13	galactosemia*.ti.
		14	exp Severe Combined Immunodeficiency/
		15	severe combined immunodeficienc*.ti.
		16	severe combined immune deficienc*.ti.
		17	or/1-16
		18	(socio-demographic* or social demographic*).tw.
		19	exp *Health Status/
		20	*Comorbidity/ or exp *Mortality/ or exp *Morbidity/
		21	exp *Prognosis/
		22	(burden adj2 (illness or disease or condition or sickness)).ti.
		23	Adaptation, psychological/
		24	(economic adj2 (outcome* or effect* or burden)).ti.
		25	*Cost of illness/
		26	exp *Health Care Costs/
		27	exp *Health Expenditures/
		28	*Quality of Life/
		29	Social Support/
		30	"Activities of Daily Living"/
		31	*Motor activity/
		32	Quality-adjusted life years/
		33	(quality of life or quality adjusted life year* or QoL or HQRL or HRQoL or QALY or self-rated health).ti.
		34	(wellbeing or well-being or quality adjusted survival).ti.
		35	Population Surveillance/
		36	Demography/
		37	Age Distribution/
		38	exp Population Groups/
		39	exp American Native Continental Ancestry Group/
		40	(incidence or prevalence).ti.
		41	*Risk Factors/
		42	*Socioeconomic Factors/
		43	Educational Status/
		44	Income/
		45	Poverty/
		46	Social Class/
		47	Social Conditions/
		48	exp Social Environment/
		49	Minority Groups/
		50	Cultural Characteristics/
		51	Age Factors/
		52	or/18-51
		53	52 and 17
		54	standard* of care.ti.
		55	Practice Guideline/
		56	(health services needs and demands).mp.

		57 Ethnic Groups/ 58 exp Socioeconomic Factors/ 59 Income/ 60 Poverty/ 61 Social Conditions/ 62 exp Social Environment/ 63 (socio-demographic* or social demographic*).mp. 64 Minority Groups/ 65 Social Support/ 66 ((patient or population or key or important or cultural or ethnic or psychological or linguistic or economic or socioeconomic or psychosocial or policy or financial or lifestyle or emotional or psychological) adj2 (disparities or factor* or barrier* or consideration* or implication* or concern* or effect* or issue* or characteristic*)).tw. 67 Cultural Characteristics/ 68 Age Factors/ 69 exp Psychology/ 70 Cultural Competency/ 71 exp Cross-Cultural Comparison/ 72 Cultural Diversity/ 73 Health Services Accessibility/ 74 (barrier* adj3 (implement* or utili?ation or "use")).tw. 75 (access not access-port).ti. 76 (system* adj2 support*).tw. 77 exp Population Characteristics/ 78 Population Surveillance/ 79 Age Distribution/ 80 Ethnic Groups/ 81 exp American Native Continental Ancestry Group/ 82 "Patient Acceptance of Health Care"/ 83 (implication* or issue*).ti. 84 adverse effect*.tw. 85 or/54-84 86 exp Neonatal Screening/ 87 (screen* or tests or test or testing or diagnos*).ti. 88 86 or 87 89 17 and 85 and 88 90 53 or 89 91 5 or 6 or 7 or 8 or 9 or 10 or 11 92 90 not 91 93 90 and 91 94 limit 93 to "review articles"
DynaMed EBSCO Licensed Resource	4 May 2015 8 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea

Note: "††", "*", "# ", and "?" are truncation characters that retrieve all possible suffix variations of the root word e.g. surg* retrieves surgery, surgical, surgeon, etc.

Appendix S.B: Alberta Care Pathways

Figure S.B.1: Classic galactosemia Alberta care pathway

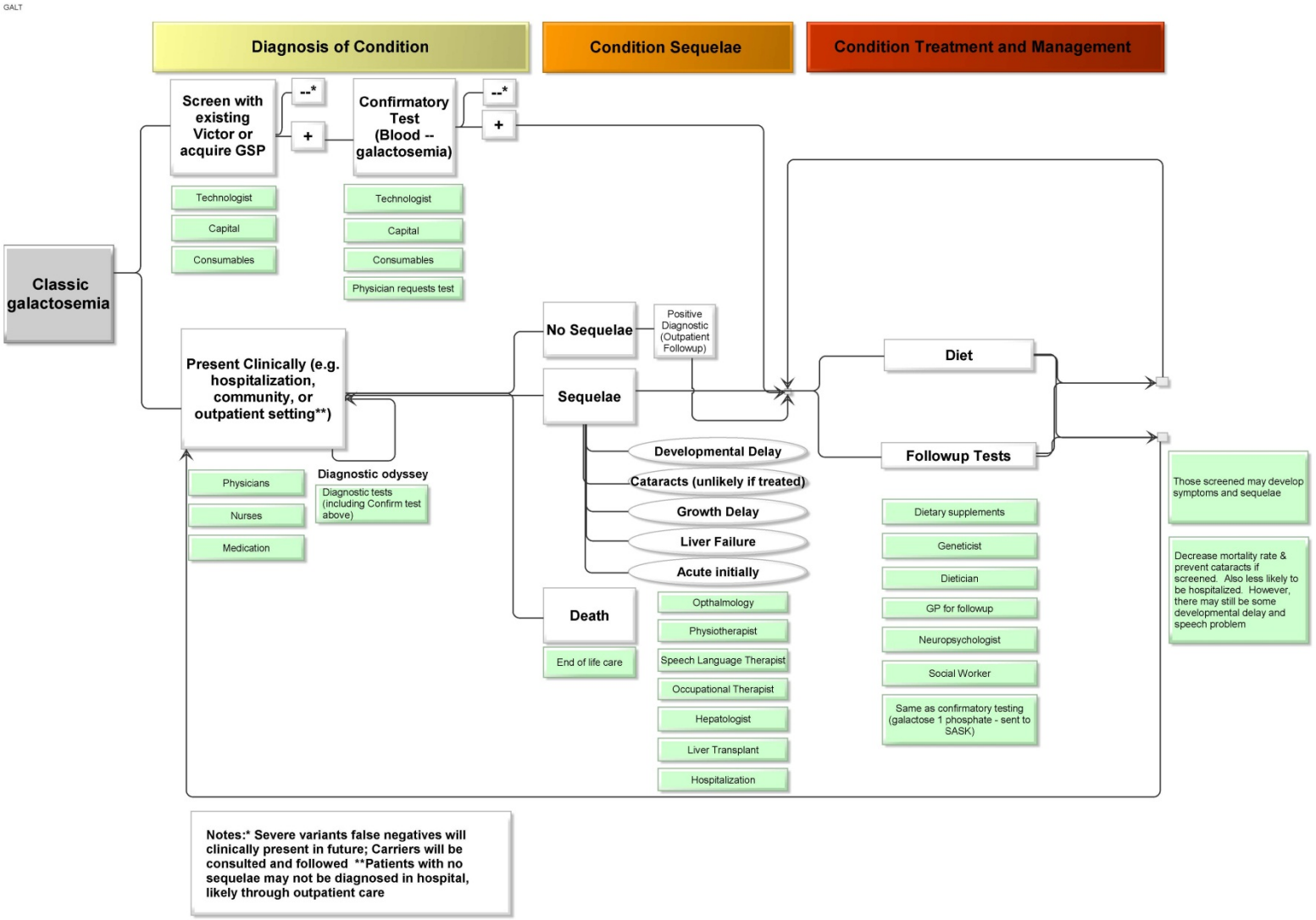


Figure S.B.2: Tyrosinemia type I Alberta care pathway

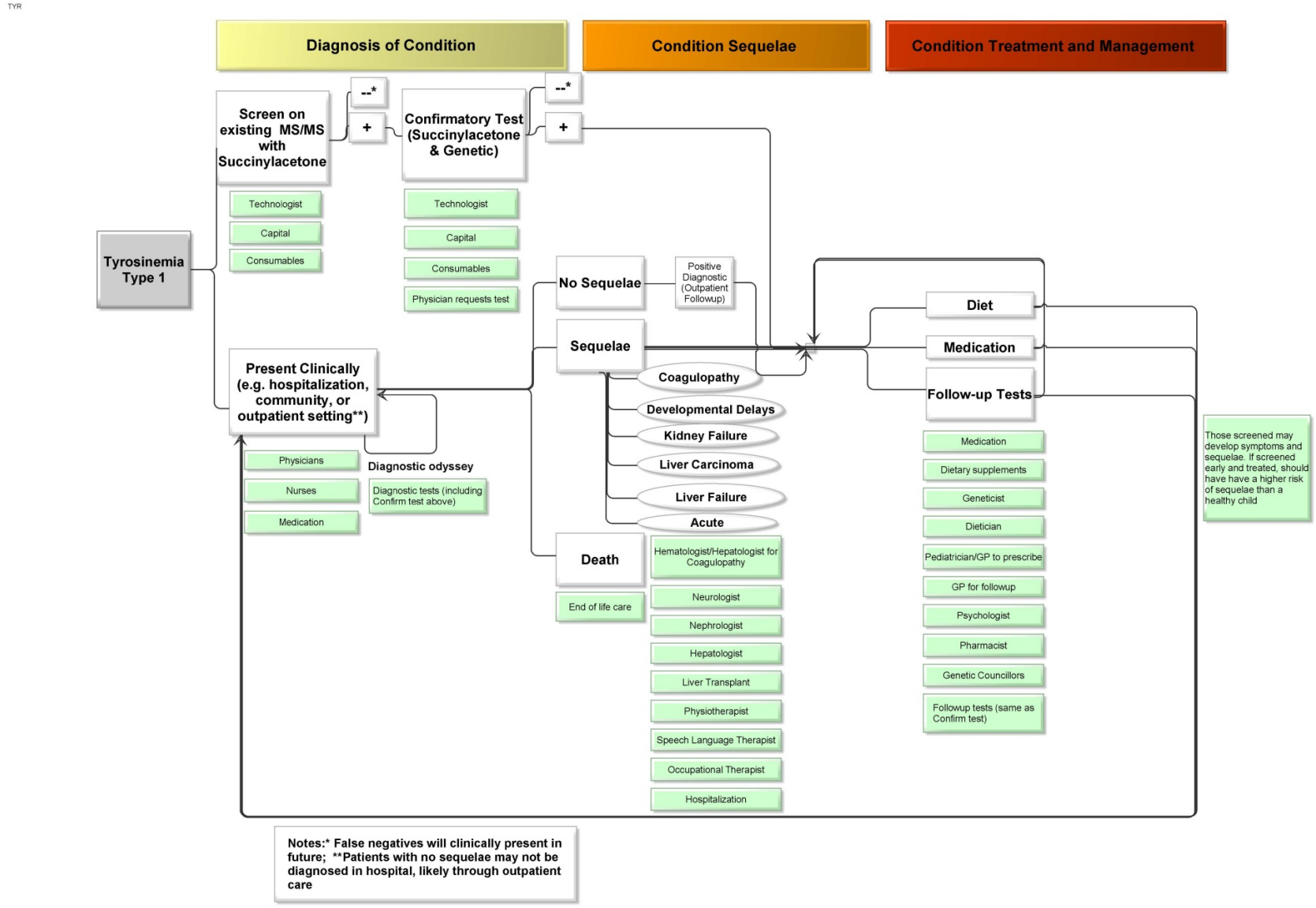


Figure S.B.3: Homocystinuria Alberta care pathway

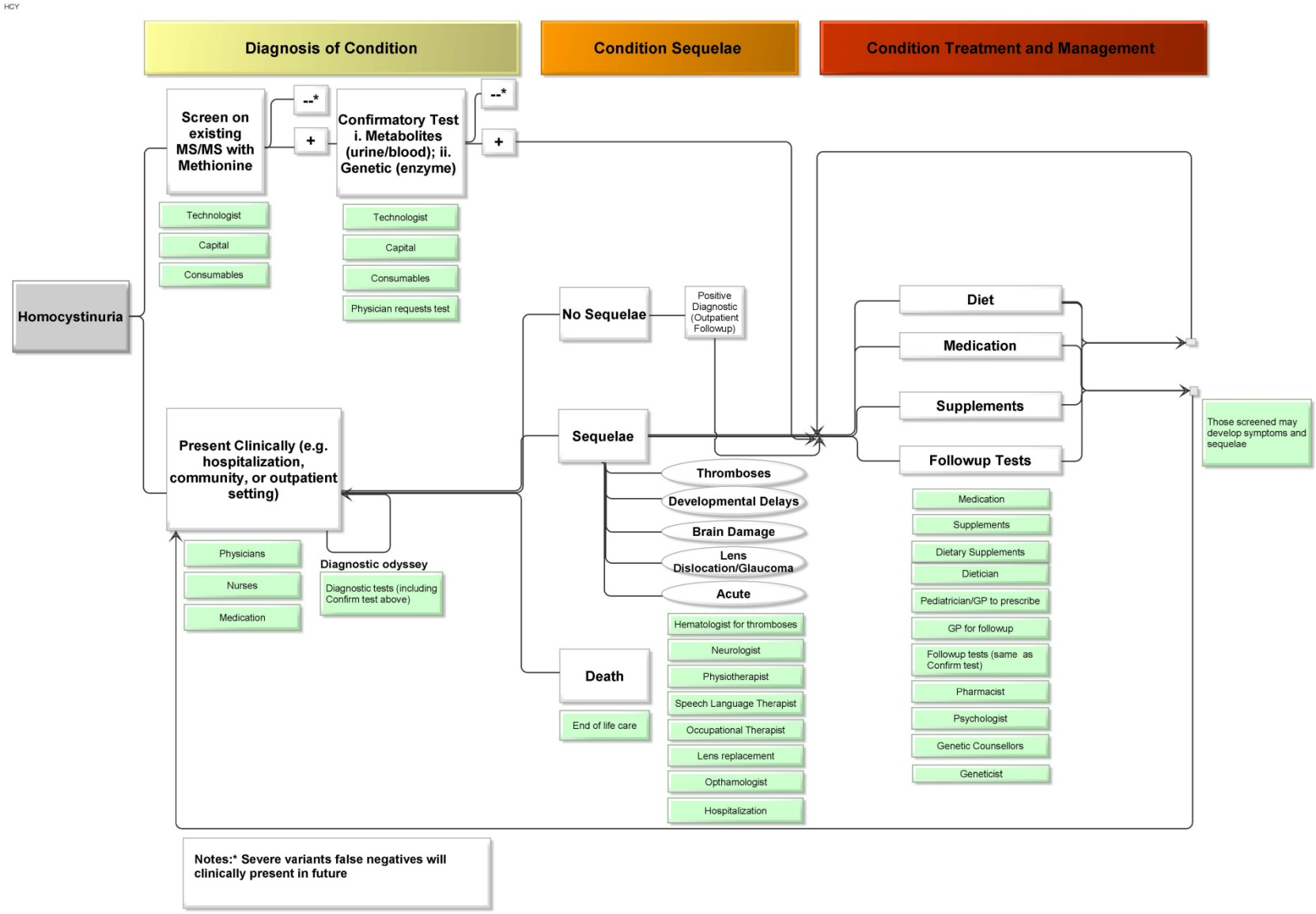


Figure S.B.4: Sickle cell disease Alberta care pathway

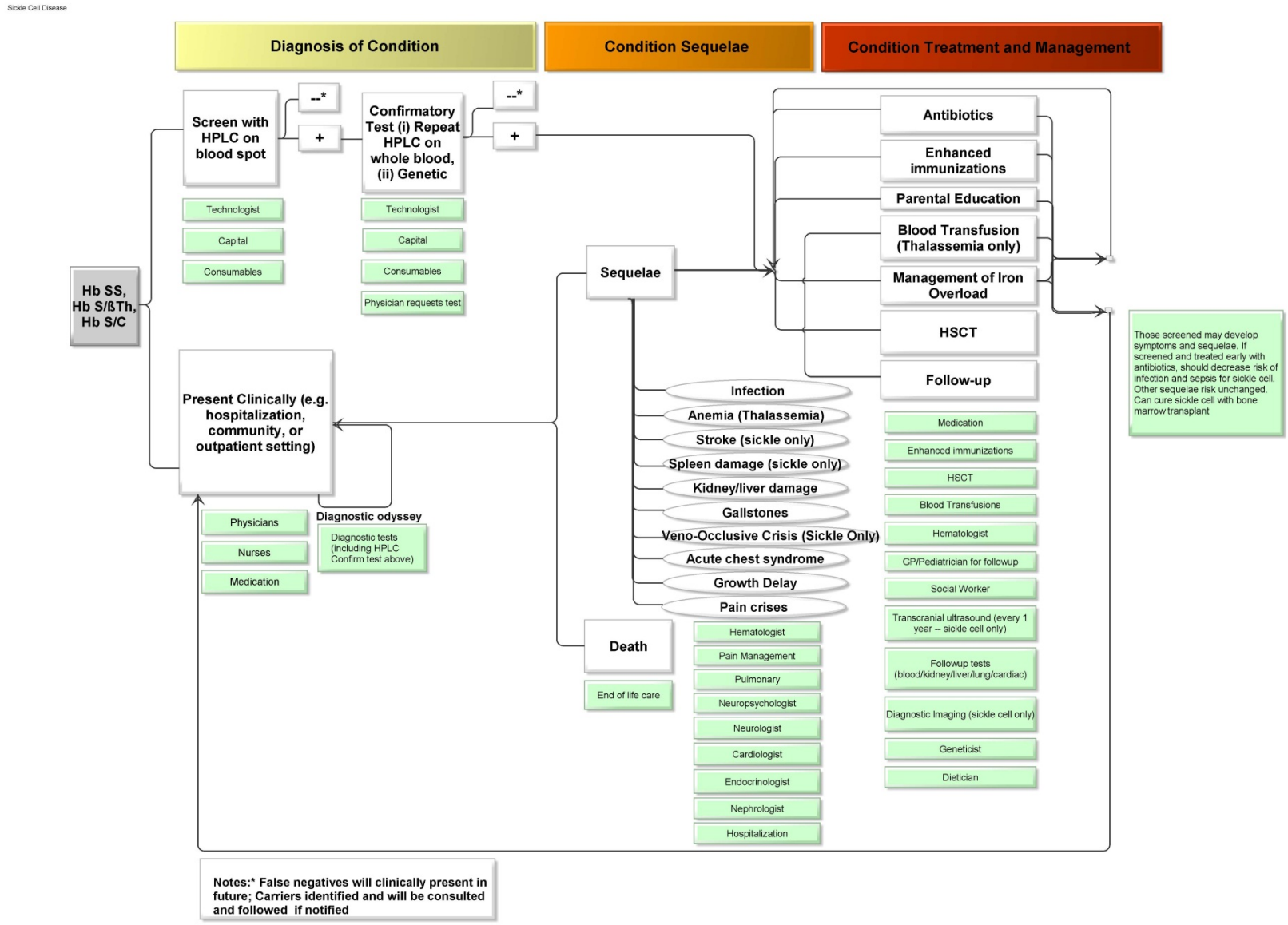
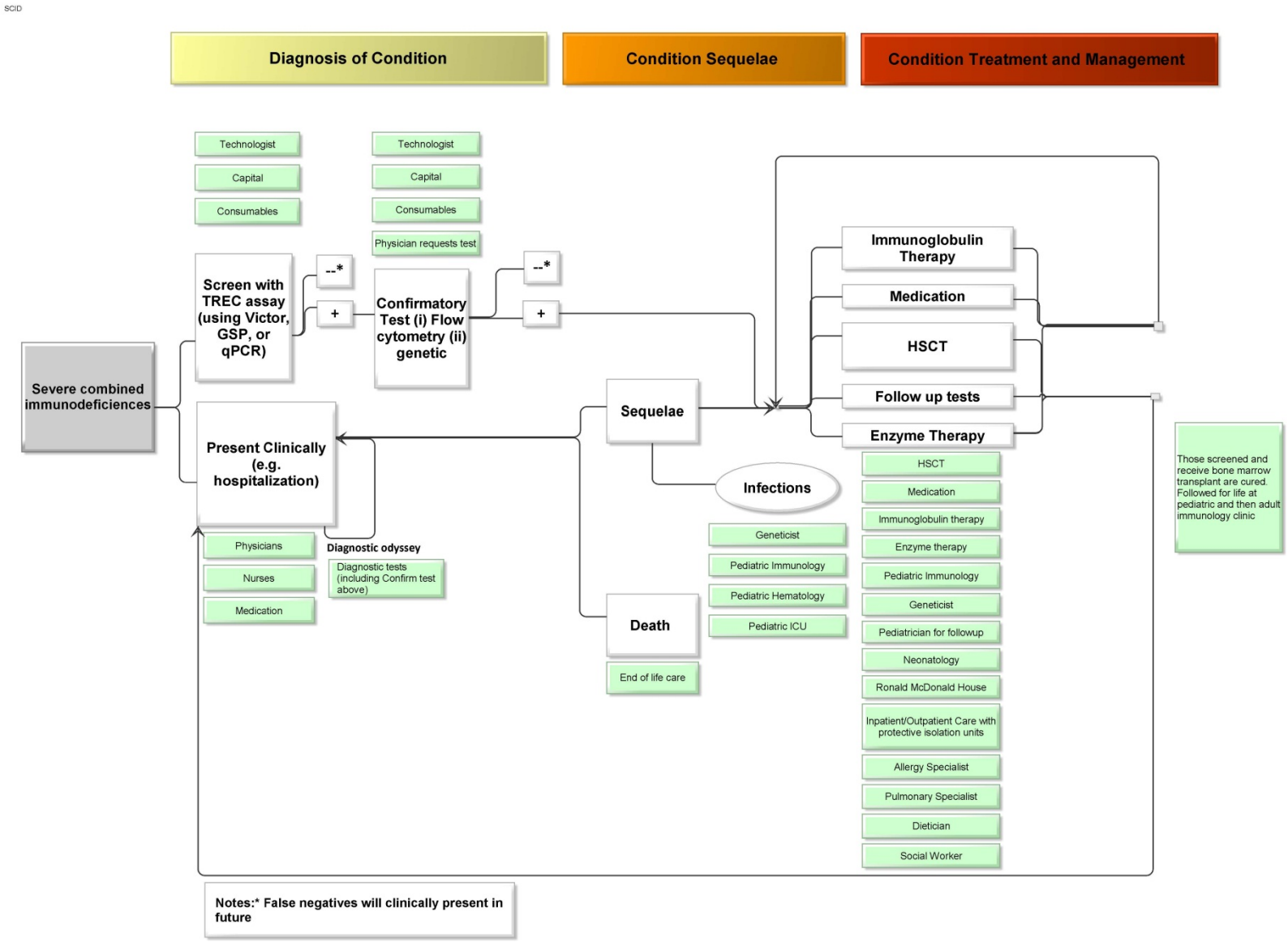


Figure S.B.5: Severe combined immunodeficiencies Alberta care pathway



Appendix S.C: Lab Resource Requirements for Expansion of Screening

Table S.C.1: Lab resource requirements for expansion of screening

Condition	Lab Resource	Implementation Cost	Annual Ongoing Cost
SCID	**Equipment**		
	Applied Biosystems QuantStudio 12K Flex	$193,033.44	$9,102.37
	UPS for QuantStudio	$3,649.36	$364.94
	Thermoshake and controller for backup Tecan Evo	$46,682.94	*
	Posid for Primary Evo	$40,181.04	*
	Various racks and holders Tecan Evo	$2,500.00	
	MCA 96, Roma, and Posid for backup Tecan Evo	$188,983.88	*
	VWR Plate spinner	$834.79	
	Axygen PlateMax sealer	$9,520.28	$952.03
	Labnet Vortemp56 (x2)	$7,975.26	$797.53
	Blood spot puncher	$29,660.00	$2,460.00
	* Service contracts for Tecan Evo upgrades of $20,130		
	Consumables		
	TREC and KREC screen	$24,368.06	$259,050.00
	TREC, KREC, and TBX1 confirmation	$16,867.07	$30,250.00
	Labour		
	Laboratory Scientist I 1.0 FTE	$50,485.00	$100,970.00
	Genetic Counsellor I 0.1 FTE	$5,336.50	$10,673.00
	Information Technology Analyst I 0.2FTE	$10,900.00	$21,800.00
	Geneticist 1.0 FTE	$105,000.00	$210,000.00
	Software		
	MedCalc-analysis software	$729.30	$729.30
	PE Specimen Gate interface	$100,000.00	
	Other		
	Flow cytometry confirmatory test		$3,000
	Blood count		$376.74
	Genetic test (US test, priced at 0.70 CAD)		$27,651.12
	Space	TBD	TBD
TYRI	**Equipment**		
	TriNest Microplate Incubator Shaker (x2)	$5,820.00	$1,128.00
	Top/Bottom freezer	$10,174.00	

	Reagent/Consumables		
	Neobase SUAC assay solution	$20,000	$32,200.00
	Miscellaneous consumables		$1,500.00
	Labour		
	Laboratory Technologist I 0.3 FTE	$14,976.15	$29,952.30
	Genetic Counsellor I 0.1 FTE	$5,336.50	$10,673.00
	Information Technology Analyst I 0.3 FTE	$16,350.00	$32,700.00
	Geneticist 0.4 FTE	$42,000.00	$84,000.00
	Geneticist Trainee (CCMG) 0.4FTE	$13,200.00	$26,400.00
	Laboratory Scientist I 0.7 FTE	$35,339.50	$70,679.00
	Software		
	Specimen Gate service contract increase		$26,200.00
	Software configuration	$35,400.00	
	Other		
	Confirmatory test – Tyr/AAQ/FAH enzyme/liver function	$2,250.00	$4,500.00
	Confirmatory test – genetic test (US test, priced at 0.70 CAD)		$14,163.39
SCD	**Equipment**		
	Flammable cabinet	$1,484.00	
	HPLC BioRad Variant (x2)	$150,000.00	
	HPLC BioRad Variant service agreement (x2)		$20,000.00
	Reagent/Consumables		
	Sickle Cell Program Reagent Pack	$14,000.00	$117,000.00
	Sickle Cell Program Wash	$1,750.00	$15,000.00
	Sickle Cell Program Buffer 1	$2,100.00	$18,000.00
	Sickle Cell Program Buffer 2	$2,100.00	$18,000.00
	External QC and microtitre plates	$100.00	$3,800.00
	Labour		
	Laboratory Technologist I 0.4 FTE	$19,968.20	$39,936.40
	Genetic Counsellor I 0.2 FTE	$10,673.00	$21,346.00
	Information Technology Analyst I 0.3 FTE	$16,350.00	$32,700.00
	Geneticist 0.4 FTE	$42,000.00	$84,000.00
	Geneticist Trainee (CCMG) 0.4 FTE	$13,200.00	$26,400.00
	Laboratory Scientist I 0.8 FTE	$40,388.00	$80,776.00
	Software		
	Software configuration	$85,000.00	

	Other		
	Confirmatory test – HPLC and CBC	$1,485.00	$2,970.00
	Confirmatory test – genetic test		$6,420.60
GALT	**Equipment**		
	GSP (x2)	$546,000.00	
	Refrigerators (x2)	$8,400.00	
	GSP service agreement (x2)		$70,000.00
	Reagent/Consumables		
	GSP Neonatal Assay Kit	$20,000.00	$130,000.00
	Miscellaneous consumables	$7,250.00	$14,500.00
	Labour		
	Laboratory Technologist I 0.3 FTE	$14,976.15	$29,952.30
	Genetic Counsellor I 0.1 FTE	$5,336.50	$10,673.00
	Information Technology Analyst I 0.2 FTE	$10,900.00	$21,800.00
	Geneticist 0.2 FTE	$21,000.00	$42,000.00
	Geneticist Trainee (CCMG) 0.2 FTE	$6,600.00	$13,200.00
	Laboratory Scientist I 0.5 FTE	$25,242.50	$50,485.00
	Software		
	Software configuration	$35,400.00	
	Other		
	Confirmatory test – liver function/GALT quant	$960.00	$1,920.00
	Confirmatory test – genetic test (US test, priced at 0.70 CAD)		$7,111.58
	Space	TBD	TBD
	Renovations	$20,000	
HCY	**Equipment**		
	No new equipment required		
	Reagent/Consumables		
	Miscellaneous consumables	$10,000.00	$1,500.00
	Labour		
	No extra labour required		
	Software		
	Software configuration	$35,400.00	
	Other		
	Confirmatory test – plasma homocysteine/CBS enzyme	$4,320.00	$8,640.00
	Confirmatory test – genetic test (US test, priced at 0.70 CAD)	$0.00	$6,312.00

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SECTION THREE: TECHNOLOGY EFFECTIVENESS AND SAFETY

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3.1 Introduction

Objective

The evidence analysis in the *Technology Effectiveness and Safety* (**T**) section of this STE report will assess the harm, screening test performance, and effectiveness outcomes of newborn screening for the seven conditions being reviewed. The specific research questions from the project charter addressed here are:

1. Is the test sensitive and specific? Does it have a relatively high positive predictive value and a relatively high negative predictive value?
2. Is screening for this condition expected to lead to over-diagnosis (identification of very mild or asymptomatic cases that would be unlikely to come to clinical attention/cause harm in the absence of screening)? If so, what is the likely extent of over-diagnosis? What harms (including psychosocial harms) are anticipated? Is there evidence regarding the degree of harm from over-diagnosis?
3. Is there evidence to support the overall benefit of screening for this condition in newborns/children (e.g. based on evaluations in other jurisdictions), in terms of clinical benefits to individuals with the screened condition? How strong is this evidence?
4. Are there any other potential harms anticipated from the screening test, diagnostic care, treatment, or other aspects of screening?
5. Is there evidence to support the acceptability of screening for this condition in newborns/children, among families of screened children, the public, and/or health professionals?
6. How do outcomes vary between cases being managed after early diagnosis and later diagnosis?

Methods

The details of the methodology used for the T section are outlined in Appendix T.A. In order to answer the above research questions, a two-step research method was used. First, a comprehensive database search was performed to identify and summarize the most recently published systematic reviews and HTA reports to evaluate adding any of the seven conditions to the current Alberta NMS Program. Second, a comprehensive systematic review of primary studies was performed to update and complement the information retrieved from the first step.

In addition, to facilitate the review process, the three metabolic conditions (GALT, TYRI, and HCY) were grouped together, and the three hemoglobinopathies (Hb SS, Hb SC, and Hb S/β-thal) were grouped together; these two groups are discussed in sections 3.2 and 3.3, respectively. SCID is addressed separately, in section 3.4.

Identifying and Summarizing Relevant Published Systematic Reviews/HTAs

In the first step, a comprehensive literature search was conducted including the following databases: Medline, EMBASE, Cochrane Database of Systematic Reviews, Databases of Abstracts of Reviews of Effects, Health Technology Assessment, CINAHL, and Web of Science. Grey literature sources such as HTA agencies' websites, clinical trial registries, and regulatory authorities' websites were also searched. Reference lists of the retrieved articles were also reviewed for further relevant references that might not have been identified by the search. The search strategy combined various medical subject headings and controlled vocabulary terms. The search was limited to systematic reviews and HTAs. The details of the search strategy and the terms used are shown in Appendix T.A (*Literature Search Summary*, Table T.A.1). Reference Manager (v.11) bibliographic software was used to combine the search results and remove duplicate citations.

One researcher (BG) screened the database for potential systematic reviews and HTAs for full-text publication retrieval. Retrieved reports were reviewed by two researchers (BG and ME) for eligibility. An article was deemed to be a systematic review if it met all of the following criteria as defined by Cook et al., 1997:[1]

- focused clinical question;
- explicit search strategy;
- use of explicit, reproducible, and uniformly applied criteria for article selection;
- formal critical appraisal of the included studies; and
- qualitative or quantitative data summary or synthesis.

Each report was independently appraised by the two researchers using the measurement tool for the assessment of multiple systematic reviews, the AMSTAR quality assessment tool.[2-4] Any disagreements were resolved by consensus, and a final quality score and rank was assigned to each review based on the consensus.

Data was extracted from the individual reports by one of the two researchers (BG or ME) using using a standard data extraction form (see Appendix T.A, *Data Extraction*). The extracted data was then synthesized and summarized.

Systematic Review of the Primary Studies

In the second step, a systematic review of primary studies published after the last search dates of the most recent systematic reviews and HTAs for the seven conditions was conducted to update and complement the information retrieved in the first step.

Literature search

A comprehensive literature search was conducted to identify primary studies on newborn screening for the seven conditions. Databases searched include Medline, EMBASE, Cochrane Central Register of Controlled Trials, CINAHL, and Web of Science. The search was limited to the English and French languages. The details of the search strategy and the terms used are shown in Appendix T.A (*Literature Search Summary*, Table T.A.2). Reference Manager (v.11) bibliographic software was used to combine the search results and remove duplicate citations.

Study selection

The titles and abstracts of the search results were screened by one of the two researchers (ME or BG). The full publications of the potentially relevant studies were retrieved and assessed independently by two researchers (ME and BG) for inclusion using the eligibility criteria listed in Table T.1.

Table T.1: Eligibility criteria of the included studies

Population	• Newborns of any ethnic origin
Intervention	• Any laboratory test conducted on a dried blood spot to screen for at least 1 of the 7 listed conditions
Comparator	• Any screening method other than the one used as intervention; or • No screening, or no comparator
Outcomes of interest	Any of the following: • Adverse events arising from the screening tests (safety), as well as other negative consequences such as psychological consequences of false positive results • Screening accuracy parameters including sensitivity, specificity, positive predictive value, negative predictive value, or any other screening accuracy parameter • Effectiveness of early compared to late treatment • Effect of newborn screening programs on morbidity, mortality, quality of life, emergency department visits, or hospital admissions
Study design	Any of the following: • Randomized control trials • Cohort studies • Case-control studies • Cross sectional studies • Case-series studies (≥5 cases)
Target condition	• GALT • TYRI • HCY • SCD (Hb SS, Hb SC, Hb S/β-thal) • SCID
Language of publication	• English • French

Excluded studies that did not fulfill one or more of the eligibility criteria are listed in Appendix T.B.

Data extraction

Data were extracted from the primary studies using a standard data extraction form (see Appendix T.A, *Data Extraction*). The extracted information included: 1) study attributes; 2) characteristics of the newborn population screened or treated; 3) details of the screening test as well as of the confirmatory test; and 4) the reported outcomes. The main outcomes of interest included the test's accuracy parameters and/or the effectiveness outcomes of early treatment and management. The extracted data was then tabulated to facilitate the analysis.

Quality assessment

Several quality assessment tools were piloted using a sample of eight randomly selected studies of different designs and conditions. Based on this piloting process, two tools were selected by the research team to be used in the project, the QUADAS-2[5] and the Effective Public Health Practice Project (EPHPP)[6, 7] (see details in Appendix T.A, *Methodological Quality Assessment*).

Each study was independently appraised by two of three researchers (ME, BG, and PC) using the QUADAS-2 tool or EPHPP tool. Any disagreements were resolved by consensus.

Data analysis and synthesis

The extracted data from the primary studies were tabulated into the previously mentioned four main domains: 1) the study's attributes; 2) the population's characteristics; 3) the test's details; and 4) the reported outcomes. Each domain was then examined and described, and the highlights of the findings were reported. Meta-analysis of the results was not applicable due to the wide heterogeneity among the studies. This heterogeneity, on the other hand, was described in the lines of the analysis and reporting of each domain.

3.2 Newborn Screening for Metabolic Conditions

Description of Technology

Screening for the three metabolic conditions (GALT, TYRI, or HCY) can be conducted by quantification of some metabolites that increase in the blood as a result of these conditions. This quantification is currently done on a tandem mass spectrometer (MS/MS) platform. The MS/MS platform is an analytical instrument that can analyze several compounds. A typical MS/MS has a collision cell or chamber where the sample is separated into different analytes. These analytes are then quantified in the second cell or chamber, using kits specific to the different analytes.

In addition to MS/MS, screening for GALT can be conducted using fluorometric spectroscopy to measure the activity of galactose-1-phosphate uridylyltransferase. This can be done on a Genetic Screening Processor (GSP®) platform, using a GALT enzyme kit (for more details, please refer to the discussion of GALT below).

Description of Included Studies

Systematic Reviews/HTAs

In total, seven reviews[8-14] were found that evaluated adding at least one of the three metabolic conditions to already existing newborn screening panels in different jurisdictions. The highlights of the results and conclusions of each report are discussed with each respective condition below.

Overall, the included reviews used frameworks similar to the Australian Framework, or weighing criteria to evaluate the inclusion of at least one of these conditions in a current screening panel. Therefore, most of the reviews grouped the studies into different categories to evaluate using the different criteria of the respective frameworks (for example, diagnostic accuracy of relevant tests, treatment availability and effect of early treatment, cost-effectiveness of screening, and epidemiology studies on incidence and/or prevalence, as well as the burden of the condition on the individuals and/or systems). In general, almost all of the reviews highlighted some important limitations of the primary studies that they included and of the evaluation process. The main limitations mentioned were: the difficulty of conducting randomized controlled trials (high levels of evidence) to evaluate

the effect of screening for these conditions due to their rarity; and the ethical concern of withholding treatment from patients with these conditions to allow comparison with those who had earlier access.

Using the AMSTAR tool to evaluate the quality of these reviews resulted in low to moderate quality levels of evidence. In general, these reviews were HTAs that used the systematic review approach to tackle the evaluation process. However, likely due to the need for an expedited decision, these reviews were not conducted in a scientifically rigorous manner. The reviews had overall low quality scores due to not conducting duplicate selection or data extraction of the studies, not consistently and explicitly conducting critical appraisal of the included studies, or not consistently providing a comprehensive list of the included studies. The detailed results of the quality appraisals of these reviews using the AMSTAR tool are shown in Appendix T.C (Table T.C.1).

Primary Studies

Upon screening the database by the two reviewers and retrieving the full-text potential publications, a total of 23 primary studies were included in this review on the metabolic conditions. The studies were selected if they were not included in one of each condition's respective systematic reviews/HTAs, or if a finding had been missed or omitted in the systematic reviews/HTAs but is of importance for our current review.

Study designs, settings, and funding sources

The number of neonates included in the primary studies varied greatly, ranging between 14 in a small case-series study and 1,495,132 in a large national newborn screening program. The highlights of the results and conclusions of the primary studies are discussed with each respective condition below, and further details (including settings, countries, and number of patients) can be found in Appendix T.C (Table T.C.4).

Among the 23 included studies, 15 were retrospective observational studies, three were case-series, two were cross-sectional, and three were case-control studies. The single arm cohort design is the best design for the diagnostic accuracy studies. However, because the cohort studies were mostly conducted within the context of a national newborn screening program, the confirmatory tests were performed only on those who tested positive in the first-tier screening test. Performing the confirmatory test only on neonates who screen positive makes the data of false negative results uncertain. The case-control design, on the other hand, is not an optimum design for this kind of study, as it tends to inflate the diagnostic accuracy parameters.

Ten of the 23 studies were conducted within a context of a routine or a pilot screening program setting. Five studies were conducted in an academic setting. The remaining studies were conducted in either a laboratory setting using archived samples, or a collaborative group/regional setting where patients' data were collected from different centres. There were two studies that looked at the effect of screening for one of the target conditions in a neonatal ICU setting.[15, 16] The studies were conducted in wide variety of countries across different continents: North America (USA and Canada), South America (Brazil), Europe (Germany, France, Ireland, Denmark, Austria, and Turkey), and Asia (China, Taiwan, and Singapore).

Among all the included studies, only four of them reported non-private funding (governmental or academic) and declared no conflict of interests.[15-18] The remaining studies did not report on either their funding source(s) or conflicts of interests.

Methodological quality

Using the QUADAS-2 tool for the assessment of the quality of the studies reporting on screening accuracy parameters (15 studies), overall, the studies were found to be of either a low or unclear quality in terms of internal validity (that is, high overall risk of bias), and high or unclear quality in terms of external validity (that is, low or unclear applicability concerns). Figures T.1 and T.2 show the overall quality of the included studies.

Figure T.1: Risk of bias (internal validity) in the included primary studies

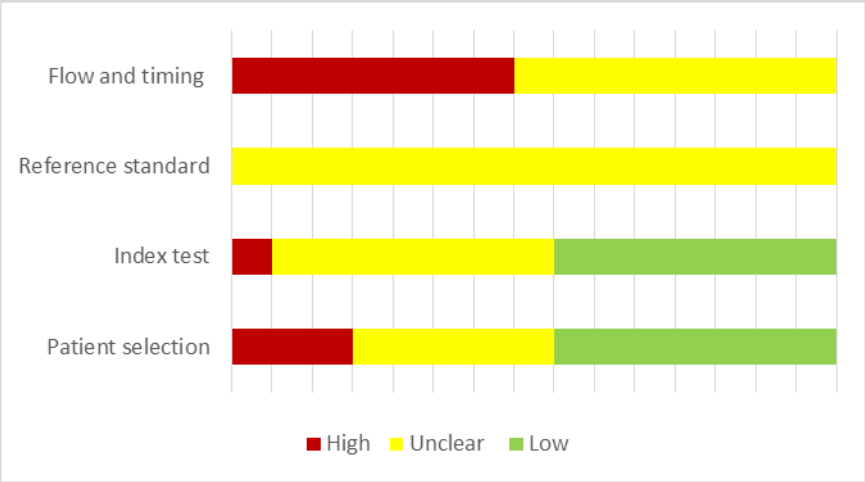
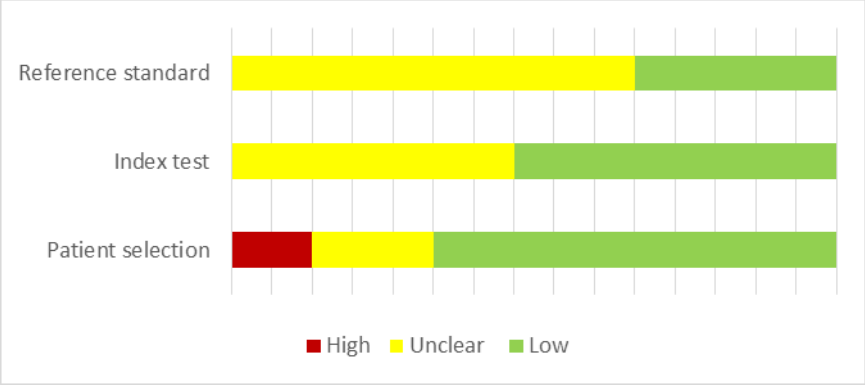


Figure T.2: Applicability concerns (external validity) in the included primary studies



The details of the scores of each study in each respective domain, together with the final ranking in internal and external validity aspects, are illustrated in Appendix T.C (Table T.C.2).

Eight studies were critically appraised using the EPHPP tool. All of them had a weak overall final rank. This was mainly due to weak study design, data collection method validation, or not clearly addressing or reporting on confounders. The results of the critical appraisal of these eight studies using the EPHPP tool are illustrated in Appendix T.C (Table T.C.3).

Results

Harms Related to Newborn Screening

The screening procedure itself (that is, the heel prick and the sample collection procedure) appeared to be safe. None of the included reports or studies reported any adverse event or safety concerns related to the screening procedure. Adverse events related to treatment are discussed in each condition's respective section below.

Effectiveness of Screening for TYRI

Systematic reviews/HTAs

There were four HTA reports found on the effectiveness of screening for TYRI. The most recent report, Makni et al., was from 2013.[8] The report's main objectives were to assess the relevance of screening for TYRI in Quebec, as well as for two more metabolic conditions, medium chain acyl-coenzyme A dehydrogenase deficiency (MCADD) and phenylketonuria (PKU). Screening for TYRI in Quebec started in the 1970s because of the founder effect, which is defined as a high frequency of a mutation in the descendants of a group of common ancestors, due to geographic isolation or high consanguinity in an ethnic group.

Four studies that looked at the diagnostic accuracy of MS/MS in the screening for TYRI were included in the HTA report by Makni et al. Among the four studies, only one study (Sander et al.) was specific to TYRI;[19] this study was included in our primary studies list because it has some treatment outcomes that were not highlighted in the HTA report. The other three studies reported on the diagnostic accuracy parameter of TYRI as one of the conditions within a screening program that included other metabolic conditions. The target metabolic marker used in these studies varied, as did their reported cut-offs. These targets were either tyrosine, or tyrosine in combination with tyrosine/phenylalanine ratio or with succinylacetone (SA). It is noteworthy that the elevation in succinylacetone is considered pathognomonic for TYRI.[20] A pathognomonic symptom or sign is defined as a very specific character for a disease or condition; SA elevation occurs only in TYRI, not in the other variants of the condition.

Despite the variation in the target metabolic markers and their cut-offs, the diagnostic accuracy parameters reported in the studies included in Makni et al. were high. Table T.2 shows the studies' reported targets, cut-offs, and diagnostic parameters.

Table T.2: Metabolic markers, cut-offs, and diagnostic accuracy parameters reported in the primary studies included in Makni et al.

Study	Metabolic marker	Cut-off(s)	Diagnostic parameters
Zytkovicz et al.[21]	Tyrosine	>442 µmol/L	Sn: NR Sp: 99.9% PPV: 0 NPV:NR
	Tyrosine Tyrosine/phenylalanine	>442 µmol/L >6	Sn: NR Sp: 99.9% PPV: 0* NPV: NR
Wilcken et al.[22]	Tyrosine	>500 µmol/L	Sn: 0* Sp: 99.9% PPV: 0* NPV: 99.9%
Schulze et al.[23]	Tyrosine SA	>200 µmol/L NR	Sn: 100% Sp: 99.9% PPV: 1.9-3.8% NPV: 100%

*Due to the rarity of the condition, some studies did not detect true positive cases, yielding unreliable results for the PPV and Sn.

NPV: negative predictive value; NR: not reported; PPV: positive predictive value; SA: succinylacetone; Sn: sensitivity; Sp: specificity

Makni et al. did not report on studies that evaluated the effect of early treatment outcomes for TYRI using 2-(2-nitro-4-triflouromethyl-benzoyl)-cyclohexane-1,3-dione (NTBC), also known as nitisinone (Orfadin®). NTBC works by reducing the level of toxic metabolites that accumulate in the body as a consequence of the condition. However, Makni et al. did indicate the presence of ongoing studies and the subsequent accumulation of data on the efficacy of NTBC, and stated that this data seems promising and corroborates the utility of screening for TYRI.

The report recommended performing a pilot study for the screening of TYRI in addition to the other two conditions included in the report (MCADD and PKU), or postponing the implementation of screening using MS/MS technology until more validation studies become available. The report also suggested that the use of MS/MS to screen for TYRI is conceivable if SA is quantified together with tyrosine, because tyrosine alone is neither sensitive nor specific.

Pandor et al. published their review in 2004.[12] The main objective of this report was to evaluate the effectiveness and cost-effectiveness of neonatal screening for inborn errors of metabolism using MS/MS in the United Kingdom. This report was an update of two previous reports from Seymour et al. (1997) and Pollitt et al. (2001). Pandor et al. did not find studies on the diagnostic accuracy of MS/MS in screening for TYRI. Only one study looked at the treatment effectiveness, and this study had a small number of patients (n=8) who were followed up for a median of 6.7 years, after either orthostatic liver transplantation or reduction hepatectomy. Only two of the eight patients were on NTBC and diet treatment; the remaining six patients were enrolled before the advent of NTBC. The 1-year survival rate was 88%, and the 5-year survival rate was 77%. The study also reported that all survivors were in mainstream school, had a reduction in annual hospital visits, and had a good quality of life.

Pandor et al. concluded that effective treatment for TYRI exists based on this study and communication with an expert; however, they also reported that screening for TYRI is labourious and not specific, as it also gives positive results with another benign condition (transient hypertyrosinemia). It is noteworthy that, at this time, most screening programs were measuring tyrosine levels using chematographic technique or bacterial inhibition assay, and the NTBC treatment was not commercially available on a wide scale.

Vallance et al., from 2007, reviewed the addition of TYRI to the screening panel for the British Columbia newborn screening advisory committee.[11] They concluded that early detection and NTBC treatment help in reducing the incidence of hepatocellular carcinoma, and advised that measuring tyrosine levels is not specific because tyrosine is also elevated with TYRII, TYRIII, and transient hypertyrosinemia. Instead of using tyrosine as a first-tier test, they advised that SA has sensitivity and specificity of almost 100% for TYRI.

In 2003, an HTA was conducted by the Medical Advisory Secretariat of Ontario's Ministry of Health and Long-Term Care.[9] This report synthesized the results of Seymour et al. and Pollitt et al. The report did not give clear conclusions or recommendations on adding TYRI. However, it illustrated a criteria weighing system that has been used for policy development, based on the following elements: incidence, treatment availability, incremental cost, and availability of testing in other jurisdictions. It is noteworthy that Ontario started screening for TYRI in 2006.[xix]

Primary studies

Test details (platform, target, and cut-offs)

Eleven primary studies were located which were not included in the most recent HTA. Among the 11 studies, three studies did not report the specifics on the diagnostic platform used, as these studies were looking only at early treatment outcomes.[24-26] All of the remaining eight studies reported using the MS/MS platform. There was wide variation in the models used among the studies, as well as the manufacturers.[15, 17, 19, 20, 27-30] There were two studies in which the authors reported that they were able to process 1,000 samples per day using MS/MS with very minimal additional manual work requirement, indicating a high throughput capacity of the MS/MS platform.[19, 20] The details of the platforms used in the included studies are shown in Appendix T.C (Table T.C.4).

Among the seven studies that reported on the screening tests used for TYRI, tyrosine (reported in five studies) and SA (reported in three studies) were the target analytes quantified in the first-tier test. The confirmatory tests reported in these studies were either repeating the same test, quantifying SA in plasma and urine, or DNA genetic testing. The latter was reported in two studies.[17, 30]

The cut-offs reported in the studies varied greatly for tyrosine (150-500 μmol/L) and SA (3-10 μmol/L). Two studies reported setting initial cut-offs at the start of the programs based on the 99th percentile of the normal range during their pilot phase, and four times the standard deviation above the mean of the population screened during the pilot phase.[17, 28] These cut-offs were then adjusted and modified to enhance the diagnostic parameters by reducing the false positive results.

[xix] For availability of TYRI screening in different Canadian jurisdictions, please refer to the S section of this report (specifically, section 2.2).

Accuracy of screening tests

In general, the screening accuracy parameters reported in the studies were high. However, the certainty and reliability of these results are questionable, due to the previously discussed limitations found in the primary studies. Sensitivity and specificity were both 100% when SA was the primary marker.[19] One study, on the other hand, reported sensitivity and specificity of 95.6% and 99.8%, respectively.[17] These values were reported for an overall screening program that included screening for TYRI and other conditions. Positive predictive value was 40% in one study that evaluated TYRI only, and ranged between 20 and 36% in two studies that looked for an overall screening program that included other conditions.[15, 17] There was one study for which the diagnostic accuracy parameters could not be mathematically calculated, as no cases with the disease were discovered.[27] Magera et al. reported 2,229, 1,177, and 430 suspect cases at cut-offs 130, 150, and 180 μmol/L, respectively, when tyrosine was the primary target for the first-tier test. However, when SA was used as a second-tier test, none of these cases were a true positive case.[29] The details of the results of the screening accuracy parameters reported in the studies are shown in Appendix T.C (Table T.C.4).

Early detection and NTBC treatment outcomes

There were three studies reporting on the early versus late NTBC treatment outcomes of TYRI.[24-26] Overall, the results reported in these studies suggest that early treatment following early diagnosis has better outcomes in terms of mortality, the need for liver transplants, the reduction in acute liver decompensation episodes, and other surrogate laboratory outcomes such as the improvement in the liver function tests. However, the number of patients enrolled in these studies was small (46, 78, and 168, respectively) due to the rarity of this condition; this small number of patients, as is the case with all rare diseases, raises some challenges around the statistical power of those studies and the certainty of the results. The largest study conducted by Myorandan et al. (n=168, with 148 evaluable patients for whom the treatment and outcomes data were available) was a collaborative work between metabolic centres in 13 different European countries.[26] The results appeared to favour early detection and NTBC treatment in terms of the incidence of hepatocellular carcinoma, the need for liver transplantation, and the development of renal dysfunction. However, most of the confidence intervals reported for the odds of these outcomes were either very wide, containing, or marginally close to the value of 1; from a statistical perspective, this reduces the certainty of these results. The details of the results of these studies are reported in Appendix T.C (Table T.C.4).

Other outcomes

In the study conducted by Lim et al., the authors reported that the screening program was voluntary and not mandated by a policy. The rate of uptake increased significantly over the years, reaching 71% at the time of publication, reflecting good acceptability of the program.[17]

Overall, the NTBC treatment appears to be well tolerated. None of the included studies reported occurrence of serious adverse events from the treatment. In the study conducted by Myorandan et al., the adverse events reported were non-serious, with eye pain and eye itching being the most frequently reported event in 6.3% and 5.6% of treated patients, respectively.[26]

Unlike tyrosine, SA is a low-level metabolite that accumulates beyond the normal levels only with the deficiency of the enzyme fumarylacetoacetate hydrolase (the deficiency of which causes TYRI). Therefore, SA is considered highly specific (pathognomonic) for TYRI. Tyrosine, on the other hand, is a non-specific target. Therefore, elevated levels of tyrosine may exist in TYRI and other variants of the disease such as TYRII, TYRIII, and another benign condition known as transient

hypertyrosinemia of the newborn. This was evident in our primary studies in that those studies which reported using SA as the first-tier test target were able to detect TYRI only, while the studies which used tyrosine as the first-tier test target were able to detect TYRI, TYRII, and TYRIII.[19, 20, 28]

Conclusions and highlights on TYRI

Despite the questionable quality of the literature due to the rarity of the disease, some conclusions could be drawn from the literature on screening for TYRI:

- Screening for TYRI is safe, no complications were reported due to the heel prick procedure.
- Overall, screening for TYRI using MS/MS appears to have high sensitivity and specificity, especially when SA is used as the primary target marker.
- Unlike tyrosine, SA is pathognomonic (highly specific) for TYRI. Using SA as the screening target marker detects only TYRI and not the other variants of the condition, and therefore reduces the recall rate and the false positive rate.
- Early treatment using NTBC seems to have good overall outcomes compared to late or no treatment, in terms of reducing the condition's complications.
- NTBC treatment appeared to have an overall good safety profile. None of the studies reported an occurrence of serious adverse events. The adverse events reported appeared to be manageable and non-serious.

Effectiveness of Screening for GALT

Systematic reviews/HTAs

There were three reports identified in the literature on the effectiveness of newborn screening for GALT.[9, 10, 14] The most recent report, Cote et al., was from 2013.[14] The main objective of the report was to advise on expanding Quebec's newborn screening panel to detect 21 inborn errors of metabolism, among which GALT was examined. Inborn errors of metabolism are a group of genetic disorders in which a single gene defect causes a block of the metabolic pathways of a certain substance, leading to an accumulation of this substance and other substrates behind the block, or deficiencies in its product.

In addition to evaluating the effectiveness of screening for GALT, Cote et al. also stressed the importance of the ethical and organizational aspects of expanding the screening panel. The report highlighted the two methods used in screening for GALT, both of which are based on the metabolism of galactose-1-phosphate by the enzyme galactose-1-phosphate uridylyltransferase:

1. measuring the elevation in total galactose and galactose-1-phosphate (as the deficiency of galactose-1-phosphate uridylyltransferase leads to accumulation of these metabolites), using an MS/MS platform; or
2. measuring the activity of galactose-1-phosphate uridylyltransferase, using the fluorometric enzyme assay method (also known as spectrofluorometry).

Because it measures a high-level metabolite, the first method can detect other rarer enzyme deficiencies in the metabolism of galactose, namely deficiencies in galactose epimerase (GALE) and galactokinase (GALK). The second method detects only GALT and the milder variant of GALT, the Duarte variant. The fluorometric enzyme assay method discussed in the report used the AutoDELFIA® kit, manufactured by PerkinElmer.

Cote et al. included only two studies that evaluated the screening tests' performance for GALT. The authors did not report diagnostic accuracy parameters of the screening test, but stressed only the false positive and false negative numbers reported in the two studies. The authors highlighted the importance of considering the time of sample collection, the method used for screening, and their relation to the false negative results. For instance, an infant who was breastfed before the sample collection might have an elevated level of the metabolite being measured. However, those infants whose sample was collected before being breastfed might not have the accumulated metabolite in their blood yet, and thus might test negative, despite having the disease. Cote et al. also highlighted a United States study that reported a 10 times reduction in mortality rate with effective management of GALT; however; they also reported another study which concluded that the effectiveness of the treatment in preventing long-term complications appears to be limited.

The report advised to include GALT in the screening panel together with biotinidase deficiency,[xx] which requires the same fluorometric enzyme assay technique. The recommendation was based on weighing criteria that included the severity of the disease, the incidence of the disease, the efficiency of the screening test, and the effectiveness of the treatment or intervention.

Seymour et al., from the United Kingdom in 1997, concluded that the literature is controversial regarding the value of neonatal screening for GALT, based on four studies on the diagnostic accuracy of screening tests and another four studies on disease outcomes. They found little evidence that early management may improve long-term outcomes, and they also stated that no effective treatment for the condition was available. Therefore, they did not recommend screening for GALT. It is noteworthy that the technique of measuring galactose-1-phosphate uridylyltransferase activity using the fluorometric enzyme assay was not yet available at the time of conducting this report.[10]

In the 2003 report by the Medical Advisory Secretariat for Ontario's Ministry of Health and Long-Term Care, the evidence shown in Seymour et al. report was revisited, criticized, and re-evaluated, based on Ontario's weighing criteria for the appropriateness of funding the screening for inborn errors of metabolism (as previously discussed in the TYRI section). This report did not give a final conclusion or recommendation on GALT; however, GALT is currently included in Ontario's screening panel.[xxi9]

Primary studies

Test details (platform, targets, and cut-offs)

Eleven studies were included on the effectiveness of screening for GALT. Among the 11 studies, eight studies reported on one or more of the diagnostic accuracy parameters of screening tests. There was wide heterogeneity in the test targets, cut-offs, and techniques used in these studies. Despite this heterogeneity, there were two main platforms reported in the studies, either MS/MS or fluorometers, based on the target marker used in each test.

In general, there were two main tests used for screening GALT. The first test, measuring total galactose and galactose-1-phosphate, was reported in five studies,[30-34] and was done on a MS/MS platform. The second test, measuring galactose-1-phosphate uridylyltransferase activity, was reported

[xx] As mentioned in the S section, biotinidase deficiency is currently in the Alberta screening panel.
[xxi] For availability of GALT screening in different Canadian jurisdictions, please refer to the S section of this report (specifically, section 2.2).

in three studies,[34-36] and was done using spectrofluorometry. The details of the platforms used (including brands and manufacturers) are shown in Appendix T.C (Table T.C.4).

The cut-offs for the total galactose measurement, as reported in four studies, ranged between >7 and >20 mg/dl.[30, 32-34] The cut-offs for measuring galactose-1-phosphate uridylyltransferase activity, as reported in two studies (using different measures), were 40 μmol/L and 10% of the mean galactose-1-phosphate uridylyltransferase fluorescence of all the samples in a plate, respectively.[34, 36]

Overall, the studies did not give details or did not report on the confirmatory tests used. However, the most frequently reported confirmatory tests were either repeating the same test, clinical confirmation, or DNA genetic testing.

Accuracy of screening tests

In general, despite the wide heterogeneity of the tests used in the studies, the sensitivities and specificities reported in these studies were high. Sensitivity was 100% among all six studies reporting on this parameter.[18, 30, 31, 33, 34, 36] Specificity, reported in five studies, ranged between 99.7 and 100%.[18, 30, 31, 33, 36]

The positive predictive values reported in the studies, on the other hand, ranged between 0.5 and 5% in the four studies reporting on this parameter.[18, 30, 32, 33] There was only one study that reported a high positive predictive value, of 83%.[34] The low positive predictive values reported in the first four studies could be the results of: first, the rarity of these diseases and the subsequent low number of true cases detected; and second, setting low cut-offs for the tests in order not to miss any cases, which is a very important aspect in any screening program.

Negative predictive value, reported in one study, was 100%.[33] The details of the diagnostic accuracy parameters of screening tests reported in the studies are shown in Appendix T.C (Table T.C.4).

Early detection and management outcomes

There were four studies reporting on the disease management outcomes.[16, 35, 37, 38] Due to the rarity of the condition, the number of patients enrolled and followed up in these studies was small (n=22 to 45). The management protocols described in the studies were based on a galactose- and lactose-free diet. To date, no drugs are available to treat this condition.

Overall, the results suggest that only cataract development and the need for cataract surgery may be reduced with early management. The impacts of early management on other complications associated with GALT (for example, speech delay and reduced IQ) were inconclusive. In only one of these four studies, comparing a group of clinically diagnosed newborns with some inborn errors of metabolism including GALT versus another group diagnosed by a newborn screening program, the results appeared to favour early detection and management in terms of reducing mental retardation and the hospitalization rate. The details of the results reported in these studies are shown in Appendix T.C (Table T.C.4).

It is noteworthy that in Alberta, two cases of GALT were detected in 2006 and 2014. The first case was diagnosed clinically in 2007, developed cataracts and required surgery, and currently has depth perception issues. The second case died due to liver failure. According to our EAG, the morbidity of the first case and the mortality of the second could have been avoided with screening (EAG members, personal communication, March 2015).

Other outcomes

Camelo et al. reported that all parents gave consent for screening their newborns (n=59,953), indicating high acceptance.[33] Lund et al. also reported that, among the 586,969 newborn families who were offered screening, 85% agreed and signed the consent form.[30] The authors also reported that parents' acceptability improved dramatically over the years, from 65% at the beginning of the pilot phase to almost 100% at the introduction of the routine program; failure to inform the parents about the pilot phase was noted as the reason for the modest acceptability at the beginning.

In Rhode et al., galactose-1-phosphate uridylyltransferase activity was measured.[36] The authors noted variation in the results among the different seasons; their interpretation was that the higher temperature in the summer caused denaturation of the enzyme protein. The authors emphasized the importance of high quality standards for the shipping of samples between the hospitals and the labs.

In a study published in 2012, Lund et al. reported results of a newborn screening program in Denmark for a number of conditions, including GALT.[30] Among 84,045 newborns screened during 2001 and 2004, 20 had positive results, but only one was true positive case of GALT, who was already clinically symptomatic by the time the screening result was available. GALT was removed from the newborn screening program in Denmark in 2005, due to the high false positive rate, the late results, and resulting failure to detect the patients before the onset of symptoms.

Reduced galactose-1-phosphate uridylyltransferase activity occurs only with classic galactosemia. Therefore, measuring the enzyme activity allows only for detection of GALT and the mild clinical variant of GALT, the Duarte variant. Quantifying total galactose, on the other hand, will detect GALT as well as other galactose inborn errors of metabolism, the GALE and GALK deficiencies. This was evident in the primary studies, as the studies that used galactose-1-phosphate uridylyltransferase activity only as the primary target of the first-tier test were able to detect only GALT and the Duarte variant;[35] the studies that used total galactose as their first-tier test primary target were able to detect GALE and GALK cases, as well as GALT and the Duarte variant.[34]

Conclusions and highlights on GALT

Despite quality concerns with the literature, mainly due to the rarity of the disease, some conclusions could be drawn from the literature on screening for GALT:

- Screening for GALT using the dried blood spot sample is safe. No incidents of adverse events due to the heel prick were reported in the literature.
- In general, despite the heterogeneity in the techniques and cut-offs used, screening for GALT appeared to have high sensitivity and specificity.
- Using the galactose-1-phosphate uridylyltransferase activity test for screening for GALT detects only classic galactosemia and the Duarte variant; unlike the measurement of total galactose (the high-level metabolite), measurement of galactose-1-phosphate uridylyltransferase activity does not detect the other rare types of galactose metabolism, GALK and GALE deficiencies.
- To date, there is no approved drug treatment for GALT. Management is based on providing a lactose- and galactose-free diet.
- The effect of early screening and management of GALT on long-term outcomes seems to be controversial. Very scarce, low-level evidence suggests that early management may have

favourable effects on mortality and cataract development, only when compared to clinical detection.

Effectiveness of Screening for HCY

Systematic reviews/HTAs

There were four HTA reports evaluating the addition of HCY to existing screening panels in four different jurisdictions.[9, 11, 13, 14] In addition to the four identified reports, a recent Cochrane systematic review published in 2013 was identified.[39] However, the selection criteria of this systematic review limited the studies to randomized controlled trials and controlled trials only, and it did not find any study of these designs. Therefore, this review did not provide any valuable data about the research question.

The most recent HTA report, Cote et al. (discussed above in the GALT section), included five primary studies, all of which used MS/MS as the platform to screen for HCY, and methionine as the target measured.[14] Methionine was either quantified alone (as reported in all five studies), or in combination with other metabolites such as phenylalanine (methionine/phenylalanine ratio, as reported in two studies); the cut-offs used for methionine measurement were not reported in any of the studies. Cote et al. did not report on any of the diagnostic accuracy parameters of screening tests in the primary studies, but only reported that the proportion of false positive results ranged between 1.5 and 11.1 per 100,000 live births. The report advised that measuring homocysteine in the plasma and urine should be the confirmatory test of choice to reduce false positive results. The report also highlighted that, in order for methionine to be detectable, the infant must have ingested milk. Therefore, the report advised that blood sample collection must be more than 24 hours after birth. Cote et al. also emphasized that, in general, HCY has no symptoms at birth; it may take up to two to three years to be clinically diagnosed, and therefore screening is of particular importance in HCY.

The authors also discussed two clinical variants of the condition based on the response to vitamin B6 (pyridoxine): 1) the vitamin B6-responsive (50%); and 2) the vitamin B6-nonresponsive (50%). Vitamin B6 together with betaine, vitamin B12, and folic acid compose the treatment that is recommended for the first variant. A low-methionine diet enriched with cysteine, together with vitamin B6, vitamin B12, and folic acid is the recommended treatment for the second variant. Cote et al. states that the treatments reduce the risk of thrombosis, developmental delay, and other complications, especially if started early. However, the report does not mention the sources of these conclusions about treatment.

Cote et al. used the same weighing criteria used for GALT to advise on HCY. These criteria included: the incidence and prevalence of the disease, the severity of the disease, the effectiveness of the treatment, the accuracy of the test, and the impact on the health system. Based on these weighing criteria, Cote et al. advised to include HCY in Quebec's screening panel, and recommended this condition be added within the first phase of implementation.

In 2010, Burton et al. reviewed evidence for five inherited errors of metabolism including HCY, specifically the vitamin B6-nonresponsive type.[13] Their evaluation of the diagnostic accuracy of the screening test included four studies, among which the MS/MS platform using methionine was the screening method used. The diagnostic accuracy parameters of screening tests reported in the four studies were very high. Sensitivity, specificity, and the negative predictive value were almost 100% in at least three of the four studies. Positive predictive value, on the other hand, ranged between 0.5 and 100%, depending on the cut-off used.

There are some concerns about the reliability of the diagnostic accuracy parameters reported in the four studies included by Burton et al. Those concerns are: 1) most of the included studies primarily reported on the diagnostic accuracy parameters of a whole screening program that includes HCY, not on individual conditions; 2) all of the studies that reported a 100% sensitivity or positive predictive value were based on only one true positive case detected; and 3) the report explicitly evaluated the clinically severe variant (the vitamin B6-nonresponsive type), which can be easily detected by screening due to high methionine levels. Because methionine elevation is not a specific measure for HCY (as methionine is elevated in many other conditions, such as MAT I/III deficiency, maternal B12 deficiency, and some other liver diseases), the authors emphasized the importance of confirmatory testing to enhance the diagnostic accuracy of the screening test. The confirmatory tests reported were total homocysteine in plasma and urine, mutational analysis, or cystathionine
β-synthase enzyme (the deficiency of which causes the condition) activity in the fibroblasts.

On top of the diagnostic accuracy of the screening test, Burton et al. also looked at other criteria for evaluating the addition of HCY to the panel, namely the availability of the treatment and the effect of early intervention. As there were no studies evaluating the outcomes in terms of these criteria, Burton et al. appear to have based their judgment on expert opinion and on what is known about the disease from the biologic and clinical background data.

Burton et al. concluded that HCY, together with the other four conditions evaluated, fulfilled their pre-set criteria, provided that these criteria be judged differently in the context of rare genetic disorders. The authors recommended that HCY and the other four conditions be added to a pilot screening program to address the gaps in knowledge and collect further data on the requirements and process of obtaining and judging evidence in these rare diseases.

In 2007, Vallance et al. reviewed and updated evidence from other United States and United Kingdom policy reports, with the consideration of the benefit versus the mitigated harm and cost of adding HCY to the panel, in the context of British Columbia.[11] Vallance et al. stated that evidence for HCY is limited, due to issues with unclear health benefit or test performance. Based on two primary studies that were included in this report, HCY seemed to have overall good health benefit if detected earlier, in terms of prevention of mental retardation and significant reduction of vascular events. However, the screening test performance in this report appeared to be inconclusive. The report stated that methionine is the traditional primary marker for HCY, and that adjusting the methionine cut-offs to improve the test sensitivity leads to a high false positive rate and a low positive predictive value. The report also included a study that was conducted in Taiwan over a 20-year period and included 1.7 million newborns. The cut-off reported in this study was 134 μmol/L, a cut-off at which 17 cases of hypermethioninemia were detected. Among these 17 cases, only one case of HCY was confirmed, while the remaining 16 cases of isolated hypermethioninemia were, in fact, another deficiency in methionine, MAT I/III deficiency. Therefore, the report emphasized the importance of confirmatory testing, using either homocysteine in plasma or urine.

In 2003, the report by the Medical Advisory Secretariat of Ontario's Ministry of Health and Long-Term Care was synthesized primarily based on Seymour et al. and Pandor et al. (both from the United Kingdom), as well as on the weighing criteria developed by experts and the authors of the report.[9-12] Those criteria were the incidence, treatment availability, and incremental cost of HCY. The report highlighted the presence of the two clinical variants of the disease (vitamin B6-responsive and -nonresponsive), as well as the availability of the treatment (methionine-restricted

diet, vitamin B6, vitamin B12, folate, and betaine) and its effectiveness in reducing the disease morbidity.

Primary studies

Test details (platform, targets, and cut-offs)

There were six studies found in the literature on the screening for HCY.[17, 27, 28, 38, 40, 41] Among them, three reported on the diagnostic aspect of HCY, among a group of other conditions.[17, 27, 28] These three studies reported using a MS/MS platform; the details of the MS/MS platforms used are listed in Appendix T.C (Table T.C.4).

Methionine was the target used to detect HCY in the three studies. The cut-offs reported in the studies were >55, >60, and >120 μmol/L, respectively. Two studies reported starting with cut-offs that are four times the standard deviation of the mean, then slightly modified them over time, and at the 99th percentile of the initial reference reported during the pilot phase.[17, 28] The latter study reported a borderline range of 60-120 μmol/L that required repeating the test. The confirmatory tests reported in the studies were either: repeating the same test, quantifying homocysteine in plasma and/or urine, DNA mutation testing, or plasma amino acids. There was one study that reported a group of amino acids ratios as a confirmatory test;[17] the ratios reported in this study were methionine/phenylalanine>1, methionine/citrulline>4, and methionine/tyrosine>0.7.

Accuracy of screening test

In general, the accuracy parameters of the screening test for HCY reported in the literature appeared to be inconclusive. Among the three studies reporting on the diagnostic aspect of HCY, one study reported the diagnostic accuracy performance of a whole program that includes HCY and other conditions.[17] This study reported a sensitivity of 95.6%, a specificity of 99.8%, and a positive predictive value of 20% for detecting HCY plus other metabolic conditions, collectively. No information was available about the accuracy of screening for HCY individually; therefore, these results cannot be accounted for, as they might be greatly confounded by condition. Another study, on the other hand, reported a sensitivity of 100% and a positive predictive value of 100% for HCY only. However, this result is based on detection of only one true positive case of the condition.[27] The third study did not report on diagnostic accuracy parameters, as no true positive cases were detected; the study only reported that there were 11 cases of suspected HCY, but all of them were false positives.[28]

Early detection and management outcomes

There were three studies on the effect of early detection of HCY on outcomes.[38, 40, 41] Two among these studies evaluated the effect of early detection and treatment via a newborn screening program compared to late diagnosis (greater than 6 weeks of age). In the first study, visual acuity and refractive errors appeared to be significantly worse in the late diagnosed and treated group than in those who were diagnosed and treated early, despite poor treatment compliance and control of homocysteine blood levels ($p=0.03$). They were also significantly worse in those diagnosed and treated late compared to the group of well controlled patients ($p=0.0002$).[40] The second study, on the other hand, reported on early diagnosis and treatment outcomes in terms of the full-scale intellectual quotient (IQ). Those who were detected early had comparable IQ to their unaffected siblings, indicating that early detection and treatment appears to have a positive impact on the mental development of HCY patients.[41] The third study looked collectively at 28 inborn errors of metabolism, and therefore the results might have been confounded by condition. The study showed

that the early detection of the inborn errors of metabolism by screening had an overall significant positive impact in reducing mental retardation and parental stress, compared to clinical diagnosis.[38]

Other outcomes

There were no other relevant outcomes reported in the included primary studies on HCY.

Conclusions and highlights on HCY

Similar to TYRI and GALT, despite the questionable quality and scarcity of evidence, some conclusions can be drawn from the literature on screening for HCY:

- Screening for HCY is safe, no complications arising from the heel prick procedure were reported in the literature.
- Diagnostic accuracy of the screening test for HCY using methionine appeared to be inconclusive due to the rarity of this condition, lack of specific screening test, high false positive rate, and lack of studies that reported on the accuracy estimates for HCY only. Methionine is a non-specific metabolite that might be elevated in HCY and another rare inborn error of metabolism (MAT I/III deficiency). Therefore, methionine is not considered the best marker for HCY. The most specific marker for the condition is the plasma total homocysteine.
- There are two clinical variants of the disease: vitamin B6-responsive and -nonresponsive. The proportion of each type appears to be 50/50.
- Management of HCY is composed of a methionine-restricted diet, vitamin B6 (pyridoxine), vitamin B12, and betaine.
- Early detection and treatment may have a good impact on the disease course in terms of reducing the disease complications, mainly thromboembolic manifestations, ocular manifestations, and developmental delay. However, the magnitude and quality of evidence is very low.

General Highlights and Conclusions on the Screening of Metabolic Conditions

- Acceptability of screening seemed to be good and increasing dramatically over time to almost 100% (reported in two studies).[17, 33]
- The time of sample collection (more than 24 hours after birth, after ingestion of the first milk meal) appears to be of great importance to allow for the accumulation of the metabolites that are measured, especially for GALT (only if total galactose is the test target) and HCY.
- Adjustment of the cut-offs has great impact on the diagnostic accuracy of the screening test, and, subsequently, the recall rate and the false positive rate. However, there are many ways such adjustments can be made, and doing so requires an expert's discretion, especially at the beginning of a program.
- Quality standards for the shipping and handling of samples between labs and hospitals should be in place to avoid variation in the test results, which might arise from the denaturation of the enzyme protein (for GALT) during high temperature seasons.
- Second sample collection is an important consideration for pre-term infants, and is advised to be at four weeks of age (reported in three studies).[17, 28, 40]

3.3 Newborn Screening for SCD (Hb SS, Hb S/β thal, Hb SC)

Description of Technology

Screening Tests

High performance liquid chromatography (HPLC)

The HPLC technology utilizes an ion exchange resin, held in a column cartridge, in conjunction with a buffer gradient. As the ionic strength and/or pH of the buffer changes, certain hemoglobins (Hbs) are eluted from the column, and the presence of Hb is detected using a spectrophotometric technique.[42] The time from injection to the point at which the Hb fraction elutes, known as the retention time of the Hb, is a reproducible measurement for a particular column, buffer exchange resin, and temperature.[42]

Fetal Hb (Hb F) is eluted separately from normal Hb (Hb A). Hbs S, C, D, E also have separate retention times and characteristic chromatographic profiles.[42] In addition, the relative proportions of the different Hbs make it possible to detect the difference between carriers and affected infants, and also to differentiate some types of compound heterozygosity of Hb S with β^+-thalassemia.[42] The key feature of the HPLC test system is its quantification of the concentration of the Hb variants, which is important for distinguishing between homozygous states and Hb variant interactions with β-thalassemia.[43] For each newborn tested, a Hb pattern presents the observed Hbs in order of relative concentration, from the highest to lowest.[43]

The HPLC method requires only a small fraction of a blood spot sample, and is preferred for population screening because it is a fully automated and high-throughput system, accommodating 1,500 samples per day with a two-minute per sample run time.[44]

Isoelectric focusing (IEF)

IEF gives separation of Hb F from Hb A and variant Hbs S, C, D-Punjab, E, and O-Arab. The separation of different Hbs is accomplished through application of a Hb sample onto a precast agarose gel containing ampholytes at pH 6-8.[42] Ampholytes are low molecular weight amphoteric molecules with varying isoelectric points (pIs), which migrate through the gel to their isoelectric points forming a stable pH gradient when an electric current is applied.[42] The Hb variants also migrate through the gel until they reach the point at which their pI equals the corresponding pH of the gel. At this point, the net charges on the variants are zero, and migration stops. The electric field counteracts diffusion and the Hb variants form discrete thin bands.[42]

IEF can be semi-automated and has high-throughput, and therefore is suitable for screening large numbers of samples.[42] Currently, in the United States, the majority of newborn screening programs use IEF because of its relatively low cost, but there is an increasing trend in the use of HPLC in newborn screening programs due to its quantitative and automated features.[45]

Interpretations of Hb patterns reported from IEF or HPLC test systems are described in Table T.3. The same Hb pattern may represent different diseases; for instance, the FS (F for fetal hemoglobin, and S for sickle hemoglobin) pattern can be sickle cell anemia (Hb SS), but also Hb S/β^0.

Table T.3: Newborn screening results for SCD

Newborn screening result	Disease
FS	Hb SS
FS	Hb S/ß^0-thalassemia
FS	Hb S/δß-thalassemia
FS	Hb S/HPFH
FSA or FS	Hb S/ß$^+$-thalassemia
FAC	Hb SC
FSD	Hb SD-Punjab
FSE	Hb SE
FSO-Arab	Hb SO-Arab

Tandem mass spectrometry (MS/MS)

MS/MS can be used as an alternative to HPLC by measuring informative peptides corresponding to the clinically relevant mutations.[46]

MS/MS is now performed routinely in Belgium. In the United Kingdom, MS/MS is used to identify Hb disease state only and not the carrier status, in order to reduce the number of confirmatory tests.[47]

Treatment Options

Preventive interventions and treatment/management for SCD include early initiation of penicillin prophylaxis, parental education, vaccination, hydroxyurea in children and adults, blood transfusion, HSCT, iron overload treatment, prevention of stroke, pain management, and other supportive treatments. These treatment options are described in S section in details. For the purpose of this project, assessment of the effectiveness of treatment and management for SCD focused on penicillin prophylaxis and parental education, because early detection of the disease by newborn screening will lead to early initiation of these preventive interventions. Assessment of the effectiveness of other treatments that are provided later in the course of the disease, although clinically relevant and important, is beyond the scope of this project.

Description of Included Studies

Details of the literature search and study selection process are outlined in section 3.1. Based on the pre-defined inclusion and exclusion criteria, four systematic reviews/HTAs[48-51] and eight primary studies[46, 47, 52-57] were included for further analysis. Excluded studies and the reasons for exclusion are listed in Appendix T.B.

Systematic Reviews/HTAs

Of the four included systematic reviews/HTAs (see Appendix T.D.1), two are Cochrane reviews.[49, 51] One of the Cochrane reviews[51] focused on penicillin prophylaxis for children with SCD; this review was conducted in 2002, and the search was updated in 2012. The other Cochrane review[49] found no study on newborn screening for SCD. Of the two HTAs, one[48] was conducted in 2000 and included studies published between 1985 and 1996. The more recent HTA by Blancquaert,[50] published in 2010, included 191 studies (of any type) for evidence on the potential benefits of newborn screening

for SCD. This HTA was conducted in response to a request from Quebec's Ministry of Health and Social Services to evaluate the appropriateness of newborn screening for SCD in Quebec. A comprehensive literature search was conducted to identify studies published between 1996 and April 2009; reference lists of relevant studies were reviewed for some early key studies, and therefore a number of studies published before 1996 were also included. Our technology assessment for newborn screening for SCD was built on this report, and thus only primary studies published after 2009 were included for further analysis.

Primary Studies

Of the eight primary studies included, seven studies reported on the results of newborn screening programs (in the United States, Brazil, France, Belgium, Germany, India, and Angola). No Canadian study on the effectiveness of newborn screening program for SCD was found. No randomized control trial on newborn screening for SCD was found.

Methodological Quality

Systematic reviews/HTAs

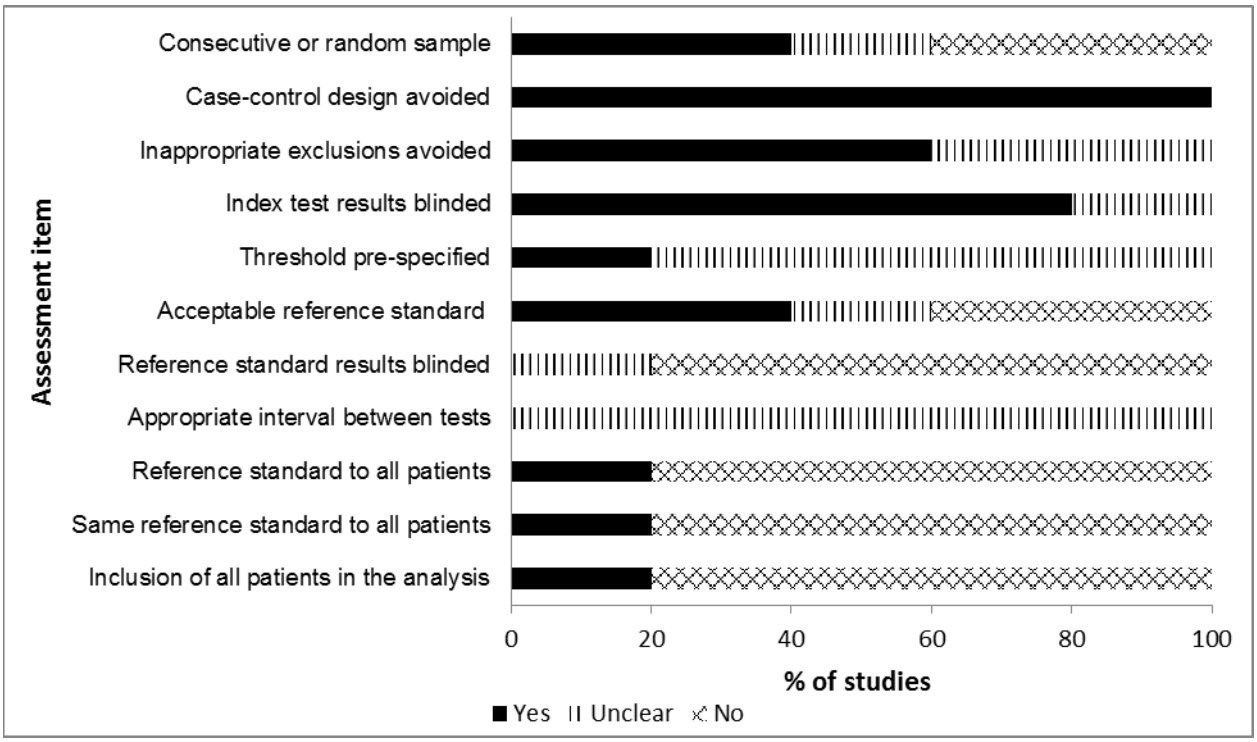
Using the AMSTAR tool independently by two of three researchers (BG, MS, and PC), one Cochrane review[51] received a medium quality rating, and the other three systematic reviews/HTAs received a low quality rating (see Appendix T.D, Table T.D.2).

The HTA by Blancquaert[50] conducted a comprehensive literature search. However, no clear set of inclusion and exclusion criteria were presented in the report. Data from included studies were extracted by a single researcher. While a formal quality assessment of the included studies was not performed, a critical analysis of the studies reviewed for each step of the causal pathway was undertaken, which provided an opportunity to discuss the quality as well as the strength and accuracy of the results.

Primary studies

The methodological quality of the studies that reported on screening test performance outcomes was assessed independently by two researchers (BG and PC) using the QUADAS-2 tool (see Figure T.3 and Appendix T.D, Table T.D.3). Overall, all the studies except one (with an unclear rating) were rated as having a high risk of bias (see Appendix T.D, Table T.D.3).

Figure T.3: Methodological quality of included studies for SCD using QUADAS-2



The methodological quality of studies that reported on treatment effectiveness outcomes was assessed independently by two researchers (BG and PC) using the EPHPP tool (see Appendix T.D, Table T.D.4). Overall, all studies were rated as weak.

Results

Harms Related to Newborn Screening

Evidence from systematic reviews/HTAs

The HTA by Blancquaert[50] did not find any evidence of physical risks related to screening or early treatment, except for possible anaphylactic reactions to penicillin, which were rare. Error rates for the standard screening techniques (IEF or HPLC) seemed minimal with respect to SCD diagnoses. Using a different second-line test on the initial blood sample reduced the number of residual false positives before test results were reported to the family. In addition, confirmatory tests were automatically requested during the first hematology consultation. Therefore, the main concerns of false positive screening results were related to parents' anxiety while awaiting confirmation of diagnosis. False negative results could delay initiation of penicillin prophylaxis and parental education, but were rare.

Evidence from primary studies

None of the included primary studies reported harms related to newborn screening for SCD in terms of harms resulting from over-diagnosis, false positive screening results, or psychosocial consequences.

Accuracy of Screening Tests

Evidence from systematic reviews/HTAs

The HTA by Blancquaert[50] found that IEF and HPLC are the two currently used tests for newborn screening for SCD. This report also included information for MS/MS whenever available.

Of all the identified studies involving newborn screening, the author found one good quality comparative study, by Campbell et al., published in 1999.[58] This study systematically analyzed more than 25,000 dried blood spots using both HPLC and IEF. The interpreter of the HPLC results was blind to the IEF results. HPLC failed to detect five rare variants, and IEF failed to detect two variants, yielding an estimate of sensitivity and specificity for HPLC at 99.45% and 99.99%, respectively.

Results from two early publications on the first four years of California's newborn screening program using HPLC were reported.[43, 59] Of more than two million newborns screened, the only two false negatives reported among affected children involved those who received blood transfusions, but with whom the required controls were not performed. Some false positives were detected with the second-line test (no details were provided), which were related to prematurity or rare variants that could not be distinguished from common variants without further testing. Including HPLC errors in detecting SCD only (two false negatives and two false positive), both sensitivity and positive predictive value were estimated at 99.81%, while both specificity and negative predictive value were estimated at 100%.

Evidence on IEF is limited, although this test has been used more widely than HPLC. Based on Campbell et al.[58] and using the same calculation used for HPLC, IEF sensitivity was 99.78%, the negative predictive value was 99.99%, for perfect specificity and positive predictive values.

Of several studies that used MS/MS, two studies involving newborn screening were designed to develop and validate a preliminary method to specifically detect certain clinically significant variants rather than all variants.[60, 61] In both studies, MS/MS results were completely consistent with IEF or HPLC results, suggesting 100% sensitivity and specificity for detecting target variants.

The author of this HTA concluded that an overall assessment of available clinical performance data indicates that both HPLC and IEF are valid tests for newborn screening of SCD, with sensitivity and specificity exceeding 99%. The choice between IEF and HPLC as first-line tests is generally based on other considerations, such as expertise, laboratory resources, or degree of automation. The limitations of these techniques, such as coelution of some variants with common variants, have implications for screening program organization and the interpretation of results. Information about pre-term infants or an infant's history of blood transfusions should be communicated to the screening laboratory to ensure that results are interpreted appropriately and necessary control samples are requested for comparison. Because some differential diagnoses cannot be made at birth, screening test results from HPLC or IEF should be considered presumptive rather than definitive. Most practice guidelines agree that any abnormal screening results must be confirmed using a different technique.

Evidence from primary studies

Of the five studies that reported screening test performance outcomes, two studies[52, 56] used HPLC for screening, one study used IEF,[55] and two studies[46, 47] used MS/MS (see Appendix T.D, Table T.D.5 and Table T.D.6). One study[52] using HPLC mainly focused on identifying SCD incidence

within Berlin, Germany, and found that all 14 SCD cases identified by HPLC were confirmed by capillary electrophoresis (CE).[xxii] The other study using HPLC[56] reported three false negatives among 1,158 newborns screened. The study using IEF[55] found the concordance between IEF and CE was 99.8% in 36,453 newborns screened.

The United Kingdom study[47] aimed to use MS/MS to detect disease state only (that is, not carrier) to reduce referrals for confirmatory testing. This study showed that MS/MS detected all SCD cases identified by HPLC. According to the authors, this protocol has been endorsed by the UK National Screening Committee for implementation in Wales, and was implemented on 1 June 2013.

The Belgium study[46] reported the results of a 3-year experience using MS/MS for the detection of the major Hb variants in East-Belgian newborns. The authors found that MS/MS appeared to be an efficient alternative approach for laboratories performing neonatal screening of hemoglobin disorders.

Effectiveness of Treatment and Management

Evidence from systematic reviews/HTAs

The HTA by Blancquaert[50] summarized evidence from systematic reviews and primary studies on the efficacy/effectiveness of early preventive interventions, including antibiotic prophylaxis and pneumococcal vaccination. This HTA also reported evidence on other preventive interventions such as the use of hydroxyurea or chronic transfusion to prevent painful episodes, stroke, and other vascular events. However, such information is not presented in our report, as it is beyond our project scope.

The Cochrane review by Hirst et al.[51] included three studies (two of which are described below) and found that all of the included trials showed a reduced incidence of infection in children with Hb SS or Hb S/β^0-thal receiving prophylactic penicillin.

Two randomized controlled trials[62, 63] examined the efficacy of penicillin prophylaxis/pneumococcal vaccine on the incidence of invasive *Streptococcus pneumonia* infections or survival. The study by Gaston et al.,[62] a double blind, placebo-controlled United States multi-centre trial conducted between 1983 and 1985, compared the outcomes of young children (aged 3 months to 3 years) with Hb SS who received prophylactic penicillin (n=105) or placebo (n=110). Sixty-seven percent of the children in the penicillin group and 72% of the children in the place group also received pneumococcal vaccine. This study demonstrated a statistically significant 84% reduction in the incidence of pneumococcal sepsis with prophylactic penicillin, compared to the placebo group (p=0.003). While no death occurred in the prophylactic penicillin group, three children in the placebo group died from pneumococcal sepsis (all three children had received a pneumococcal vaccine). The authors of this study did not provide any information about how many children were diagnosed during the neonatal period.

[xxii] CE utilizes a combination of ion migration and electro-osmotic flow to separate protein molecules. With this method, it is possible to detect the difference between carriers and affected infants, and also to differentiate some types of compound heterozygosity of Hb S with β+-thalassemia.[42] No details about CE are provided in this report as this method is not used in Canada, and is not relevant to the Alberta context.

The randomized controlled trial conducted in Jamaica by John et al.[63] between 1978 and 1983 compared the efficacy of pneumococcal vaccine and prophylactic penicillin in 242 young children with Hb SS (aged 6 months to 3 years). Seventy-six percent of participants were identified through newborn screening. This study was not double-blind and had a high rate of early dropouts and of patients lost to follow-up. In addition, 16 children switched from the penicillin group to the vaccine group after randomization. No pneumococcal infections occurred in the penicillin group while receiving penicillin, although four infections occurred within one year after discontinuing penicillin.

According to the author of the HTA, clinical practice guidelines generally recommended that prophylactic penicillin be initiated at 2 months of age and continued until 5 years of age. All children with SCD should be provided with penicillin prophylaxis, although evidence on the efficacy of penicillin prophylaxis has not been established in children with Hb SC disease or Hb S/β^+-thal.

The HTA by Blancquaert[50] found no controlled trials on the efficacy/effectiveness of parental education for early detection and quick treatment of splenic sequestration.

Evidence from primary studies

No new primary studies published after 2009 were found that focused on the effectiveness of treatment and management for SCD.

Effectiveness of Newborn Screening Programs for SCD

A comprehensive newborn screening program for SCD includes the introduction of various screening tests, parental education, initiation of prophylactic penicillin, and the administration of additional vaccinations, and follow-up.[64]

Evidence from systematic reviews/HTAs

The HTA by Blancquaert[50] found no randomized control trials that compared short- or long-term clinical outcomes of SCD with or without a newborn screening program, suggesting no direct evidence of the highest quality was available to support the effectiveness of newborn screening in improving outcomes of children with SCD.

The HTA found two cohort studies that reported on the effectiveness of newborn screening programs. A study by Vichinsky et al. in California,[65] published in 1988, demonstrated that, during a 10-year follow-up of a regional newborn screening program with a strong focus on parental education but without the use of penicillin prophylaxis, the overall mortality rate for children with Hb SS diagnosed in the neonatal period was 1.8%, compared with 8% for children with Hb SS diagnosed at a mean age of 21 months.

In a French study published in 2002, complications of Hb SS were compared between a group of 38 children diagnosed at birth by newborn screening and a control group of 69 children diagnosed after the newborn period (at a mean age of 24 months). Penicillin prophylaxis and parental education were initiated once the diagnosis was confirmed. Only children who survived at least two years were included in the study, and therefore the effectiveness of newborn screening on early mortality could not be assessed. The study showed significantly fewer painful events and splenic sequestration episodes in the newborn screening group compared to the control group ($p=0.04$). There was no significant difference between the groups in acute chest pain, acute exacerbation of anemia, meningitis, septicemia, or length of hospital stay.

The HTA also identified several trend and longitudinal studies. The study by Yanni et al.,[66] published in 2009, compared the mortality rates between 1983 to 2002 in black children with SCD in the United States, and found that mortality rate decreased significantly by 68% (95% CI: 58 to 75%) from 1983-1986 to 1999-2002 for children aged 3 years or younger. Mortality caused by infection decreased from 57 to 23% in children younger than 4 years of age. As noted by the authors, it is not possible to determine whether the expansion of newborn screening for SCD after 1987 contributed to the reduction of mortality rates.

Frempong and Pearson[67] assessed the impact of newborn screening on SCD-related mortality between 1990 and 2002 in Connecticut. Based on the death certificates, there were 13 deaths between 1970 and 1988 when no newborn screening was available, five deaths (who were not screened at birth) between 1988 and 1990 when limited newborn screening was available, and no deaths between 1990 and 2002 when universal newborn screening coupled with provision of penicillin prophylaxis and vaccination was available. These results may suggest that the newborn screening program with comprehensive follow-up care greatly reduced mortality in children with SCD.

Two studies reported experiences with Jamaica's newborn screening programs, which were provided in the same cultural and geographical context a few years apart.[68, 69] Penicillin prophylaxis, parental education in early diagnosis of acute splenic sequestration, and close monitoring in sickle cell clinics were part of the screening program. In the study by Lee et al.,[68] published in 1995, 100,000 newborns were screened between 1973 and 1981, and 307 of 315 children diagnosed with Hb SS were followed. In the study by King et al.,[69] 150,803 newborns were screened between 1995 and 2006, and 395 children with Hb SS were followed. Among children with Hb SS born between 1973 and 1975, 14.2% died before the age of 2 years and 23.8% before the age of 10 years, compared to 0.8% and 8.6%, respectively, for children with Hb SS born between 1995 and 2006. King et al. also compared mortality caused by acute splenic sequestration before and after a parental education program was introduced in 1978. Mortality caused by acute splenic sequestration decreased from 28% for the period 1974-1978 to 3% for 1979-1983, and then to 0.53% for the period 1995-2006.

Several longitudinal studies (Gill et al. (1995),[70] Quinn et al. (2004),[71] Telfer et al. (2007),[72] and Lerner et al. (2009)[73]) reported on birth cohorts over 20 to 27 years. The estimated survival based on mortality related to SCD ranged from 95.9 and 99.0% at 10 years of age, and between 93.6 and 99.0% at 18 years of age, but the average or median follow-up is generally shorter. Although the authors of these longitudinal studies observed reduced childhood mortality from SCD, an increase in the mean age at death, and a reduced proportion of deaths from infection, as noted by Blancquaert, the temporal association revealed in longitudinal studies cannot be viewed as evidence of causation. It is impossible to determine the extent to which the reduction in specific mortality rates is attributable to the gradual introduction of newborn screening programs. Interpretation of these results should take into account the ecological nature of the data (aggregate data) and the quality of information gathered from death certificates.

Evidence from primary studies

The five studies, conducted in Brazil,[53] France,[54] the United States,[57] India,[56] and Angola[55] reported short- or long-term outcomes of newborn screening programs for SCD (Appendix T.D, Table T.D.6)

The study by Lobo et al.,[53] published in 2014, reported the results of the first 10 years of the newborn screening program for SCD in Brazil (HEMORIO). Over 10 years, 1,217,833 infants were screened for SCD using HPLC. This study reported a mortality rate of 3.7% during 10 years, and a survival rate of 94% (95% CI: 0.918 to 0.914) for SCD. The survival rate was 92.4% (95% CI: 0.894 to 0.995) for Hb SS only. Data collected during the 10 years of the program showed a reduction in the mortality of patients with SCD, in comparison to available historical statistical data before the implementation of the national screening program.

The study by Quinn et al.[57] followed 940 children with SCD identified by the newborn screening program from 1983 to 2007. The study found that, with early detection of SCD and the resulting improved medical care (early initiation of penicillin prophylaxis, initial visit to the sickle cell centre, and pneumococcal vaccination at ages 2 and 5 years), the majority of children with Hb SS (93.9%) and nearly all children with mild forms of SCD (98.4%) lived into adulthood. Although the combination of penicillin and vaccination did not prevent all fatal pneumococcal infections, bacterial sepsis was no longer the leading cause of death. The investigators noted that, while mortality in young children with SCD has been decreasing, young adults seem to be at high risk for death shortly after the transition to adult medical care.

Two other studies[55, 56] also reported reduced early mortality following the initiation of newborn screening programs for SCD. The study conducted in France[54] reported reduced delays in the management of children diagnosed with SCD.

Specific Issues with Newborn Screening for SCD

Carrier status

There is a difference in how jurisdictions deal with infants that are carriers of SCD. In the United States and Brazil,[53] infants with carrier status are not sent for confirmatory tests, but education packages are sent to parents and parents are offered the test free of charge. In the United Kingdom, infants with carrier status are sent for confirmatory testing.[47] From 2011 to 2012, approximately 10,000 sickle carrier infants were identified and referred for follow-up in England.[47]

Pre-term infants and low birth weight

A confounding problem in newborn screening for SCD is the low expression of the Hb A β-chain in the first week of life, particularly in pre-term infants.[47] In addition, the history of blood transfusion in newborns also affects the screening results obtained.[47] Therefore, it is essential that the screening facility know that the infants are pre-term or have had a transfusion.[47] The majority of the included studies did not provide information in this regard.

Clinical Practice Guidelines

According to the United Kingdom clinical practice guidelines published in 2010,[74] the National Screening Committee and National Health Service (NHS) Policy is that all newborns should be screened for SCD. Such screening should be extended to infants under 1 year of age newly arrived in the United Kingdom. Newborn screening and, when necessary, follow-up testing and referral, should be carried out according to the guidelines of the NHS Sickle Cell and Thalassemia Screening Program. The main objective of the newborn screening program is to improve outcomes in SCD through early treatment and care.

The guidelines state that sensitivity and specificity are approximately 99% for the methods used (HPLC or IEF, cellulose acetate electrophoresis). Unequivocal identification of Hb variants can only be achieved by either protein analysis (for example, mass spectrometry) or DNA analysis.[74]

In the case of suspected SCD, confirmatory testing and clinical follow-up should be performed in a timely manner so that penicillin prophylaxis can be started by 3 months of age; conjugate pneumococcal vaccine is provided to all infants from 8 weeks of age, but is particularly important if the child has SCD.

Discussion

In 1975, New York was the first state in the Unites States to begin newborn screening for SCD; by 2006, all 50 states have implemented universal newborn screening programs for SCD.[45] As such, a substantial amount of evidence in this review came from the reporting of United States newborn screening programs with short- or long-term follow-up. No Canadian studies were found on newborn screening for SCD, although newborn screening has been provided universally in Ontario since 2006,[64] and other jurisdictions in Canada also have newborn screening programs for SCD (as described in the S section).

The purpose of newborn screening for SCD is to reduce morbidity and mortality by ensuring early detection of SCD and early initiation of penicillin prophylaxis to prevent pneumococcal infections (that is, from 12 weeks of age), aggressive vigilance for routine infant immunizations and pneumococcal vaccination at the age of 2 years, and preventive parental education prior to the appearance of the first symptoms of the disease.[54, 75, 76] The assessment of the technology effectiveness of newborn screening for SCD in this T section was guided by the Australian Framework, with a focus on the screening test performance, treatment effectiveness, and effectiveness of newborn screening programs.

Methodological Issues

Some newborn screening programs used historical comparison when assessing effectiveness of the treatment or program. Use of historical controls presents methodological problems, because treatment and management may have altered and presumably improved over time, and, more importantly, the natural history of the disease has not been understood accurately.[78]

Main Findings

The main findings of this review are summarized below:

- Physical harms associated with the procedure of newborn screening for SCD (that is, the heel prick) and treatment with prophylactic penicillin seems to be minimal. Although no study was found that specifically focused on psychosocial harms of newborn screening for SCD, it has been well recognized in general that false positive results of newborn screening tests may cause parents anxiety and stress while waiting for the results of confirmatory testing, or prompt further investigations and unnecessary treatments with no proven benefits, thus causing family distress and increase use of healthcare services.
- Based on the published results, both HPLC and IEF are valid tests for newborn screening of SCD, with estimated sensitivity and specificity exceeding 99%. Reported positive predictive value and negative predictive value are also high. MS/MS may have potential for newborn screening for SCD, but it can only detect certain variants of Hb, and has not been fully validated.

- Two randomized control trials published in the 1980s demonstrated that prophylactic penicillin significantly reduced the incidence of life-threatening infections and early mortality. Limited evidence from observational studies also suggests that early initiation of parental education was associated with reduced mortality caused by acute splenic sequestration.
- Based on the included systematic reviews/HTAs and additional primary studies published after 2009 that reported short- or long-term outcomes of a number of newborn screening programs conducted worldwide, newborn screening programs for SCD that encompass early identification of SCD, early initiation of penicillin prophylaxis and parental education, and follow-up comprehensive care are associated with reduced early mortality and improved survival in children diagnosed with SCD. These outcomes are primarily based on retrospective observational studies, and no randomized control trial has been conducted to demonstrate the overall benefit of newborn screening programs.

Conclusion

Research evidence accrued over the last 40 years has demonstrated that screening tests for SCD with IEF or HPLC are highly sensitive and specific, and harms related to the screening procedure and treatment with prophylactic penicillin appeared to be minimal. The efficacy of penicillin prophylaxis in preventing pneumococcal infections and resulting sepsis in young children was demonstrated in a randomized control trial. However, no randomized control trial was found to examine the effectiveness of newborn screening programs in reducing mortality and morbidity for children with SCD. The overall benefits of newborn screening programs comprising early detection of SCD, early initiation of penicillin prophylaxis and parental education, and comprehensive follow-up have been demonstrated by short- or long-term follow-up studies of a number of national or regional programs, in terms of reduced early mortality and improved survival in children diagnosed with SCD at birth.

3.4 Newborn Screening for SCID

Description of Technology

Screening Tests

TREC by real-time PCR

T-cell receptor excision circles (TRECs), a biomarker of newly generated circulating T-cells, can be measured by real-time/quantitative polymerase chain reaction (qPCR) using DNA extracted from infant dried blood spots collected for newborn screening.[80] TRECs are small, circular pieces of DNA that are formed during the differentiation of T-cells in the thymus as a result of the rearrangement of T-cell receptor genes, and do not replicate with cellular division. One particular TREC, the δRec-φJα TREC, is produced by approximately 70% of all T-cells that express the α/β T-cell receptor.[81] qPCR amplification across the joined ends of the δRec-φJα TREC reflects the number of recently formed T-cells in peripheral blood.[82] A normal number of TRECs is a good biomarker for new autologous T-cell production, provided the DNA is adequate for PCR.[83] Low or absent TRECs in a dried blood spot may be indicative of an underlying T-cell deficiency.[84]

The use of TRECs as a newborn screening analyte has several advantages, including: the ability to use dried blood spots, low cost, high throughput, and high sensitivity (that is, the avoidance of false

negative results from infants with SCID who have high numbers of B-cells, maternal T-cell engraftment, or oligoclonally expanded T-cells).[85]

Conditions that can be detected by TREC assay include SCID regardless of genetic cause, a variety of forms of T-cell lymphopenia, and other serious immune defects in infants.[81]

IL-7 by ELISA

Other assays for SCID newborn screening include immunoassays (for example, ELISA) for interleukin 7 (IL-7) and T-cell specific proteins. These protein-based assays might become first-line or second-line screening tests; or they could be used in combination with the TREC assay in a two-tier system.[86] The level of IL-7 is elevated in the absence of T-cells.[87]

Lymphocyte count

Healthy newborns have an average of 3,100/μL (range 2,500 to 5,500/μL) T-cells.[80] A total lymphocyte count has been suggested as a screening test for SCID that could be performed on cord blood locally in nurseries. Around 10% of SCID samples have lymphocyte counts near normal values because of the presence of high numbers of T-cells or maternal lymphocytes.[82] Universal screening by lymphocyte counts would also require costly liquid blood collection.[88] Overlap between healthy infants with the lowest lymphocyte counts and SCID infants with the highest lymphocyte counts will produce high rates of false negatives and false positives, making absolute lymphocyte counts alone problematic as a screening test.[86, 88]

Tandem mass spectrometry (MS/MS)

For some variants such as T+ SCID or delayed onset ADA SCID, TREC assay may not be a good test as TRECs are within normal range at birth and thus are undetectable at diagnosis, causing delay in treatment. However, many newborn screening programs worldwide currently use TREC assay as method to evaluate ADA SCID.[89]

ADA SCID can be detected by either ADA enzyme activity or accumulation of metabolites due to ADA deficiency. MS/MS has been suggested for these cases,[89, 90] specifically to measure adenosine and 2'-deoxyadenosine levels.

Confirmatory Test

Infants with abnormal TREC results can have a flow cytometry test to enumerate lymphocyte subsets: T-cells, B-cells, natural killer cells, and naïve and memory phenotype T-cells.[80] Diagnosis of SCID can be performed using flow cytometry by measuring T-cell enumeration and T-cell proliferation after stimulation with antigens or mitogens.[88, 91] T-cell measurements can then be compared to age-matched controls. A negative response to mitogens is typical of SCID.[88]

Treatment Options

The standard treatment for SCID is HSCT,[92] preferably from an HLA-matched relative, or HLA-matched unrelated donors (in the absence of related donors).[93] For certain genetic subtypes (for example, ADA SCID and XL-SCID), gene therapy may be considered when no fully matched donors available.[94, 95] Enzyme replacement therapy is another potential option for ADA SCID, which is often used as an interim treatment while waiting for definitive therapy by HSCT or gene therapy.[95]

Description of Included Studies

Details of the literature search and study selection process are outlined in section 3.1. According to the pre-defined inclusion and exclusion criteria, one systematic review[87] and 15 primary studies are included for further analysis.[81, 84, 89, 90, 96-98 80, 99-105] The majority of included studies measured TREC by qPCR on a dried blood spot. Our literature search did not identify any full-text studies on the use of the combination of TREC/KREC that met our predefined inclusion criteria. All relevant publications on TREC/KREC identified from our literature search were conference abstracts, which were excluded from further analysis due to the lack of details. All of the excluded studies and the reasons for exclusion are listed in Appendix T.B.

Systematic review

The objective of the systematic review by Lipstein et al.[87] was to examine evidence for newborn screening for SCID in terms of test characteristics, treatment efficacy, and cost-effectiveness (see Appendix T.E.1). English-language articles published between January 1988 and October 2008 were searched. Reference lists of selected articles were reviewed, and a Medline search was conducted for publications by key SCID investigators. The authors of the systematic review also conducted interviews with American experts (researchers, clinicians, and parent advocates) in the field of SCID.

The original full report by Lipstein et al.[106] addressed a broader range of questions including the natural history of the disease, variations in genotype and phenotype, and prevalence of SCID, whereas the journal publication by Lipstein et al.,[87] the main source of evidence for our project, focused on screening, treatment, and cost-effectiveness.

A total of 26 primary studies were included in this systematic review: five cohort studies, three case-control studies, five large case series (over 50 patients), 12 small case series studies, and one cost-effectiveness analysis. No randomized controlled trial was found.

For each included study, two reviewers independently assessed the quality of the study design and the category of evidence (such as treatment or cost-effectiveness) using the tools originally developed by the NHS Centre for Reviews and Dissemination and adapted in two previous HTAs of newborn screening.[12, 107]

Our technology assessment for newborn screening for SCID was built on this review, only primary studies published after 2008 were included in our assessment.

Primary studies

Of the 15 primary studies published after 2008, the last search date of the systematic review by Lipstein et al.,[87] six studies reported screening test performance outcomes,[81, 84, 89, 90, 96, 97] seven on treatment effectiveness,[98-104] and two on the effectiveness of a newborn screening program.[80, 105] Most studies are retrospective observational studies, and no randomized controlled trial was found.

Methodological Quality

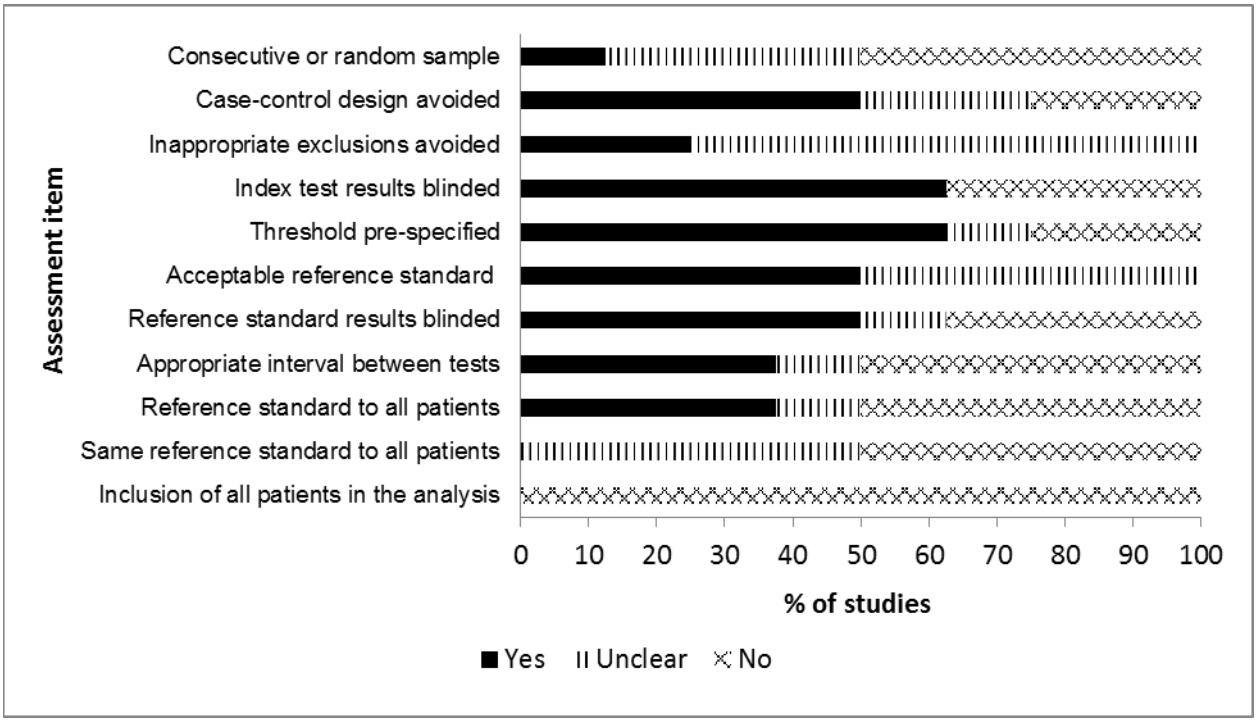
Systematic review

Using the AMSTAR tool independently by two researchers (BG and MS), the systematic review by Lipstein et al.[87] received a medium quality rating (see Appendix T.E, Table T.E.2).

Primary studies

The methodological quality of studies that reported on screening test performance outcomes was assessed using the QUADAS-2 tool independently by two researchers (BG and PC) (see Appendix T.E, Table T.E.3). Overall, all the studies were rated as having high risk of bias (see Figure T.4).

Figure T.4: Methodological quality of included studies for SCID using QUADAS-2



The methodological quality of studies that reported on treatment effectiveness was assessed using the EPHPP tool independently by two researchers (BG and PC) (see Appendix T.E, Table T.E.4). Overall, all studies except one (with a moderate rating) were rated as weak.

Results

Harms Related to Newborn Screening

Evidence from systematic review

The systematic review found no study that addressed the safety issue of newborn screening for SCID.

Evidence from primary studies

None of the included primary studies reported harms related to newborn screening for SCID in terms of harms resulted by over-diagnosis, false positive screening results, or psychosocial consequences.

Accuracy of Screening Tests

Evidence from systematic reviews/HTAs

The systematic review included three case-control studies and one cohort study that examined screening test outcomes (see Appendix T.E, Table T.E.5). As seen in Table T.4, three methods are used for newborn screening for SCID, including: 1) lymphocyte counts on whole blood; 2) qPCR for TRECs on dried blood spots; and 3) ELISA for IL-7 on dried blood spots. None of these three methods distinguish between the various SCID genotypes and phenotypes.

Table T.4: Screening test performance results from systematic review for SCID

Screening tests	Cut-off	Sensitivity (%)	Specificity (%)
Lymphocyte counts on whole blood n=2 studies	<2.8 × 10^9/L	86	94
	<5.0 × 10^9/L	56	100
qPCR for TRECs on dried blood spot n=1 case-control study	< 30 copies/µL	100	97
Two-tier (on dried blood spot): ELISA for IL-7+PCR for TRECs n=1 case-control study	IL-7	85	96
	TRECs	100	92

Based on the data provided in one case-control study using qPCR for TRECs on dried blood spots, the authors of the systematic review calculated sensitivity of 100% and specificity of 97%, at a cut-off of <30 copies/µl.

Another case-control study evaluated a two-tiered approach in which IL-7 was measured first and then TRECs, measured from those samples with elevated IL-7. This study included samples from 13 children with SCID and 183 anonymized dried blood spots that were assumed to be without SCID, but did not have confirmatory tests. For the first-tier (IL-7 measurement), 85% sensitivity (55-98% CI) and 96.1% specificity were reported, and for the second-tier (TREC count), 92.3% specificity and a sensitivity that was "approaching 100%" were reported.

The authors noted some issues that deserve specific attention. First, for a condition with a published incidence of approximately 1 in 100,000 live births, it will take several years for states like Wisconsin or Massachusetts to gather sufficient data to evaluate the test's accuracy, even if the actual incidence of SCID was significantly higher. Second, some children with abnormal or inconclusive results were found to have clinically significant immunodeficiencies other than SCID. Although these children had false positive screening results, these children likely benefited from early identification of another immunodeficiency. These special situations should be considered when determining the utility of newborn screening for SCID.

Evidence from primary studies

Six studies reported screening performance outcomes, including: two United States newborn screening programs using TREC assay (New York,[84] Wisconsin[81]); one United Kingdom newborn screening program using TREC assay;[97] one Canadian study using the TREC assay on T^{++} SCID;[96] and two Italian studies using MS/MS on ADA SCID.[89,90] The study characteristics and findings from these studies are presented in Appendix T.E (Table T.E.6).

The New York study[84] reported a positive predictive value of 2.1% for SCID, and 20.3% for clinically significant conditions. Negative predictive value was reported to be 100%, as no false negative case was found. There was a strong correlation between the TREC value and the absolute CD3 T-cell count (flow cytometry) (R^2=0.423, p≤0.0001).

The Wisconsin study[81] demonstrated that the TREC assay is a highly sensitive test that detects known causes of SCID, as well as persistent severe T-cell lymphopenia and other defects of the hematopoietic system. This assay is also highly specific (with reported specificity of 99.98%), as there were only 38 false positive tests out of over 200,000 infants, giving a false positive rate of 0.018%. One important contributing factor for the low false positive rate is the algorithm whereby premature infants have repeat TREC assays performed until they reach an adjusted gestational age of 37 weeks, and the repeat testing rate was only 0.19%, which is well below the repeat testing rate of other newborn screening assays.

The United Kingdom retrospective study[97] analyzed 5,081 newborn screening cards and 18 confirmed cases of SCID. No confirmatory tests were done for screening test positive infants, and thus no accuracy information was available. Different TREC cut-offs (20, 25, 30, 35, and 40 copies/μL) were examined, resulting in a 1% screening positive rate with a TREC cut-off of <40 copies/μL, and a 0.04% screening positive rate with a TREC cut-off of <20 copies/μL. A TREC cut-off of <20 copies/μL detected all 18 SCID cases; the 0.04% screening positive rate with this cut-off means a referral of 208 samples for confirmatory tests per year in the United Kingdom, which was considered appropriate for the national newborn screening program.

The Canadian study[96] found that the TREC assay did not detect the majority of T^+ SCID cases, a variant that is predominant in SCID patients in Manitoba, particularly in First Nations of Northern Cree ancestry and Mennonites. This study found that the TREC assay was capable of working with dried blood spots stored for more than 20 years. The authors suggested including supplemental targeted or multiplexed screening for ethnic-specific mutations to ensure that the highest proportion of affected infants is identified.

Of the two Italian studies that focused on MS/MS for ADA SCID, one study[89] showed that the use of MS/MS to measure adenosine and 2'-deoxyadenosine on dried blood spots can detect ADA SCID. The other study[90] compared MS/MS and TREC assay, and found that MS/MS but not TREC assay was able to detect delayed onset ADA SCID.

Effectiveness of Treatment and Management

No attempt was made to compare different modes of HSCT (for example, preconditioned or not), due to the scope of this project. Assessment of treatment effectiveness focused on the comparison between early versus late treatment.

Evidence from systematic review

The systematic review found no randomized controlled trial on treatment effectiveness. While this review included more studies that compared different treatment modality (for example, donor-recipient matching or the use of pre-transplant conditioning), for the purposes of this project, only information from studies that compared early versus late treatment was extracted (Appendix T.E, Table T.E.5) and presented here.

Four studies that specifically addressed HSCT in neonates or infants were included for the comparison between early versus late treatment: one cohort (117 patients) and three case series

studies (475, 89, and 13 patients, respectively). These studies showed that infants who received early HSCT (that is, before 28 days of age) consistently showed better survival rates than those who received later treatment, with recipients from matched related donors having the best survival rate. The results in this regard were not presented in this review in detail, because comparison of different treatment strategies warrant another systematic review with this focus.

Evidence from primary studies

Seven primary studies examined the effectiveness of treatments for SCID (see Appendix T.E, Table T.E.7).[98-104] The majority of these studies were case series studies, and no randomized controlled trial was found. All studies focused primarily on HSCT, and two studies also included enzyme replacement or gene therapy. The number of patients included in these studies ranged from 90 to 699. In five studies, patients were followed up for more than 10 years, with the longest follow-up being over 30 years.[98, 99, 101-103]

Mortality and survival

Overall, the included studies showed higher survival rates in children who received HSCT early, compared to children who received treatment at a later time.

A European multicenter study[101] with the largest number of HSCT cases to date (n=699) demonstrated a statistically significantly higher 10-year survival rate in patients who received HSCT before 6 months of age (68%), compared to patients who received HSCT between 6 to 11 months of age (59%) or after 12 months of age (51%) (p=0.0008). Factors such as HSCT performed after the year 1995, a younger age, a B+ phenotype, geno-identical and pheno-identical donors, an absence of respiratory impairment, or viral infection before HSCT were associated with better prognosis.

The study by Brown et al.[99] showed significantly improved outcomes in 60 SCID infants diagnosed at birth because of a positive family history, compared with those of 48 family members (most siblings) diagnosed at older ages. The overall improved survival of more than 90% is related to a reduced rate of infection and significantly improved transplantation outcomes, irrespective of donor choice, conditioning regimen used, or underlying genetic diagnosis.

Chan et al.[100] reported an infant mortality rate of 42% for 138 neonates who were not tested at birth, compared to a 15% mortality rate for 20 neonates who were tested at birth.

Buckley et al.[102] analyzed 166 patients who received HSCT and were followed for up to 29 years. The overall survival rate was 76%. The survival rates are similar for the different genetic types of SCID.

Long-term outcomes

Despite remarkable improvements in survival rates, few data are available on the long-term clinical status and quality of life of patients with SCID who received a transplant. There are some concerns about possible impairment of T-cell function later in life, as a consequence of thymopoiesis (the process in the thymus by which thymocytes differentiate into mature T-cells) exhaustion.[103]

Buckley et al.[102] analyzed long-term outcomes of 166 patients who received HSCT. They found that, when compared to children who received HSCT after 3.5 months of age, infants who received HSCT before 3.5 months showed a statistically significantly higher survival rate; furthermore, there

was a higher percentage with no problems, fewer required boost transplantation, and fewer had a height below the 3rd percentile.

Neven et al.[103] analyzed long-term (median follow-up 14 years, ranging from 2 to 34 years) outcomes of 90 SCID patients who survived at least two years after HSCT; therefore, short-term survival rate was not available. Almost half the patients have experienced one or more significant clinical events, including persistent chronic GvHD, autoimmune and inflammatory manifestations, opportunistic and non-opportunistic infections, chronic human papilloma virus (HPV) infections, and a requirement for nutritional support. With the notable exception of severe HPV infection, these complications tended to become less common 15 years after HSCT. In most cases, HSCT enables long-term survival with infrequent sequelae. However, the occurrence of relatively late-onset complications is a concern that requires specific means of prevention, and justifies careful patient follow-up.

Effectiveness of Newborn Screening Programs

Of the four recently published United States studies, one[80] reported experience and outcomes of newborn screening programs from 11 states, while the other three[81, 84, 105] reported outcomes of newborn screening programs from California, New York, and Wisconsin.

In the larger multistate study, the primary targets of SCID screening included typical SCID, leaky SCID, and Omenn syndrome, which require immune system restoration for survival. Additional diagnoses were detected as secondary targets. TREC assay was used in all participating programs, and results of abnormal TREC test were reported within the first 3 weeks of life, followed by flow cytometry by 4 to 5 weeks of age.

TREC cut-offs varied considerably between newborn screening programs in different states, ranging from ≤7 to <252 copies/μL. TREC cut-offs were ≤25 copies/μL for three states including California, where the assay protocol by PerkinElmer Genetics, Inc. was used. Other states used local protocols, with a TREC cut-off of <30 copies/μL for two states including Wisconsin, <125 copies/μL for New York and ≤150 copies/μL for Texas.

While the overall referral rate for flow cytometry was 42 per 100,000 infants screened, the referral rates varied considerably between programs, ranging from 14.9 per 100,000 infants in California to 136 per 100,000 infants in Texas, due to the use of different TREC cut-offs. Furthermore, the definitions for T-cell lymphopenia by flow cytometry also varied, ranging from <1,500 to <2,500/μL or more.

Screening of over 3 million infants in 11 states from 2008 through 2013 identified 52 cases of SCID, yielding a population-based incidence of SCID of 1 in 58,000 births, which is higher than the incidence of 1 in 100,000 estimated from previous clinical studies. No program identified a false negative test for SCID. Different TREC and T-cell lymphopenia cut-offs resulted in variable false positive rates, defined as abnormal TREC results that require a follow-up flow cytometry test that, when performed, shows T-cells above the program cut-off for T-cell lymphopenia.

A total of 411 infants with non-SCID T-cell lymphopenia were identified by newborn screening programs, which is almost eight times more frequent than SCID. The diagnoses included syndromes with T-cell impairment (DiGeorge, trisomy 21, ataxia telangiectasia, trisomy 18, CHARGE, Jacobsen, CLOVES, ECC, Fryns, Nijmegen breakage, Noonan, Rac2 defect, Renpenning, TAR, not specified, or cytogenetic abnormalities) in 136 infants, secondary T-cell impairment (cardiac anomalies, multiple congenital anomalies, loss into third space, gastrointestinal anomalies, neonatal

leukaemia, or not specified) in 117 infants, pre-term birth alone in 29 infants (7% of non-SCID T-cell lymphopenia cases, or 1 in 104,000 births), variant SCID in 12 infants (3% of non-SCID T-cell lymphopenia cases, or 1 in 250,000 births), and unspecified T-cell lymphopenia in 117 infants.

Of the 52 infants diagnosed with SCID, 49 infants received immunity restoring treatments (44 HSCT, 4 gene therapy, and 2 ADA enzyme injection therapy). Of the seven deaths, four infants died after transplantation. Overall survival was 87% in infants with SCID (45 out of 52) and 92% (45 out of 49) among those infants who received immunity restoring treatments.

As mentioned by the authors, the major limitation of this study was the lack of uniformity of TREC assay methodology and rules for retesting among the individual newborn screening programs, despite general adherence to the Clinical and Laboratory Standards Institute Guidelines. There was a lack of information regarding the ages at which blood samples were obtained for TREC assay and for flow cytometry testing.

The authors also pointed out that, although unsuspected non-SCID immunodeficiency syndromes were identified and some of these were serious enough to require HSCT or thymus transplantation, these benefits must be weighed against the burdens of increased parental anxiety and costs of additional testing in infants with less profound T-cell lymphopenia.

In the California newborn screening program,[105] primary targets include typical SCID, leaky SCID, Omenn syndrome, and complete DiGeorge syndrome that require immune-restoring treatment. Clinically significant T-cell lymphopenia with less than 1,500 autologous T-cells/μL is the secondary target. Infants with an initial TREC level of ≤40/μL had a second TREC testing, and those infants with a TREC level of ≤25/μL were referred to flow cytometry.

Fifteen SCID cases were identified from 993,724 infants screened during the first two years of California's newborn screening program, yielding a population-based SCID incidence of 1 in 66,250 births. As to the secondary target, the incidence was 1 in 19,900 (0.005%) for significant T-cell lymphopenia.

The overall flow cytometry referral rate was 0.016%, and reported false positive rate was 0.01%. The survival rate was 93% in the 15 SCID patients who received HSCT (13 patients), gene therapy, or thymus transplantation.

The authors noted that there is a great variety in the clinical, genotypic, and demographic characteristics of infants with SCID and T-cell lymphopenia in California, and thus that continued screening and monitoring will reveal the full phenotypic spectrum. To complement newborn screening for SCID, there is a need to continue to improve rapid, high-throughput genotyping to delineate the underlying genetic defects in patients with SCID and T-cell lymphopenia, so that genotype-specific treatment strategies can be tailored to these patients to improve clinical outcomes.

Specific Issues with Newborn Screening for SCID

Pre-term infants

The New York study[84] found that TREC level averages varied by gestational age. The mean TREC level in the overall pre-term population was 1,521/μL (95% CI: 1,494 to 1,548/μL), and there was a trend towards fewer TRECs in infants born at earlier gestational age. For pre-term infants (<37 weeks gestation), if their TREC level was less than 200/μL, a repeat TREC assay was performed at an age equivalent to at least 37 weeks; if >200/μL TRECs were present, the specimen was considered to be within acceptable limits and no further follow-up was requested. The Wisconsin

newborn screening program[81] also used a similar approach for pre-term infants, which reduced the number for flow cytometry referrals. In contrast with the New York study, the United Kingdom study found no difference in TREC levels by gestational age, with mean TREC levels of 110, 115, and 120/μL in the <32 weeks, 32 to 36 weeks, and >36 weeks gestational age categories, respectively.

Gender and race/ethnicity

In the New York study,[84] among infants referred for confirmatory tests, the male to female ratio was 1.72 ($p<0.001$; chi-square test). Males had fewer TRECs than females. Race/ethnicity also differed between screen negative and screen positive infants ($p<0.001$; chi-square test) and the difference appeared to be primarily driven by an increased frequency of infants of African descent in the screen positive referral population, who had lower TREC levels than other race/ethnicities. It is possible that lower TREC levels are a consequence of white blood cell counts, which are known to be 10 to 20% lower in African Americans, compared to European Americans.

Screening protocol

Various TREC protocols have been developed and validated.[111, 112] However, there is no consensus on the method used for SCID screening, and laboratories currently use different, non-standardized, assays for detection of TRECs in newborn dried blood spots.[97] Depending on the assay parameters (such as DNA isolation method, plasmid, qPCR primers and probes, or singleplex or multiplex mode), the absolute number of TRECs varies greatly, with the difference between laboratories being several-fold.[96, 105, 113] Therefore, positive screen results rather than actual numbers are used to compare data between laboratories.[105] Of the 11 states in the United States with newborn screening programs, while three programs used protocols developed by PerkinElmer, all other programs used local protocols.

The CDC recently reported that there was an incorrect reference range with the EnLite™ Neonatal TREC kit, indicating that the method may be returning inconsistent results for the reference gene (EAG members, personal communication, August 2015). A new lab-developed test method developed in conjunction with the CDC may be an alternative to the currently available commercial kit. This may have implications to Alberta as to what screening technology should be chosen, if SCID were added to the provincial core panel.

Discussion

Methodological Issues

The methodological quality of included studies that reported screening performance outcomes, as assessed by the QUADAS-2 tool, was rated poor, in general. The QUADAS tool was originally developed for diagnostic accuracy studies, where subjects usually receive both index test and reference standard so a 2×2 table can be constructed. However, it is almost impossible to apply this cross-sectional approach to studies of a screening test for a very rare disease like SCID, because individuals with negative screening results usually will not receive the reference standard, making it difficult to obtain the actual number of false/true negatives.

Information about screening test accuracy on detecting SCID came from two types of studies, case control or newborn screening programs. In studies with a case control design, samples from patients with confirmed SCID and from healthy controls were tested with the TREC assay. Previous studies of other tests with case control design using healthy controls showed inflated estimates of test

accuracy compared to studies using a cohort of consecutive patients, possibly due to spectrum effects and limited-challenge bias.[108] This type of study is useful in the early phase of the development of the test, but estimates of test accuracy based on this type of study should be interpreted with caution.

Since SCID was added to the United States newborn screening core panel of 29 genetic disorders in 2010,[109] 25 states currently screen for SCID, making available results based on aggregated data from 11 newborn screening programs[80] and information obtained from individual state screening programs. False negatives were not reported in any of included studies, indicating a sensitivity of 100%. To arrive at a true value for the sensitivity, there must be a systematic search for "missed cases", which should be a key component of any screening program.[110] However, some of the included studies did not provide details in this regard.

Main Findings

The main findings of this review are summarized below:

- Evidence on harms related to the screening test is limited. While only the United States multistate study[80] reported no over-diagnosis after screening over 3 million infants, information regarding over-diagnosis or other psychosocial harms was not available in the systematic review[87] or primary studies. Although no study was found that specifically focused on psychosocial harms of newborn screening for SCID, it has been well recognized in general that false positive results of a newborn screening test may cause parents anxiety and stress while waiting for the results of confirmation diagnosis, or prompt further investigations and unnecessary treatments with no proven benefits, thus causing family distress and increase use of healthcare services.
- Identification of T-cell lymphopenia rather than true SCID through screening, where the nature of the underlying diagnosis or the appropriate pathway for management is not clear, may create worry in families and result in over-diagnosis. This concern can be minimized by the use of appropriate cut-offs that decrease the number of T-cell lymphopenia cases identified.
- Based on the published results from the systematic review and primary studies, the TREC assay using qPCR is highly sensitive and specific, with reported sensitivity and specificity exceeding 99%. Because positive and negative predictive values are dependent on prevalence of the target disease, for a rare condition like SCID, the positive predictive value will not be high. One newborn screening study reported that, for typical and leaky SCID, the positive predictive value was 2.1% and the negative predictive value was 100% (because of no known false negative). For clinically significant T-cell lymphopenia, which has a higher incidence, the positive predictive value increased to 20.3%.
- Consistent evidence from the systematic review and primary studies suggests that SCID infants who received early HSCT before the onset of infections had statistically significant higher survival than those who received late treatment or no treatment. HSCT as a potentially curative treatment option changed the clinical course of SCID from a fatal disease to a chronic disease. However, there are some concerns about the long-term complications following HSCT.
- As SCID was added to the United States newborn screening core panel in 2010, recent reports of state-wide newborn screening programs are primarily focused on the screening

performance outcomes and incidence data, with very limited information on the effectiveness of newborn screening (early identification and early treatment with HSCT) on mortality and survival.

Conclusion

SCID screening is the first addition to the United States national Recommended Uniform Screening Panel that followed a new evidence-based review process. It is also the first newborn screening test to use DNA as the primary analyte.

Evidence gathered from systematic reviews and recently published primary studies, particularly reporting on newborn screening programs from several states in the United States, indicate that the TREC assay is highly sensitive and specific in detecting SCID in infants. Early identification of SCID provides the opportunity for early initiation of immunity restoring treatments (HSCT, enzyme replacement therapy, or gene therapy), and currently available evidence consistently shows better survival with early treatment with HSCT when compared to late or no treatment. Findings from longer-term follow-up of children with SCID identified by screening will help further explore the overall benefit of newborn screening programs for SCID.

Appendix T.A: Methodology

Literature Search Summary

Systematic Reviews/HTAs

The literature search was conducted by the IHE Research Librarian from 11-15 September 2014. The search was limited to systematic reviews and HTAs.

Table T.A.1: Literature search summary – systematic reviews/HTAs

Database	Edition or date searched	Search Terms††	
MEDLINE (includes in process and other non-indexed citation) OVID Licensed Resource	11 September 2014 330 results	1	Metabolic Diseases/
		2	exp Metabolism, Inborn Errors/
		3	(metabolic adj2 (condition* or disease* or disorder* or deficienc*)).mp.
		4	(organic acid adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		5	(fatty acid oxidation adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		6	(amino acid adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		7	exp Amino Acid Metabolism, Inborn Errors/
		8	inborn errors of metabolism.tw.
		9	Endocrine System Diseases/
		10	(endocrine adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		11	hemoglobinopath*.mp.
		12	(hemoglobin adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		13	Hemoglobinopathies/
		14	Propionic Acidemia/
		15	(propionic acidemia* or propionic aciduria*).tw.
		16	(methylmalonyl* adj2 mutase).mp.
		17	(methylmalonic acidemia* or methylmalonic aciduria*).mp.
		18	cobalamin.mp.
		19	adenosylcobalamin synthesis.mp.
		20	isovaleric acidemia*.mp.
		21	exp Isovaleryl-CoA Dehydrogenase/
		22	(isovaleryl* adj2 dehydrogenase).mp.
		23	(3-methylcrotonyl* adj2 carboxylase).mp.
		24	3-methylcrotonylglycinuria.mp.
		25	(3-Hydroxy-3-methylglutaryl* adj2 lyase).mp.
		26	3-hydroxy-3-methyglutaric aciduria.mp.
		27	(HMG* adj2 lyase).mp.
		28	(hydroxymethalglutaryl adj2 lyase).mp.
		29	exp Holocarboxylase Synthetase Deficiency/
		30	exp Multiple Carboxylase Deficiency/
		31	((multiple adj2 carboxylase) or holocarboxylase).mp.

		32	beta ketothiolase.mp.
		33	exp Acetyl-CoA C-Acyltransferase/
		34	(acetyl* adj2 acyltransferase).mp.
		35	(glutaric adj (acidemia* or aciduria*)).mp.
		36	Glutaryl-CoA Dehydrogenase/
		37	(glutaryl* adj2 dehydrogenase).mp.
		38	Carnitine.mp.
		39	exp Carnitine/
		40	exp Acyl-CoA Dehydrogenases/
		41	(acyl* adj2 dehydrogenase).mp.
		42	Acyl-CoA Dehydrogenase, Long-Chain/
		43	(hydroxyacyl* adj2 dehydrogenase).mp.
		44	(hydroxy-acyl* adj2 dehydrogenase).mp.
		45	Long-Chain-3-Hydroxyacyl-CoA Dehydrogenase/
		46	trifunctional protein.mp.
		47	exp Mitochondrial Trifunctional Protein/
		48	Argininosuccinic Aciduria/
		49	argininosuccinic aciduria*.mp.
		50	argininosuccinate lyase*.mp.
		51	Argininosuccinate Lyase/
		52	Citrullinemia/
		53	citrullinemia.tw.
		54	Maple Syrup Urine Disease/
		55	maple syrup urine disease.tw.
		56	Homocystinuria/
		57	(homocystinuria or homocysteinemia or homocysteinurea).tw.
		58	exp Phenylketonurias/
		59	phenylketonuria*.tw.
		60	pku.tw.
		61	Tyrosinemias/
		62	Tyrosinemia*.tw.
		63	Congenital Hypothyroidism/
		64	congenital hypothyroidism.tw.
		65	Adrenal Hyperplasia, Congenital/
		66	congenital adrenal hyperplasia.tw.
		67	21 hydroxylase.tw.
		68	exp Anemia, Sickle Cell/
		69	sickle cell*.tw.
		70	ss disease.tw.
		71	beta-Thalassemia/
		72	beta thalassemia.tw.
		73	exp Hemoglobin SC Disease/
		74	sc disease.tw.
		75	Biotinidase Deficiency/
		76	biotinidase deficienc*.tw.
		77	Cystic Fibrosis/
		78	cystic fibrosis.tw.

		79	Galactosemias/
		80	galactosemia*.tw.
		81	exp Severe Combined Immunodeficiency/
		82	severe combined immunodeficienc*.tw.
		83	severe combined immune deficienc*.tw.
		84	or/1-83
		85	exp Infant/
		86	(neonat* or newborn* or infan* or baby or babies).mp.
		87	85 or 86
		88	84 and 87
		89	meta-analysis.pt.
		90	(meta-anal$ or metaanal$).mp.
		91	((quantitativ$ adj3 review$1) or (quantitativ$ adj3 overview$)).mp.
		92	((systematic$ adj3 review$) or (systematic adj3 overview$)).mp.
		93	((methodologic adj3 review$1) or (methodologic adj3 overview$)).mp.
		94	(integrat$ adj5 research).mp.
		95	(quantitativ$ adj3 synthes$).mp.
		96	or/89-95
		97	review.pt. or (review$ or overview$).mp.
		98	(medline or medlars or pubmed or index medicus or embase or cochrane).mp.
		99	(scisearch or web of science or psycinfo or psychinfo or cinahl or cinhal).mp.
		100	(excerpta medica or psychlit or psyclit or current contents or science citation index or sciences citation index).mp.
		101	(hand search$ or manual search$).mp.
		102	((((electronic adj3 database$) or bibliographic) adj3 database$) or periodical index$).mp.
		103	(pooling or pooled or mantel haenszel).mp.
		104	(peto or der simonian or dersimonian or fixed effect$).mp.
		105	((combine$ or combining) adj5 (data or trial or trials or studies or study or result or results)).mp.
		106	or/98-105
		107	97 and 106
		108	96 or 107
		109	(hta$ or health technology assessment$ or biomedical technology assessment$).mp.
		110	technology assessment, biomedical/ or biomedical technology assessment/
		111	109 or 110
		112	108 or 111
		113	112 and 88
EMBASE	11 September 2014 600 results	1	metabolic disorder/
		2	exp "inborn error of metabolism"/
		3	(metabolic adj2 (condition* or disease* or disorder* or deficienc*)).mp.
		4	(organic acid adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		5	(fatty acid oxidation adj2 (condition* or disorder* or disease* or

			deficienc*)).mp.
		6	(amino acid adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		7	"disorders of amino acid and protein metabolism"/
		8	inborn errors of metabolism.tw.
		9	endocrine disease/
		10	(endocrine adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		11	hemoglobinopath*.mp.
		12	(hemoglobin adj2 (condition* or disorder* or disease* or deficienc*)).mp.
		13	exp hemoglobinopathy/
		14	propionic acidemia/
		15	(propionic acidemia* or propionic aciduria*).tw.
		16	(methylmalonyl* adj2 mutase).mp.
		17	methylmalonyl coenzyme A mutase/
		18	(methylmalonic acidemia* or methylmalonic aciduria*).mp.
		19	methylmalonic acidemia/
		20	methylmalonic aciduria/
		21	"disorders of carboxylic acid metabolism"/
		22	cobalamin/
		23	cobalamin.mp.
		24	adenosylcobalamin synthesis.mp.
		25	isovaleric acidemia*.mp.
		26	isovaleryl coenzyme A dehydrogenase/
		27	(isovaleryl* adj2 dehydrogenase).tw.
		28	(3-methylcrotonyl* adj2 carboxylase).mp.
		29	methylcrotonoyl coenzyme A carboxylase/
		30	(methylcrotonoyl adj2 carboxylase).mp.
		31	3-methylcrotonylglycinuria.mp.
		32	(3-Hydroxy-3-methylglutaryl* adj2 lyase).mp.
		33	3-hydroxy-3-methyglutaric aciduria.mp.
		34	(HMG* adj2 lyase).mp.
		35	hydroxymethylglutaryl coenzyme A lyase/
		36	(hydroxymethalglutaryl adj2 lyase).mp.
		37	"disorders of carbohydrate metabolism"/
		38	((multiple adj2 carboxylase) or holocarboxylase).mp.
		39	beta ketothiolase.mp.
		40	(acetyl* adj2 acyltransferase).mp.
		41	acetyl coenzyme A acyltransferase/
		42	(glutaric adj (acidemia* or aciduria*)).mp.
		43	glutaryl coenzyme A dehydrogenase/
		44	(glutaryl* adj2 dehydrogenase).mp.
		45	carnitine.mp.
		46	carnitine/
		47	acyl coenzyme A dehydrogenase/
		48	(acyl* adj2 dehydrogenase).mp.
		49	medium chain acyl coenzyme a dehydrogenase/ or medium chain

			acyl coenzyme a dehydrogenase deficiency/
		50	long chain acyl coenzyme A dehydrogenase/
		51	(hydroxyacyl* adj2 dehydrogenase).mp.
		52	long chain 3 hydroxyacyl coenzyme A dehydrogenase/
		53	(hydroxy-acyl* adj2 dehydrogenase).mp.
		54	3 hydroxyacyl coenzyme A dehydrogenase/
		55	trifunctional protein.mp.
		56	mitochondrial trifunctional protein/
		57	argininosuccinic aciduria/
		58	argininosuccinic aciduria*.mp.
		59	argininosuccinate lyase*.mp.
		60	argininosuccinate lyase/
		61	citrullinemia/
		62	citrullinemia.tw.
		63	maple syrup urine disease/
		64	maple syrup urine disease.tw.
		65	homocystinuria/
		66	(homocystinuria or homocysteinemia or homocysteinurea).tw.
		67	phenylketonuria/
		68	phenylketonuria*.tw.
		69	pku.tw.
		70	tyrosinemia/
		71	tyrosinemia*.tw.
		72	congenital hypothyroidism/
		73	congenital hypothyroidism.tw.
		74	congenital adrenal hyperplasia/
		75	congenital adrenal hyperplasia.tw.
		76	21 hydroxylase.tw.
		77	sickle cell anemia/ or sickle cell/ or sickle cell beta thalassemia/
		78	sickle cell*.tw.
		79	ss disease.tw.
		80	beta thalassemia/
		81	beta thalassemia.tw.
		82	hemoglobin sc disease/
		83	sc disease.tw.
		84	biotinidase deficiency/
		85	biotinidase deficienc*.tw.
		86	exp cystic fibrosis/
		87	cystic fibrosis.tw.
		88	galactosemia/
		89	galactosemia*.tw.
		90	exp severe combined immunodeficiency/
		91	severe combined immunodeficienc*.tw.
		92	severe combined immune deficienc*.tw.
		93	or/1-92
		94	exp infant/
		95	(neonat* or newborn* or infan* or baby or babies).mp.

		96	94 or 95
		97	93 and 96
		98	(meta-anal$ or metaanal$).mp.
		99	((quantitativ$ adj3 review$1) or (quantitativ$ adj3 overview$)).mp.
		100	((systematic$ adj3 review$) or (systematic adj3 overview$)).mp.
		101	((methodologic adj3 review$1) or (methodologic adj3 overview$)).mp.
		102	(integrat$ adj5 research).mp.
		103	(quantitativ$ adj3 synthes$).mp.
		104	or/98-103
		105	review.pt. or (review$ or overview$).mp.
		106	(medline or medlars or pubmed or index medicus or embase or cochrane).mp.
		107	(scisearch or web of science or psycinfo or psychinfo or cinahl or cinhal).mp.
		108	(excerpta medica or psychlit or psyclit or current contents or science citation index or sciences citation index).mp.
		109	(hand search$ or manual search$).mp.
		110	((((electronic adj3 database$) or bibliographic) adj3 database$) or periodical index$).mp.
		111	(pooling or pooled or mantel haenszel).mp.
		112	(peto or der simonian or dersimonian or fixed effect$).mp.
		113	((combine$ or combining) adj5 (data or trial or trials or studies or study or result or results)).mp.
		114	or/106-113
		115	105 and 114
		116	104 or 115
		117	(hta$ or health technology assessment$ or biomedical technology assessment$).mp.
		118	technology assessment, biomedical/ or biomedical technology assessment/
		119	117 or 118
		120	116 or 119
		121	97 and 120
EBM Reviews - Cochrane Database of Systematic Reviews 2005 to July 2014 EBM Reviews - Database of Abstracts of Reviews of Effects 3rd Quarter 2014 EBM Reviews - Health Technology Assessment 3rd Quarter 2014 Health	15 September 2014 338 results	1	exp Metabolism, Inborn Errors/
		2	(metabolic adj2 (condition* or disease* or disorder* or dificienc*)).mp.
		3	(organic acid adj2 (condition* or disorder* or disease* or dificienc*)).mp.
		4	(fatty acid oxidation adj2 (condition* or disorder* or disease* or dificienc*)).mp.
		5	(amino acid adj2 (condition* or disorder* or disease* or dificienc*)).mp.
		6	exp Amino Acid Metabolism, Inborn Errors/
		7	inborn errors of metabolism.mp.
		8	Endocrine System Diseases/
		9	(endocrine adj2 (condition* or disorder* or disease* or dificienc*)).mp.
		10	hemoglobinopath*.mp.
		11	(hemoglobin adj2 (condition* or disorder* or disease* or dificienc*)).mp.
		12	Hemoglobinopathies/

Technology Assessment		13	Propionic Acidemia/
		14	(propionic acidemia* or propionic aciduria*).mp.
		15	(methylmalonyl* adj2 mutase).mp.
		16	(methylmalonic acidemia* or methylmalonic aciduria*).mp.
		17	cobalamin.mp.
		18	adenosylcobalamin synthesis.mp.
		19	isovaleric acidemia*.mp.
		20	exp Isovaleryl-CoA Dehydrogenase/
		21	(isovaleryl* adj2 dehydrogenase).mp.
		22	(3-methylcrotonyl* adj2 carboxylase).mp.
		23	3-methylcrotonylglycinuria.mp.
		24	(3-Hydroxy-3-methylglutaryl* adj2 lyase).mp.
		25	3-hydroxy-3-methyglutaric aciduria.mp.
		26	(HMG* adj2 lyase).mp.
		27	(hydroxymethalglutaryl adj2 lyase).mp.
		28	exp Holocarboxylase Synthetase Deficiency/
		29	exp Multiple Carboxylase Deficiency/
		30	((multiple adj2 carboxylase) or holocarboxylase).mp.
		31	beta ketothiolase.mp.
		32	exp Acetyl-CoA C-Acyltransferase/
		33	(acetyl* adj2 acyltransferase).mp.
		34	(glutaric adj (acidemia* or aciduria*)).mp.
		35	Glutaryl-CoA Dehydrogenase/
		36	(glutaryl* adj2 dehydrogenase).mp.
		37	Carnitine.mp.
		38	exp Carnitine/
		39	exp Acyl-CoA Dehydrogenases/
		40	(acyl* adj2 dehydrogenase).mp.
		41	Acyl-CoA Dehydrogenase, Long-Chain/
		42	(hydroxyacyl* adj2 dehydrogenase).mp.
		43	(hydroxy-acyl* adj2 dehydrogenase).mp.
		44	Long-Chain-3-Hydroxyacyl-CoA Dehydrogenase/
		45	trifunctional protein.mp.
		46	exp Mitochondrial Trifunctional Protein/
		47	Argininosuccinic Aciduria/
		48	argininosuccinic aciduria*.mp.
		49	argininosuccinate lyase*.mp.
		50	Argininosuccinate Lyase/
		51	Citrullinemia/
		52	citrullinemia.mp.
		53	Maple Syrup Urine Disease/
		54	maple syrup urine disease.mp.
		55	Homocystinuria/
		56	(homocystinuria or homocysteinemia or homocysteinurea).tw.
		57	exp Phenylketonurias/
		58	phenylketonuria*.mp.
		59	pku.mp.

		60	Tyrosinemias/
		61	Tyrosinemia*.mp.
		62	Congenital Hypothyroidism/
		63	congenital hypothyroidism.mp.
		64	Adrenal Hyperplasia, Congenital/
		65	congenital adrenal hyperplasia.mp.
		66	21 hydroxylase.mp.
		67	exp Anemia, Sickle Cell/
		68	sickle cell*.mp.
		69	ss disease.mp.
		70	beta-Thalassemia/
		71	beta thalassemia.mp.
		72	exp Hemoglobin SC Disease/
		73	sc disease.mp.
		74	Biotinidase Deficiency/
		75	biotinidase deficienc*.mp.
		76	Cystic Fibrosis/
		77	cystic fibrosis.mp.
		78	Galactosemias/
		79	galactosemia*.mp.
		80	exp Severe Combined Immunodeficiency/
		81	severe combined immunodeficienc*.mp.
		82	severe combined immune deficienc*.mp.
		83	or/1-82 189
		84	exp Infant/
		85	(neonat* or newborn* or infan* or baby or babies).mp.
		86	84 or 85
		87	83 and 86
CINAHL	11 September 2014 153 results	S77	S74 OR S76
		S76	S72 AND S75
		S75	(meta-analysis OR metaanalysis OR pubmed OR medline OR cinahl OR search* OR (systematic* AND review*))
		S74	S72 AND S73
		S73	
		S72	S70 AND S71
		S71	(neonat* or newborn* or infan* or baby or babies)
		S70	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39 OR S40 OR S41 OR S42 OR S43 OR S44 OR S45 OR S46 OR S47 OR S48 OR S49 OR S50 OR S51 OR S52 OR S53 OR S54 OR S55 OR S56 OR S57 OR S58 OR S59 OR S60 OR S61 OR S62 OR S63 OR S64 OR S65 OR S66 OR S67 OR S68 OR S69
		S69	severe combined immune deficienc*
		S68	severe combined immunodeficienc*
		S67	(MH "Severe Combined Immunodeficiency")

		S66	galactosemia*
		S65	(MH "Galactosemia")
		S64	cystic fibrosis
		S63	(MH "Cystic Fibrosis")
		S62	biotinidase deficienc*
		S61	sc disease
		S60	beta thalassemia
		S59	(MH "beta-Thalassemia")
		S58	ss disease
		S57	sickle cell*
		S56	(MH "Anemia, Sickle Cell+")
		S55	21 hydroxylase
		S54	congenital adrenal hyperplasia
		S53	congenital hypothyroidism
		S52	(MH "Congenital Hypothyroidism")
		S51	tyrosinemia*
		S50	pku
		S49	phenylketonuria*
		S48	(MH "Phenylketonuria+")
		S47	(homocystinuria or homocysteinemia or homocysteinurea)
		S46	maple syrup urine disease
		S45	(MH "Maple Syrup Urine Disease")
		S44	citrullinemia
		S43	argininosuccinate lyase*
		S42	argininosuccinic aciduria*
		S41	trifunctional protein
		S40	(hydroxy-acyl* N2 dehydrogenase)
		S39	(hydroxyacyl* N2 dehydrogenase)
		S38	(acyl* N2 dehydrogenase)
		S37	carnitine
		S36	(MH "Carnitine")
		S35	(glutaryl* N2 dehydrogenase)
		S34	(glutaric N1 (acidemia* or aciduria*))
		S33	(acetyl* N2 acyltransferase)
		S32	beta ketothiolase
		S31	((multiple N2 carboxylase) or holocarboxylase)
		S30	(hydroxymethalglutaryl N2 lyase)
		S29	(HMG* N2 lyase)
		S28	(3-hydroxy-3-methyglutaric aciduria)
		S27	(3-Hydroxy-3-methylglutaryl* N2 lyase)
		S26	3-methylcrotonylglycinuria
		S24	(3-methylcrotonyl* N2 carboxylase)
		S23	(isovaleryl* N2 dehydrogenase)
		S22	(isovaleryl* N2 dehydrogenase)
		S21	isovaleric acidemia*
		S20	adenosylcobalamin synthesis
		S19	cobalamin

		S18 (MH "Vitamin B12 Deficiency+") S17 (carboxylic acid N2 metabolism) S16 methylmalonic acidemia* S15 methylmalonic aciduria* S14 (methylmalonyl* N2 mutase) S13 propionic acidemia* OR propionic aciduria* S12 (MH "Hemoglobinopathies+") S11 (hemoglobin N2 (condition* or disorder* or disease* or deficienc*)) S10 hemoglobinopath* S9 (endocrine N2 (condition* or disorder* or disease* or deficienc*)) S8 (MH "Endocrine Diseases") S7 errors N2 metabolism S6 (amino acid N2 (condition* or disorder* or disease* or deficienc*)) S5 (fatty acid oxidation N2 (condition* or disorder* or disease* or deficienc*)) S4 (organic acid N2 (condition* or disorder* or disease* or deficienc*)) S3 (metabolic N2 (condition* or disease* or disorder* or deficienc*)) S2 (MH "Metabolism, Inborn Errors+") Search modes - Find all my search terms Interface S1 (MH "Metabolic Diseases") Search modes - Find all my search terms
Web of Science	9 September 2014 1,458 results	# 54 #52 AND #51 Refined by: PUBLICATION YEARS: (2012 OR 2001 OR 2013 OR 2000 OR 2011 OR 1998 OR 2010 OR 1997 OR 2009 OR 1994 OR 2014 OR 1996 OR 2007 OR 1992 OR 2006 OR 1991 OR 2005 OR 1995 OR 2004 OR 1989 OR 2008 OR 1993 OR 2002 OR 1990 OR 2003 OR 1999) # 53 #52 AND #51 # 52 TOPIC: ((meta-analysis OR metaanalysis OR search OR pubmed OR medline OR cinahl OR HTA OR "technology assessment" OR (systematic* SAME review*))) # 51 #50 AND #49 # 50 #48 OR #47 OR #46 OR #45 OR #44 OR #43 OR #42 OR #41 OR #40 OR #39 OR #38 OR #37 OR #36 OR #35 OR #34 OR #33 OR #32 OR #31 OR #30 OR #29 OR #28 OR #27 OR #26 OR #25 OR #24 OR #23 OR #22 OR #21 OR #20 OR #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1 # 49 TOPIC: ((neonat* or newborn* or infan* or baby or babies)) # 48 TOPIC: (severe combined immune deficienc*) # 47 TOPIC: (severe combined immunodeficienc*) # 46 TOPIC: (galactosemia*) # 45 TOPIC: (cystic fibrosis) # 44 TOPIC: (biotinidase deficienc*) # 43 TOPIC: (sc disease) # 42 TOPIC: (beta thalassemia) # 41 TOPIC: (ss disease) # 40 TOPIC: (sickle cell*) # 39 TOPIC: (21 hydroxylase) # 38 TOPIC: (congenital adrenal hyperplasia)

		# 37 TOPIC: (congenital hypothyroidism) # 36 TOPIC: (pku) # 35 TOPIC: (homocystinuria or homocysteinemia or homocysteinurea) # 34 TOPIC: (maple syrup urine disease) # 33 TOPIC: (citrullinemia) # 32 TOPIC: (argininosuccinate lyase*) # 31 TOPIC: (argininosuccinic aciduria*) # 30 TOPIC: (trifunctional protein) # 29 TOPIC: ((hydroxy-acyl* NEAR/2 dehydrogenase)) # 28 TOPIC: ((hydroxyacyl* NEAR/2 dehydrogenase)) # 27 TOPIC: ((acyl* NEAR/2 dehydrogenase))\ # 26 TOPIC: (carnitine) # 25 TOPIC: ((glutaryl* NEAR/2 dehydrogenase)) # 24 TOPIC: (glutaric acidemia*) OR TOPIC: (glutaric aciduria*) Search language=Auto # 23 TOPIC: ((acetyl* NEAR/2 acyltransferase)) # 22 TOPIC: (beta ketothiolase) # 21 TOPIC: (((multiple NEAR/2 carboxylase) or holocarboxylase)) # 20 TOPIC: (hydroxymethylglutaryl NEAR lyase) # 19 TOPIC: ((HMG* NEAR/2 lyase)) # 18 TOPIC: (3-hydroxy-3-methyglutaric aciduria) # 17 TOPIC: (3-Hydroxy-3-methylglutaryl* NEAR/2 lyase) # 16 TOPIC: (3-methylcrotonylglycinuria) # 15 TOPIC: ((methylcrotonyl* NEAR/2 carboxylase)) # 14 TOPIC: ((isovaleryl* NEAR/2 dehydrogenase)) # 13 TOPIC: (isovaleric acidemia*) # 12 TOPIC: (adenosylcobalamin synthesis) # 11 TOPIC: (cobalamin) # 10 TOPIC: ((methylmalonyl* NEAR/2 mutase)) # 9 TOPIC: (propionic acidemia*) OR TOPIC: (propionic aciduria*) # 8 TOPIC: ((hemoglobin NEAR/2 (condition* or disorder* or disease* or deficienc*))) # 7 TOPIC: (hemoglobinopath*) # 6 TOPIC: ((endocrine NEAR/2 (condition* or disorder* or disease* or deficienc*))) # 5 TOPIC: ((metabolism NEAR/2 error*)) # 4 TOPIC: (("amino acid" NEAR/2 (condition* or disorder* or disease* or deficienc*))) # 3 TOPIC: (("fatty acid oxidation" NEAR/2 (condition* or disorder* or disease* or deficienc*))) # 2 TOPIC: (("organic acid" NEAR/2 (condition* or disorder* or disease* or deficienc*))) # 1 TOPIC: ((metabolic NEAR/2 (condition* or disease* or disorder* or deficienc*)))

Note: "††", "*", "# ", and "?" are truncation characters that retrieve all possible suffix variations of the root word e.g. surg* retrieves surgery, surgical, surgeon, etc.

Primary Studies

The literature search was conducted by the IHE Research Librarian from 27 November to 1 December 2014. The search was limited to primary studies in English or French. Date limits for each condition varied on when the last published systematic review was done.

Table T.A.2: Literature search summary – primary studies

Database	Edition or date searched	Search Terms††
MEDLINE (includes in process and other non-indexed citation) OVID Licensed Resource	27 November 2014 12,341 results	1 Homocystinuria/ 2 (homocystinuria or homocysteinemia or homocysteinurea).tw. 3 Tyrosinemias/ 4 tyrosinemia*.tw. 5 exp Anemia, Sickle Cell/ 6 sickle cell*.tw. 7 ss disease.tw. 8 beta-Thalassemia/ 9 beta thalassemia.tw. 10 exp Hemoglobin SC Disease/ 11 sc disease.tw. 12 Galactosemias/ 13 galactosemia*.tw. 14 exp Severe Combined Immunodeficiency/ 15 severe combined immunodeficienc*.tw. 16 severe combined immune deficienc*.tw. 17 limit 1 to yr="2009 -Current" 18 limit 2 to yr="2009 -Current" 19 17 or 18 20 limit 3 to yr="2002 -Current" 21 limit 4 to yr="2002 -Current" 22 20 or 21 23 limit 5 to yr="1996 -Current" 24 limit 6 to yr="1996 -Current" 25 limit 7 to yr="1996 -Current" 26 limit 8 to yr="1996 -Current" 27 limit 9 to yr="1996 -Current" 28 limit 10 to yr="1996 -Current" 29 limit 11 to yr="1996 -Current" 30 23 or 24 or 25 or 26 or 27 or 28 or 29 31 limit 12 to yr="1996 -Current" 32 limit 13 to yr="1996 -Current" 33 31 or 32 34 limit 14 to yr="2008 -Current" 35 limit 15 to yr="2008 -Current" 36 limit 16 to yr="2008 -Current" 37 34 or 35 or 36

		38	19 or 22 or 30 or 33 or 37
		39	limit 38 to (english or french)
		40	animals/
		41	humans/
		42	40 not (40 and 41)
		43	39 not 42
		44	meta-analysis.pt.
		45	(meta-anal$ or metaanal$).mp.
		46	((quantitativ$ adj3 review$1) or (quantitativ$ adj3 overview$)).mp.
		47	((systematic$ adj3 review$) or (systematic adj3 overview$)).mp.
		48	((methodologic adj3 review$1) or (methodologic adj3 overview$)).mp.
		49	(integrat$ adj5 research).mp.
		50	(quantitativ$ adj3 synthes$).mp.
		51	or/44-50
		52	review.pt. or (review$ or overview$).mp.
		53	(medline or medlars or pubmed or index medicus or embase or cochrane).mp.
		54	(scisearch or web of science or psycinfo or psychinfo or cinahl or cinhal).mp.
		55	(excerpta medica or psychlit or psyclit or current contents or science citation index or sciences citation index).mp.
		56	(hand search$ or manual search$).mp.
		57	((((electronic adj3 database$) or bibliographic) adj3 database$) or periodical index$).mp.
		58	(pooling or pooled or mantel haenszel).mp.
		59	(peto or der simonian or dersimonian or fixed effect$).mp.
		60	((combine$ or combining) adj5 (data or trial or trials or studies or study or result or results)).mp.
		61	or/53-60
		62	52 and 61
		63	51 or 62
		64	(hta$ or health technology assessment$ or biomedical technology assessment$).mp.
		65	technology assessment, biomedical/ or biomedical technology assessment/
		66	64 or 65
		67	63 or 66
		68	43 not 67
		69	limit 68 to (addresses or autobiography or bibliography or biography or case reports or clinical conference or comment or congresses or consensus development conference or consensus development conference, nih or dataset or dictionary or directory or editorial or festschrift or interactive tutorial or interview or lectures or legal cases or legislation or letter or meta analysis or news or newspaper article or patient education handout or periodical index or portraits or "review" or systematic reviews or video-audio media or webcasts)
		70	68 not 69
EMBASE OVID Licensed Resource	27 November 2014 17,482 results	1	homocystinuria/
		2	(homocystinuria or homocysteinemia or homocysteinurea).tw.
		3	1 or 2
		4	limit 3 to yr="2009 -Current"

		5 tyrosinemia/ 6 tyrosinemia*.tw. 7 5 or 6 8 limit 7 to yr="2002 -Current" 9 sickle cell anemia/ or sickle cell/ or sickle cell beta thalassemia/ 10 sickle cell*.tw. 11 ss disease.tw. 12 hemoglobin sc disease/ 13 (sickle adj2 beta thalassemia).tw. 14 s beta thalassemia.tw. 15 sc disease.tw. 16 or/9-15 17 limit 16 to yr="1996 -Current" 18 galactosemia/ 19 galactosemia*.tw. 20 18 or 19 21 limit 20 to yr="1996 -Current" 22 exp severe combined immunodeficiency/ 23 severe combined immunodeficienc*.tw. 24 severe combined immune deficienc*.tw. 25 22 or 23 or 24 26 limit 25 to yr="2008 -Current" 27 4 or 8 or 17 or 21 or 26 28 limit 27 to (english or french) 29 animal/ 30 human/ 31 29 not (29 and 30) 32 28 not 31 33 meta-analysis.pt. 34 (meta-anal$ or metaanal$).mp. 35 ((quantitativ$ adj3 review$1) or (quantitativ$ adj3 overview$)).mp. 36 ((systematic$ adj3 review$) or (systematic adj3 overview$)).mp. 37 ((methodologic adj3 review$1) or (methodologic adj3 overview$)).mp. 38 (integrat$ adj5 research).mp. 39 (quantitativ$ adj3 synthes$).mp. 40 or/33-39 41 review.pt. or (review$ or overview$).mp. 42 (medline or medlars or pubmed or index medicus or embase or cochrane).mp. 43 (scisearch or web of science or psycinfo or psychinfo or cinahl or cinhal).mp. 44 (excerpta medica or psychlit or psyclit or current contents or science citation index or sciences citation index).mp. 45 (hand search$ or manual search$).mp. 46 ((((electronic adj3 database$) or bibliographic) adj3 database$) or periodical index$).mp. 47 (pooling or pooled or mantel haenszel).mp. 48 (peto or der simonian or dersimonian or fixed effect$).mp. 49 ((combine$ or combining) adj5 (data or trial or trials or studies or study or

		result or results)).mp. 50 or/42-49 51 41 and 50 52 40 or 51 53 (hta$ or health technology assessment$ or biomedical technology assessment$).mp. 54 technology assessment, biomedical/ or biomedical technology assessment/ 55 53 or 54 56 52 or 55 57 32 not 56 58 limit 57 to (book or book series or editorial or letter or note or "review" or short survey or trade journal) 59 57 not 58
EBM Reviews - Cochrane Central Register of Controlled Trials October 2014	1 December 2014 785 results	**# Searches** 1 Homocystinuria/ 2 (homocystinuria or homocysteinemia or homocysteinurea).tw. 3 Tyrosinemias/ 4 Tyrosinemia*.mp. 5 exp Anemia, Sickle Cell/ 6 sickle cell*.mp. 7 ss disease.mp. 8 beta-Thalassemia/ 9 beta thalassemia.mp. 10 exp Hemoglobin SC Disease/ 11 sc disease.mp. 12 Galactosemias/ 13 galactosemia*.mp. 14 exp Severe Combined Immunodeficiency/ 15 severe combined immunodeficienc*.mp. 16 severe combined immune deficienc*.mp. 17 1 or 2 18 limit 17 to yr="2009 -Current" 19 3 or 4 20 limit 19 to yr="2002 -Current" 21 5 or 6 or 7 or 8 or 9 or 10 or 11 22 limit 21 to yr="1996 -Current" 23 12 or 13 24 limit 23 to yr="1996 -Current" 25 14 or 15 or 16 26 limit 25 to yr="2008 -Current" 27 18 or 20 or 22 or 24 or 26
CINAHL	1 December 2014 2,346 results	S18 S1 OR S2 OR S14 OR S15 OR S16 Limiters - Human; Language: English, French S17 S1 OR S2 OR S14 OR S15 OR S16 S16 S11 OR S12 OR S13 Limiters - Published Date: 20080101-20141231 S15 S9 OR S10 Limiters - Published Date: 19960101-20141231 S14 S3 OR S4 OR S5 OR S6 OR S7 OR S8 Limiters - Published Date:

		19960101-20141231 S13 severe combined immune deficienc* S12 severe combined immunodeficienc* S11 (MH "Severe Combined Immunodeficiency") S10 galactosemia* S9 (MH "Galactosemia") S8 sc disease S7 beta thalassemia S6 (MH "beta-Thalassemia") S5 ss disease S4 sickle cell* S3 (MH "Anemia, Sickle Cell+") S2 tyrosinemia*Limiters - Published Date: 20020101-20141231 S1 (homocystinuria or homocysteinemia or homocysteinurea)Limiters - Published Date: 20090101-20141231
Web of Science	1 December 2014	#15 #12 not #13 Refined by: **LANGUAGES:** (ENGLISH OR FRENCH) #14 #12 not #13 #13 TS=(animal or animals or pisces or fish or fishes or catfish or catfishes or sheatfish or silurus or arius or heteropneustes or clarias or gariepinus or fathead minnow or fathead minnows or pimephales or promelas or cichlidae or trout or trouts or char or chars or salvelinus or salmo or oncorhynchus or guppy or guppies or millionfish or poecilia or goldfish or goldfishes or carassius or auratus or mullet or mullets or mugil or curema or shark or sharks or cod or cods or gadus or morhua or carp or carps or cyprinus or carpio or killifish or eel or eels or anguilla or zander or sander or lucioperca or stizostedion or turbot or turbots or psetta or flatfish or flatfishes or plaice or pleuronectes or platessa or tilapia or tilapias or oreochromis or sarotherodon or common sole or dover sole or solea or zebrafish or zebrafishes or danio or rerio or seabass or dicentrarchus or labrax or morone or lamprey or lampreys or petromyzon or pumpkinseed or pumpkinseeds or lepomis or gibbosus or herring or clupea or harengus or amphibia or amphibian or amphibians or anura or salientia or frog or frogs or rana or toad or toads or bufo or xenopus or laevis or bombina or epidalea or calamita or salamander or salamanders or newt or newts or triturus or reptilia or reptile or reptiles or bearded dragon or pogona or vitticeps or iguana or iguanas or lizard or lizards or anguis fragilis or turtle or turtles or snakes or snake or aves or bird or birds or quail or quails or coturnix or bobwhite or colinus or virginianus or poultry or poultries or fowl or fowls or chicken or chickens or gallus or zebra finch or taeniopygia or guttata or canary or canaries or serinus or canaria or parakeet or parakeets or grasskeet or parrot or parrots or psittacine or psittacines or shelduck or tadorna or goose or geese or branta or leucopsis or woodlark or lullula or flycatcher or ficedula or hypoleuca or dove or doves or geopelia or cuneata or duck or ducks or greylag or graylag or anser or harrier or circus pygargus or red knot or great knot or calidris or canutus or godwit or limosa or lapponica or meleagris or gallopavo or jackdaw or corvus or monedula or ruff or philomachus or pugnax or lapwing or peewit or plover or vanellus or swan or cygnus or columbianus or bewickii or gull or chroicocephalus or ridibundus or albifrons or great tit or parus or aythya or fuligula or streptopelia or risoria or spoonbill or platalea or leucorodia or blackbird or turdus or merula or blue tit or cyanistes or pigeon or pigeons or columba or pintail or anas or starling or sturnus or owl or athene noctua or pochard or ferina or cockatiel or nymphicus or hollandicus or skylark or alauda or tern or sterna or teal or crecca or oystercatcher or haematopus or ostralegus or shrew or shrews or sorex or araneus or crocidura or russula or european mole or talpa or chiroptera or bat or bats or eptesicus or serotinus or myotis or dasycneme or daubentonii or pipistrelle or pipistrellus or cat or cats or felis or catus or feline or

		dog or dogs or canis or canine or canines or otter or otters or lutra or badger or badgers or meles or fitchew or fitch or foumart or foulmart or ferrets or ferret or polecat or polecats or mustela or putorius or weasel or weasels or fox or foxes or vulpes or common seal or phoca or vitulina or grey seal or halichoerus or horse or horses or equus or equine or equidae or donkey or donkeys or mule or mules or pig or pigs or swine or swines or hog or hogs or boar or boars or porcine or piglet or piglets or sus or scrofa or llama or llamas or lama or glama or deer or deers or cervus or elaphus or cow or cows or bos taurus or bos indicus or bovine or bull or bulls or cattle or bison or bisons or sheep or sheeps or ovis aries or ovine or lamb or lambs or mouflon or mouflons or goat or goats or capra or caprine or chamois or rupicapra or leporidae or lagomorpha or lagomorph or rabbit or rabbits or oryctolagus or cuniculus or laprine or hares or lepus or rodentia or rodent or rodents or murinae or mouse or mice or mus or musculus or murine or woodmouse or apodemus or rat or rats or rattus or norvegicus or guinea pig or guinea pigs or cavia or porcellus or hamster or hamsters or mesocricetus or cricetulus or cricetus or gerbil or gerbils or jird or jirds or meriones or unguiculatus or jerboa or jerboas or jaculus or chinchilla or chinchillas or beaver or beavers or castor fiber or castor canadensis or sciuridae or squirrel or squirrels or sciurus or chipmunk or chipmunks or marmot or marmots or marmota or suslik or susliks or spermophilus or cynomys or cottonrat or cottonrats or sigmodon or vole or voles or microtus or myodes or glareolus or primate or primates or prosimian or prosimians or lemur or lemurs or lemuridae or loris or bush baby or bush babies or bushbaby or bushbabies or galago or galagos or anthropoidea or anthropoids or simian or simians or monkey or monkeys or marmoset or marmosets or callithrix or cebuella or tamarin or tamarins or saguinus or leontopithecus or squirrel monkey or squirrel monkeys or saimiri or night monkey or night monkeys or owl monkey or owl monkeys or douroucoulis or aotus or spider monkey or spider monkeys or ateles or baboon or baboons or papio or rhesus monkey or macaque or macaca or mulatta or cynomolgus or fascicularis or green monkey or green monkeys or chlorocebus or vervet or vervets or pygerythrus or hominoidea or ape or apes or hylobatidae or gibbon or gibbons or siamang or siamangs or nomascus or symphalangus or hominidae or orangutan or orangutans or pongo or chimpanzee or chimpanzees or pan troglodytes or bonobo or bonobos or pan paniscus or gorilla or gorillas or troglodytes) #12 #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1 Refined by: **[excluding]: DOCUMENT TYPES:** (EDITORIAL MATERIAL OR NEWS ITEM OR BOOK OR BOOK CHAPTER OR REVIEW OR BIOGRAPHICAL ITEM OR LETTER) #11 #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1 #10 **TOPIC:** (severe combined immune deficienc*) #9 **TOPIC:** (severe combined immunodeficienc*) #8 **TOPIC:** (galactosemia*) #7 **TOPIC:** (sc disease) #6 **TOPIC:** (s beta thalassemia) #5 **TOPIC:** (sickle SAME beta thalassemia) #4 **TOPIC:** (ss disease) #3 **TOPIC:** (sickle cell*) #2 **TOPIC:** (Tyrosinemia*) #1 **TOPIC:** (homocystinuria or homocysteinemia or homocysteinurea)

Note: "††", "*", "# ", and "?" are truncation characters that retrieve all possible suffix variations of the root word e.g. surg* retrieves surgery, surgical, surgeon, etc.

Grey Literature

The grey literature search was conducted by the IHE Research Librarian from 29 October 2014 to 11 June 2015.

Table T.A.3: Literature search summary – grey literature

Source	Edition or date searched	Search Terms††
Systematic reviews and HTAs		
CADTH *http://www.cadth.ca/*	29 October 2014 2 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
INESS *http://www.inesss.qc.ca/en/home.html*	29 October 2014 6 results	Newborn screening or neonatal screening or infant screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
CHSPR *http://www.chspr.ubc.ca/*	29 October 2014 0 results	Newborn screening or neonatal screening or infant screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
ICES *http://www.ices.on.ca/*	29 October 2014 0 results	Newborn screening or neonatal screening or infant screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
OHTAC *http://www.hqontario.ca/evidence/publications-and-ohtac-recommendations/ontario-health-technology-assessment-series*	29 October 2014 0 results	Browsed list
NLCAHR: Newfoundland and Labrador Centre for Applied Health Research. (CHRSP) *http://www.nlcahr.mun.ca/CHRSP/*	29 October 2014 0 results	Browsed list
THETA *http://theta.utoronto.ca/*	29 October 2014 0 results	Browsed list
Adelaide Health Technology Assessment *http://www.adelaide.edu.au/ahta/pubs/*	29 October 2014 0 results	Browsed list

MSAC *http://www.msac.gov.au/internet/msac/publishing.nsf/Content/completed-assessments*	29 October 2014 0 results	Browsed list
Euroscan *http://www.euroscan.org.uk/*	29 October 2014 2 results	Newborn screening or neonatal screening or infant screening or immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
UK National Health Technology Assessment Programme *http://www.nets.nihr.ac.uk/projects?collection=netscc&meta_P_sand=Project*	29 October 2014 9 results	Newborn screening or neonatal screening or infant screening or immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
NICE *http://www.nice.org.uk/*	29 October 2014 0 results	Browsed list
AHRQ *http://ahrqpubs.ahrq.gov/OA_HTML/ibeCZzpHome.jsp*	30 October 2014 6 results	Newborn screening or neonatal screening or infant screening or immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or or homocysteinemia or homocysteinurea
Aetna *http://www.aetna.com/health-care-professionals/clinical-policy-bulletins/medical-clinical-policy-bulletins.html*	30 October 2014 0 results	Browsed list
BlueCross and Blue Shield (BCBS) Association *http://www.bcbs.com/blueresources/tec/vols/*	30 October 2014 0 results	Browsed list
Google *http://www.google.com*	30 October 32014 19 results	immunodeficiencies OR "immune deficiency" OR SCID OR galactosemia OR thalassemia OR "sickle cell" OR tyrosinemia OR homocystinuria OR homocysteinemia OR homocysteinurea "health technology assessment" filetype:pdf
Guidelines		
Guidelines Clearinghouse *http://www.guideline.gov/*	2 February 2015 12 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
CMA Infobase	13 February	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or

	2015 2 results	thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Clinical Trials		
Clinical trials.gov	9 March 2015 478 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Websites		
Google	2 April 2015	severe combined immunodeficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea severe combined immunodeficiency OR SCID therapy OR treatment OR screening OR diagnosis OR testing -pubmed filetype:pdf galactosemia therapy OR treatment OR screening OR diagnosis OR testing -pubmed filetype:pdf thalassemia OR sickle cell OR ss disease OR sc disease therapy OR treatment OR screening OR diagnosis OR testing -pubmed filetype:pdf homocystinuria OR homocysteinemia OR homocysteinurea therapy OR treatment OR screening OR diagnosis OR testing -pubmed filetype:pdf Newborn screening or neonatal screening
Government of BC *http://www2.gov.bc.ca/*	5 April 2015 2 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Alberta Health *http://www.health.alberta.ca/*	5 April 2015 4 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of Saskatchewan *http://www.saskatchewan.ca/*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of Manitoba *https://www.gov.mb.ca/index.html*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of Ontario Ministry of Health *http://www.health.gov.on.ca/en/*	15 April 2015 3 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of New Brunswick *http://www2.gnb.ca/*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Government of Nova Scotia *http://novascotia.ca/*	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea

Government of PEI http://www.gov.pe.ca/	15 April 2015 1 result	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Newfoundland Ministry of Health http://www.gov.nl.ca/	15 April 2015 0 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Yukon Government http://www.gov.yk.ca/	15 April 2015 See BC	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
NWT Government http://www.gov.nt.ca/	15 April 2015 See Alberta	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Nunavut Government http://www.gov.nu.ca/	15 April 2015 0 results	Newborn screening or neonatal screening or severe combined immunodeficiencies or immune deficiency or SCID or galactosemia or thalassemia or sickle cell or ss disease or sc disease or tyrosinemia or homocystinuria or homocysteinemia or homocysteinurea
Regulatory Information		
Health Canada Medical Devices Active Licence Listing (MDALL) http://www.hc-sc.gc.ca/dhp-mps/md-im/licen/mdlic-eng.php	11 June 2015 7 results	Hb variants OR HPLC VARIANT OR Bio-Rad Laboratories OR TRECs OR Qiagen OR IL-7 OR ELISA OR R&D system OR Tandem mass spectrometry OR Fluorescence spectrometry OR fluorescence spectroscopy OR spectrofluorometry OR Victor fluorometer OR PerkinElmer

Selection of Key Studies

Titles and abstracts were screened by one researcher (BG or ME) and relevant articles were retrieved. Eligibility of key studies was determined by two researchers (ME and BG) according to the following inclusion and exclusion criteria. Disagreement will be resolved by consensus.

Inclusion criteria

Study design

Stage 1

Systematic reviews and HTAs – A literature review was considered systematic if it met all of the following criteria:

- focused clinical question;
- explicit search strategy;
- use of explicit, reproducible, and uniformly applied criteria for article selection;
- formal critical appraisal of the included studies; and

- qualitative or quantitative data summary or synthesis (i.e., a meta-analysis).

Stage 2

Primary studies – Randomized controlled trials, cohort studies, case-control studies, cross-sectional studies, or case series studies.

Population: newborns, any ethnic origin

Index test: any laboratory tests conducted on dried blood spots to screen for the seven conditions

Comparator: any screening method or platform, no screening, or no comparator

Reference standard (gold standard): varies, depending on the condition

Target condition: seven core conditions (GALT, TYRI, HCY, Hb SS, Hb SC, Hb S/β-thal, SCID)

Outcome of interest: at least one of the following:

- **Safety:** adverse events associated with blood tests and diagnostic procedures, psychological consequences of false positive results
- **Effectiveness:** screening accuracy (sensitivity, specificity, positive predictive value, negative predictive value, and false positive rate); effects of early diagnosis and treatment compared to delayed diagnosis and treatment on clinical outcomes such as mortality, morbidity, quality of life, emergency room visits, or hospital admissions

Language: limited to English and French

Publication period: for systematic reviews/HTAs: from 1990 onwards; for primary studies: from the last search dates of the most recent systematic reviews/HTAs to present

In the case of duplicate publications, the most recent or most comprehensive version was included.

Exclusion criteria

Studies were excluded if they met any of the following criteria:

- reviews that do not meet the criteria for systematic reviews;
- conference abstracts, letters, news, and editorial comments, case reports;
- tests that are not performed on the dried blood spots;
- target conditions other than the seven core conditions defined for this project;
- studies that covered a broad range of conditions but outcomes were not reported separately for the primary target (e.g., studies focused on primary immunodeficiency, but outcomes were not presented separately for SCID); or
- animal studies.

Methodological Quality Assessment

Methodological quality were formally assessed independently by two of three researchers (BG, ME, and PC) using appropriate quality assessment tools. Assessment results from the two researchers were compared, and consensus was reached through discussion or arbitration by a third party. Quality assessment results were not used as inclusion or exclusion criteria.

For systematic reviews/HTAs: A Measurement Tool to Assess Systematic Reviews (AMSTAR) (*www.amstar.ca*) was used.

For screening accuracy studies: The Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist (*www.bris.ac.uk/quadas/quadas-2/*) was used to appraise the quality of the primary studies that report on screening accuracy parameters.

The QUADAS-2 tool evaluates the studies in two aspects:

1. the internal validity of the study (that is, the risk of internal bias) in four domains (patient selection, the index test, the reference standard, and flow and timing); and
2. the external validity of the study (that is, the degree to which the results of the study are generalizable to the general population) in all of the domains listed above except flow and timing.

Each study was evaluated and assigned with a score of high, unclear, or low quality for every domain. Based on the score of each domain, an overall rank of high, low, or unclear for the whole study was assigned in relation to the internal validity and external validity. The final quality appraisal results were then tabulated and presented according to the recommendations of the authors of the QUADAS-2 tool.[5]

For treatment effectiveness studies: The Effective Public Health Practice Project (EPHPP) tool was used to assess the quality of primary studies reporting on the effectiveness of early treatment/management following screening. The EPHPP tool was developed by a Canadian research group to be applied to studies in the public health area to support the decision-making process.[6, 7] The EPHPP tool assesses the quality of a study in six parameters: selection bias, study design, confounders, blinding, data collection method, and the withdrawals/dropouts.

The studies appraised with the EPHPP tool were assigned a rating of strong, moderate, or weak in each of the mentioned parameters. Based on the score of each parameter, a global rating of strong, moderate, or weak was assigned to each study. The final quality assessment results were then tabulated to facilitate the evaluation of the overall evidence.

Data Extraction

One researcher (ME or BG) extracted the data from the selected systematic reviews or primary studies according to predetermined data extraction forms.

The following general categories of data were extracted:

For systematic review/HTAs:

- report context (author, year, type of data synthesis, and objectives)
- literature search strategy
- study selection criteria
- quality assessment, data extraction, data analysis and synthesis
- outcomes reported (safety, screening accuracy, treatment effectiveness, and any other relevant clinical or epidemiological outcome such as incidence or prevalence)

For primary studies:

- study attributes (author, year, country, setting, study design, period)

- characteristics of the newborn population screened or treated
- descriptions of screening tests (time of sample collection, platform, target, cut-off) and confirmatory tests
- reported outcomes (harms, screening performance parameters, effectiveness of treatment and management, or effectiveness of newborn screening program)

Data Analysis and Synthesis

A narrative and tabular summary of the selected studies were produced. Potential limitations in the applicability and relevance of study results to the Alberta context were identified and discussed.

External Review

The draft report was reviewed by the members of the provincial Expert Advisory Group (EAG) assembled for this project.

Appendix T.B: Excluded Studies

Below is a list of excluded studies, grouped by reason for exclusion.

Inapplicable study design (n=27)

(1) Wilcken B. The consequences of extended newborn screening programmes: Do we know who needs treatment? *Journal of Inherited Metabolic Disease* 2008;(2):173-177.

(2) Yeh E, Theriault M, Higgins L, Chakraborty P, Bulman DE. Severe combined immunodeficiency (SCID) screening in Ontario - Methodology and workflow used by Newborn Screening Ontario (NSO). *Clinical Biochemistry* 2014;47(15):142.

(3) Turgeon C, Magera MJ, Allard P, Tortorelli S, Gavrilov D, Rinaldo P, et al. Effective and affordable 1st tier newborn screening (NBS) for tyrosinemia type I (TYR-I). *Molecular Genetics and Metabolism* 2008;93(3):256-257.

(4) Tanyalcin T, Kopish G, Tanyalcin I, Baker M, Hoffman G, Laessig R, et al. The results of biochemical and genetical approach to exclusive galactosemia cases in Turkey through selective screening. *Journal of Inherited Metabolic Disease* 2010;33:S64.

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Appendix T.C: Results for Metabolic Conditions

Table T.C.1: Quality assessment results for the main included systematic reviews/HTAs for metabolic conditions, using the AMSTAR tool

Criterion	Burton et al.[13]	Pandor et al.[12]	Seymour et al.[10]	Makni et al.[8]	Cote et al.[14]
Was a priori design provided?	Yes	Yes	Cannot answer	Cannot answer	No
Was there duplicate study selection and data extraction?	Cannot answer	No	Yes	No	No
Was a comprehensive literature search performed?	Yes	Yes	Yes	Yes	Cannot answer
Was the status of publication (e.g. grey literature) used as an inclusion criteria?	Yes	Yes	Yes	Yes	Cannot answer
Was a list of studies (included and excluded) provided?	No	Yes	No	No	Yes
Were the characteristics of the included studies provided?	No	Yes	Not applicable	Yes	Yes
Was the scientific quality of included studies assessed and documented?	Cannot answer	Yes	Yes	Yes	Yes
Was the scientific quality of the included studies used appropriately in formulating conclusions?	Yes	Yes	Yes	Yes	No
Were the methods used to combine the findings of the studies appropriate?	Not applicable	Not applicable	Not applicable	Cannot answer	Not applicable
Was the likelihood of publication bias assessed?	No	No	No	Cannot answer	No
Was the conflict of interest included?	Yes	Cannot answer	No	No	Yes
Total score (out of 11)	**5**	**7**	**5**	**5**	**4**
Rating (high/medium/low)	**Medium**	**Medium**	**Medium**	**Medium**	**Low**

Table T.C.2: Quality assessment results for metabolic studies reporting on diagnostic accuracy parameters, using the QUADAS-2 tool

Study	*Risk of bias*				*Overall risk of bias*	*Applicability*			*Overall applicability concerns*
	Patient selection	Index test	Reference standard	Flow and timing		Patients selection	Index test	Reference standard	
Sander et al.[19]	☺	☹	?	?	High	☺	☺	?	Unclear
Tu et al.[15]	?	☺	?	☹	High	☹	☺	?	Low
Matern et al.[27]	☺	?	?	?	High	☺	?	?	Unclear
Niu et al.[28]	?	☺	?	☹	High	☺	☺	☺	Low
Morrissey et al.[20]	☺	?	?	☹	High	☺	?	?	Unclear
Magera et al.[29]	☹	?	?	?	High	☺	?	?	Unclear
Lim et al.[17]	☺	☺	?	?	Unclear	☺	☺	☺	Low
Badawi et al.[35]	?	?	?	☹	High	?	?	?	Unclear
Jensen et al.[31]	☹	?	?	☹	High	☹	?	?	Unclear
Item et al.[32]	☺	☺	?	?	Unclear	☺	☺	?	Unclear
Kwon et al.[18]	?	?	?	?	Unclear	?	?	?	Unclear
Camelo et al.[33]	?	☺	?	☹	High	?	?	?	Unclear
Rhode et al.[36]	☹	?	?	?	Unclear	☺	☺	☺	Low
Freer et al.[34]	☺	☺	?	☹	High	☺	☺	☺	Low
Lund et al.[30]	☺	☺	?	?	Unclear	☺	☺	☺	Low

Table T.C.3: Quality assessment results for metabolic studies reporting on effectiveness, using the EPHPP tool

Study	Selection bias	Study design	Confounders	Blinding	Data collection	Withdrawals and drop-outs	Final rating
Larochelle et al.[24]	Strong	Moderate	Weak	Moderate	Weak	Strong	Weak
Masurel-Paulet et al.[25]	Weak	Weak	Weak	Moderate	Weak	Strong	Weak
Myoranda et al.[26]	Weak	Weak	Weak	Moderate	Weak	Weak	Weak
Hughes et al.[37]	Moderate	Weak	Weak	Moderate	Weak	Weak	Weak
Waisbren et al.[38]	Weak	Weak	Weak	Moderate	Weak	Not applicable	Weak
Karadag et al.[16]	Moderate	Weak	Weak	Moderate	Strong	Not applicable	Weak
Mulvihill et al.[40]	Weak	Moderate	Weak	Moderate	Weak	Not applicable	Weak
Yap et al.[41]	Weak	Weak	Weak	Moderate	Weak	Not applicable	Weak

Table T.C.4: Data reported in the included primary studies on the metabolic conditions

Study Author/year/country/ setting/design	Newborn population screened or treated/ period/condition	Time of sample collection/ platform/target/cut-off/ confirmatory test	Outcomes reported	Notes
Badawi et al.[35] 1996 Ireland Newborn screening program Retrospective observational study	n=1,200,000 *Age at diagnosis*: 6.9 days, and 2.5 days for the high risk group 32 patients followed up for complications, age range 2 weeks to 20 years *Period:* 1972-1992 *Condition:* GALT	*Time of sample collection:* NR *Platform/target/cut-off:* E coli bacterial inhibition assay BIA of Beutler test *Confirmatory test:* NR	*Diagnostic accuracy:* FN: 5 FNR: 11% *Treatment (diet) outcomes:* Out of 32 patients for whom data was available, 13/32 (41%) had no complications. The remaining 19/32 (59%) had 1 or 2 of the following complications: cataract, speech problems, tremors, delayed mental development or infections. 3 patients had persistent cataracts, and 8 developed lens opacity that regressed completely with diet. *Mortality:* 9/62 (15%) died (unlike diet outcomes data, mortality data was available for the 62 patients)	The authors also reports incidence of 1:23,000 A total of 55 cases of classic galactosemia, and 7 Duarte variants were detected The treatment (diet) outcomes results did not break down the Duarte variant and the classic cases
Camelo et al.[33] 2009 Brazil Academic Retrospective observational study	n=59,953 *Period:* NR *Condition:* GALT	*Time of sample collection:* NR *Platform:* colourimetric assay using automatic DIAS plate reader *Target:* T galactose *Cut-off:* either 2 readings >7 mg/dl or 1 reading >20 mg/dl *Confirmatory test:* Repeat the screening test galactose-1-phosphate uridylyltransferase enzyme activity using fluorometric method on platforms: Hitachi F-2000 fluorometer and Hitachi	*Diagnostic accuracy:* Comparing 1st to 2nd sample Sn: 100% Sp: 99.73% PPV: 1.9% NPV: 100% FPR: 158/59,950=0.26%	The authors report incidence of 1:19,984 (95% CI 1:7,494, 1:59,953) Acceptability: the authors report that all parents gave consent

		F-2001 spectrophotometer Cut-off: for GALT activity test < 37 µmol/hr/1gm Hb Normal range for GALT activity reported 37-66 µmol/hr/1gm Hb Genetic mutation testing		
Freer et al.[34] 2010 USA, Philadelphia Newborn screening program Retrospective observational study	n=1,320,000 for the 1st phase (2001-2006) n=274,960 for the 2nd phase (2007 and 2008) *Period:* 2001-2008, outcomes traced from January 2001-March 2006 *Condition:* GALT	*Time of sample collection:* NR *Platform:* Astoria Pacific Automated SPOTCHCK continuous flow assay system for T galactose measurement Astoria Pacific Automated SPOTCHCK continuous flow system "modified", for GALT activity measurement *Target:* T galactose, GALT *Cut-off:* T galactose >1.1 mmol/L (>20 mg/dl), changed later to >1.66 mmol/L (>30mg/dl) with the range of 1.1-0.8 considered inconclusive and requires repeating the test GALT activity ≤40 µmol/L/L <32-41 µmol/L was considered typical for GALT 41-120 µmol/L suggesting clinically benign mutations 150-500 µmol/L normal *Confirmatory test:* DNA genetic mutation testing and clinical confirmation	*Diagnostic accuracy:* At GALT cut-off <40 µmol/L Sn: 100% PPV: 83% Using the algorithm illustrated by the authors among 209 infants who had T galactose >1.1 mmol/L and/or GALT≤40 µmol/L the authors were able to detect 10 cases of suspected GALK deficiency, 20 cases of suspected GALE deficiency, 11 homozygotes of GALT cases and 3 compound heterozygotes	The screening involved 2 tests (T galactose and GALT activity) A newborn was considered having the condition if T galactose and/or GALT were positive The authors also described their technique to detect GALE and GALK deficiency: 1) T galactose is measured 2) free galactose is measured the same way without adding alkaline phosphatase 3) gal-1-p is calculated based on this equation *Gal-1-p= T gal – free gal* 4) gal-1-p/T gal ratio is calculated 5) if this ratio is <25%, GALK is suspected, and if the ratio is >25%, GALE is suspected The authors reported that changing the cut-off, dramatically reduced the referral rates from 41 in 2005 to 23 and 24 in the following 2 years
Hughes et al.[37] 2009 Ireland Academic	14 families (30 patients) Cohort A: older siblings diagnosed clinically and treated within a week of birth Cohort B: younger siblings	NR	*Treatment outcomes:* (early (since birth) versus late (within a week) treatment) The two cohorts collectively had high complications rate	Treatment is restricted galactose intake > or <20 mg/day

Case series	diagnosed and treated since birth as part of the guidelines being travellers or high risk population *Age:* range 6-26 months *Period:* NR *Condition:* GALT		77% language delay 71% low IQ In most cases cerebral white matter degeneration was evident in MRI There was no statistically significant difference between the two groups in language and speech complications 9/13 vs. 11/13, IQ 9/13 vs. 11/15, neurological exam 3/14 vs. 4/16, brain MRI 5/5 vs. 5/7, and gal-1-p levels in the blood N.B: different outcomes data were not available for all patients on all outcomes, and therefore reported denominators are different	
Item et al.[32] 2002 Austria National newborn metabolic screening program Retrospective observational study	n=43,688 *Period:* over 5 months *Condition:* GALT	*Time of sample collection:* 3-5 days *Platform:* NR *Target:* T galactose *Cut-off:* >12 mg/dl was considered borderline that requires repetition (the mainly used cut-off) >20 mg/dl urgently contacted, requires repetition and GALT activity measurement *Confirmatory test:* Repeat T galactose test and genetic mutation	*Diagnostic accuracy:* PPV (calculated as TP/(TP+FP)): 4/110=3.6%	4 true positive cases were detected, 1 was classic galactosemia, and 3 were compound heterozygotes Duarte/galactosemia The authors conclude that their DGGE/CFLPA method of detecting gene mutations, have significantly detected more carrier than routine biochemical follow-up
Jensen et al.[31] 2001 Denmark Laboratory Case-control of archived samples	n=12 patients and 2,055 random controls *Age at screening:* samples were collected 4-8 days after birth *Period:* NR *Condition:* GALT	*Time of sample collection*: 4-8 days after birth *Platform:* Negative ion mode electrospray MS/MS API, PE-Sciex) with an electrospray source *Target:* total hexose monophosphate as a marker	*Diagnostic accuracy:* Sn: 100% Sp: 100%	The authors reports receiving grant from MDS Sciex, the manufacturer, and academia

		for gal-1-p with setting biased to gal-1-p detection *Cut-off:* 1.2 mmol/L *Confirmatory test:* Alkaline phosphatase-galactose dehydrogenase method used by the Swedish NBS program PCR amplified DNA for galactose-1-phosphate uridylyltransferase enzyme mutations		
Karadag et al.[16] 2013 Turkey Case series	22 neonates with GALT from 10,099 admitted to the NICU *Age:* Median 13 days, range 3-23 days *Gender:* 18 (81%) males and 4 (19%) females *Consanguinity*: 13 (59%) *Family history:* 1 (4%) *Pre-term:* 4 (18%) *Median gestational age for the whole cohort:* 38 weeks, range (31-42) weeks *Presenting symptoms:* Hepatomegaly 22 (100%) Jaundice 19 (86%) Vomiting 17 (77%) Cataract 15 (68%) Feeding difficulty 13 (59%) Poor weight gain 5 (22%) E coli sepsis 10 (45%) Long QT interval 1 (4%) Hemophagocytosis 1 (4%) *Period:* January 2005-January 2011 *Conditions:* GALT	NR Clinical symptoms were confirmed with a low GALT activity in 22 (100%) of the patients	*Treatment outcomes:* The 22 patients followed a lactose free diet Improved live functions in 22 (100%) Resolved signs of cataract in 13/17 (77%) Learning disabilities 5 (22%) Operated for cataract 4 (18%) Speech deficits 4 (18%) Developmental delays 3(13%) Psychomotor retardation 1 (4%) Cataract completely resolved in 11 (73%) of the patients who started the galactose restricted diet in the first month Among 18 patients diagnosed before 17 days, 13/18 developed cataract. While among the 4 diagnosed after 17 days, 4/4 (all) developed cataract Patients diagnosed before 17 days did not require cataract surgery compared to those diagnosed later, p=0.01	The authors declare no private funding and no conflict of interests The authors conclude that early diagnosis and treatment of galactosemia with a galactose-restricted diet could partially prevent complications, nut not all of them Cataracts can develop early in the first few days of life, early diagnosis seems important in the prevention of severe cataracts

Kwon et al.[18] 2000 USA, Wisconsin Council of Regional Networks for Genetic Services (CORN) Retrospective observational study	n=9,221 positive cases, and 53 confirmed in 1993 n=10,210 positive cases, and 54 confirmed in 1994 The total number of screened newborns was not reported *Period:* 1993 and 1994 *Condition:* GALT	*Time of sample collection*: NR *Platform:* NR *Target:* NR *Cut-off:* NR *Confirmatory test:* NR	*Diagnostic accuracy:* 1993: Sn: 100% Sp: 99.7% PPV: 0.57% 1994: Sn: 100% Sp: 99.7% PPV: 0.53%	The CORN collects information from all USA regions on NBS results, at the time, 48 states were screening for classic galactosemia The authors report an incidence of 1:54,900 (95% CI 1:44,200, 1:72,400) for 1993 and 1:62,800 (95% CI 1:55,500, 1:72,400) in 1994 Source of funding was governmental (NIH)
Larochelle et al.[24] 2012 Canada, Quebec Academic Retrospective observational study Retrospective chart review for events before 1994 and prospective data collection thereafter	n=78 24 patients treated before 1 month 26 patients treated after 1 month 28 patients never treated *Period:* Patients born with TYRI between 1984 and 2004 *Condition:* TYRI	*Time of sample collection*: NR *Platform:* NR *Target:* SA in blood or urine *Cut-off:* NR	*Treatment outcomes:* *Early versus late versus no treatment* The pre NTBC group spent 7% of their lives in the hospital, including 3.7% in an active neurologic crisis. Conversely, none of the patients on treatment had acute decompensation Among non-treated patients, 8/28 (29%) died before transplant versus no patients in the treated group, p <0.01 Liver transplantation in was performed in 20/28 (71%) of the never treated group, versus 7 patients in those treated late, and 0 in the early treated group, p <0.01 for both respectively The study also reports improvement in some liver function tests (ALT, INR, and AFP) in all patients after 4 months of treatment	NTBC was made available in Quebec since 1994 Patients were followed until transplantation, death, or end of study SA was also used to monitor the treatment The time of follow-up was categorized into months without NTBC or pre NTBC and months on NTBC Safety of the treatment: 1 patient had ocular crystals after 8 months of treatment 1 patients had a hypoglycemic episode 12 patients had episodes of increased ALT >60 IU/L

Lim et al.[17] 2014 Singapore National newborn screening program Governmental/ Academic Retrospective observational study	n=177,267 *Period:* July 2006-April 2014 *Condition:* TYRI and HCY, however no cases of the two conditions were detected	*Time of sample collection*: 48 hours after birth; for pre-term infants, samples are also collected at 2 and 4 weeks of age *Platform:* One ESI MS/MS API 2000, PerkinElmer, Ohio, USA; Two ESI MS/MS API 3200, Applied Biosystems, USA *Targets:* tyrosine for TYRI; methionine for HCY *Cut-offs:* Tyrosine >150 µmol/L, changed to >300 µmol/L to improve specificity Methionine >55 µmol/L *Confirmatory test:* Amino acids in plasma and urine, and DNA testing (no more details reported)	*Diagnostic accuracy:* reported for the overall performance of the program, not for individual conditions Sn: 95.6 Sp: 99.8% PPV: 20% including maternal conditions and 18% without it FPR: 15% *Acceptability:* The program is voluntary not mandated by a policy. The rate of uptake increased significantly over time with an uptake of 71% at the time of publication *Recall rate:* Was significantly decreased from 1.5% to 0.12% due to the switching of the absolute cut-off from 150 to 300 µmol/L	The authors reports that results of the testing were available within 1.64 days and 3.8 days if the 2nd-tier test is required The authors also indicated that measuring tyrosine is of low specificity in detecting TYRI No cases of TYRI or HCY were detected to the date of publication Cut-offs of different targets were set at the 99th percentile of the initial reference range during the pilot phase of the project. This has led to a high recall rate as it prioritized sensitivity over specificity. Large proportion of the recalled samples were due to elevated tyrosine levels The authors reports on 2nd-tier testing using SA >5 µmol/L and tyr/phe ratio >2 for TYRI and; met/phe ratio >1, met/cit >4 µmol/L, and met/tyr >0.7 for HCY The authors report TYRII and 3 as secondary targets for TYRI and hypermethioninemia for HCY Genetic tests were sent out of the country because of lack of local resources (typically takes 4-6 weeks) Sources of funding of the study are the Singapore MOH and universities. The authors declared no conflict of interest
Lund et al.[30] 2012	n=504,049 for both the pilot and routine programs n=84,054 screened for	*Time of sample collection:* 4-9 days during the pilot phase 2-3 days upon implementing	*Diagnostic accuracy:* For the whole program: FPR: 0.038	The pilot phase extended from 2002 to 2009 in included multiple metabolic conditions, followed by the routine

Denmark Pilot and routine newborn screening program Retrospective observational study	galactosemia from 2001 to 2004 n=140,565 screened for TYRI between February 2009 to the end of the study 2011 *Median age at screening:* 5 days for the pilot phase, and 2.5 days for the routine program *Period:* 2002-2011 *Condition:* GALT, TYRI, and other IEM	the routine program *Platform:* MS/MS SciEx API 365 or SciEx API 2000 was used for GALT; Non Derivatized MS/MS PerkinElmer was used for TYRI *Target:* SA for TYRI; gal-1-p for GALT *Cut-off:* SA >2.1 U gal-1-p >1.2 mM^3 *Confirmatory test:* For GALT: GALT activity, DNA genetic mutation testing For TYRI: urine organic acids, plasma amino acids and DNA testing	PPV: 37% Sp: 99.99% Sn: 92% For GALT: (between 2001 and 2004) Sn:100% calculated Sp: 99.98% calculated PPV: 5% FPR: 0.0226 Using gal-1-p, the authors were able to detect 1 TP case and 19 FP. The later were a mix of Duarte and Duarte plus GALT compound heterozygotes For TYRI: (introduced in February 2009) FPR: 0 PPV: 100% Both TYRI and GALT screening yielded 1 TP case with each condition, however, the GALT patient was already symptomatic at the time of screening. On the other hand, the TYRI patient was clinically free Screening for GALT has stopped due to failure to detect the patient before the onset of symptoms *Acceptability:* 586,969 newborn family were offered screening. Among them 504,049 (85%) agreed and signed the consent form The authors also report that parents' acceptability increased dramatically over the years from 65% at the beginnings of the pilot phase to almost 100% at the	program Screening was part of a whole continuum of care program. Infants with positive results were followed up in the center for inherited metabolic disorders in Copenhagen Results were made available during 2-7 days in the pilot phase and 2-3 days when the routine program was implemented All pre-term infants had the test repeated at gestational age 32 weeks or when oral feeding was established Shifting from the pilot to the routine program, the following factors were considered for changing the panel (disease spectrum and frequency, availability of efficient treatment, reliable confirmatory tests, prevention of early death and absence of clinical signs at birth Cut-offs were initially set according to literature, then adjusted over time to optimize the test performance and improve discriminatory power The authors lists two challenges in optimizing the cut-off: 1) hyperalimentation, and 2) variations in the amount of blood in the filter paper They also report that the FPR of 0.0226 for GALT screening could have been reduced to 0.003 if gal-1-p was 2mM^3 instead of 1.2 mM^3

			introduction of the routine program. Failure to inform the parents about the pilot study was the main drive for the modest rate at the beginnings.	
Magera et al.[29] 2006 USA, Minnesota Lab quantitative determination of SA for TYRI in a newborn screening program Retrospective observational study	n=124,780 *Period*: 15 months *Condition:* TYRI	*Time of sample collection:* NR *Platform:* LC MS/MS a Triple Quadrupole mass spectrometer, API 3000, applied Biosystems, MDS Sciex, Toronto, Ontario, Canada *Target:* tyrosine *Cut-off:* 150 µmol/L *Confirmatory test:* SA following elevated tyrosine levels, with a cut-off of 3 µmol/L	The authors report 2,229, 1,177, and 430 suspected cases at tyrosine cut-offs 130, 150, and 180 µmol/L, respectively Using SA as a 2nd-tier test no TP cases were discovered	The authors reports on some correlation coefficients based on confirmed cases data The authors also report that there are no false negative results discovered clinically to the date of publication
Masurel-Paulet et al.[25] 2008 France Governmental Case series	n= 46 *Patients' age at the start of the NTBC treatment:* <6 months 31 (67%) 6-24 months 10 (22%) >24 months 5 (11%) *Period:* NR *Condition:* TYRI	NR	*Long-term outcomes of TYRI patients treated with NTBC* With NTBC treatment, 31 patients showed normalized AFP levels versus 15 patients had persistently elevated levels, no p values reported No statistically significant difference was found for age at the onset of the treatment, NTBC dosage, or compliance between the normalized and persistently elevated groups Of 42 long-term treated patients, 7/42 (17%) didn't take the drug regularly because of poor compliance, on the other hand, 15/42 (36%) patients had poor compliance with dietary treatment	Mean duration of treatment follow-up was 4 years and 9 months, range (3 months to 12 years and 9 months) 42 patients diagnosed clinically 4 diagnosed via screening (3 patients were screened due to family history, 1 patient diagnosed via screening for PKU) All patients were given low protein diet with supplementation of special amino acids mixtures, without phenylalanine or tyrosine Baseline data of the 3 groups of some live enzymes appeared to be comparable; however, there was no statistical significance parameters reported.

Matern et al.[27] 2007 USA, Minnesota Newborn screening program and Academic Retrospective observational study	n=6,479 for TYRI n=516 for HCY *Period:* 2004-2007 *Condition:* TYRI, HCY, and others	*Time of sample collection:* NR *Platform:* MS/MS *Target:* tyrosine for TYRI and methionine for HCY *Cut-off:* 150 µmol/L for tyrosine, 60 µmol/L for methionine 2nd tier test: *Platform:* MS/MS *Target:* SA for TYRI and homocysteine for HCY *Cut-off:* SA >5 µmol/L and homocysteine >15 µmol/L	*Diagnostic accuracy:* PPV for HCY using methionine is 1/1= 100% Sn for HCY using methionine is 1/1=100% No cases of TYRI were discovered, therefore calculating the diagnostic accuracy for TYRI was not possible	The main objective of this study is to evaluate the effect of adding a 2nd tier test on FPR
Morrissey et al.[20] 2011 USA, New York State newborn screening program Retrospective observational study	n=500,000 *Period:* December 2007-December 2009 *Condition:* TYRI	*Time of sample collection*: NR *Platform:* 2 Micro LC MS/MS spectrometers, and HPLC, Waters Corp., Manchester, UK To accommodate the big number of samples, the authors added TQD MS/MS, and Acquity UPLC system, Waters Corp., Manchester, UK *Target:* SA *Cut-off:* 5 µmol/L, (3-5 µmol/L) was considered borderline result that requires test repeat *Confirmatory test:* SA and amino acids in plasma and urine; and liver function tests	*Diagnostic accuracy:* FP: 3 TP: 2 PPV: 2/5=40%	According to the authors, SA is pathognomonic to TYRI. The cut-off of 5, reported in this study is considered very low, also lower than the lowest value proposed by Allard et al. The authors report processing 1,000 samples/day
Myorandan et al.[26] 2014 13 European countries including 21 centers Orphanet collaboration Cross-sectional Surveys were used to collect data on general information as principles of the treatment in the	n=168 *Age at diagnosis for the whole group:* mean 12.9± SD 23.8 months *Age at diagnosis for those diagnosed by NBS:* mean 0.58± SD 0.73 months *Age at diagnosis for those diagnosed via selective screening:* mean 15.5± SD 24.9 months	*Time of sample collection:* NR *Platform:* NR *Target:* 132 patients diagnosed by selective screening using the following targets: 30 SA 2 tyrosine 82 SA + tyrosine 18 unknown 28 patients diagnosed via NBS: 12 tyrosine	114/168 (68%) had symptoms at diagnosis, mostly liver failure or liver dysfunction. Among them, 74 patients had combined liver and kidney dysfunction Age at diagnosis was significantly associated with symptoms. Renal dysfunction was more frequent in 2-6 and >6 months group compared to those diagnosed <2 months,	Patients were followed up for 9.1±6.3 years All patients tolerated NTBC without serious side effects. Non serious side effects reported were: thrombocytopenia 8 (5%) eye pain 10 (6.3%) eye itching 9 (5.6%) keratitis and conjunctivitis (did not exceed 3%) Without details, the authors

different centers, and individual patients data	*Consanguinity*: 49/168 (29%) *Affected family members*: 56/168 (35%) *Gender*: 100 males, 68 females *Period:* NR *Condition:* TYRI	4 SA 4 SA + tyrosine 8 unknown 3 patients were diagnosed prenatally 5 patients have no data on their diagnostic procedure *Cut-off:* NR	p=0.007 and p=0.013, respectively The combination of symptoms was also more frequent in the 2-6 months group and the >6months group compared to those <2 months, p<0.001 for both comparisons respectively HCC was more frequent in those diagnosed at >6 months compared to <6 months group, p=0.47 Genotyping was performed in 58 patients. There was no significant correlation between the genotype and the phenotype (clinical symptoms) In 148 evaluable patients, for whom both treatment and outcomes data were available, early diagnosis and treatment (in neonatal period) <1 month, showed lower rate of complications, especially for HCC, and requirement for live transplantation compared to those diagnosed >1 month HCC Ref <1 month 1-6 months OR=2.5 (95% CI 0.2, 25.8) n=45 7-12 months OR=6.3 (95% CI 0.6, 65.6) n=20 >12 months OR=12.7 (95% CI 1.5, 103) n=46 Liver transplantation Ref <1 month 1-6 months OR=2.5 (95% CI 0.2, 25.8) 7-12 months OR=4 (95% CI 0.3, 47.1) >12 months OR=12.7 (95% CI 1.5, 103)	reported that, to their knowledge, there were no false negative results when SA, measured mostly in urine, was used for screening Monitoring NTBC treatment was performed using SA measurement in blood or urine This study was supported by an educational grant from Milupa Metabolics All authors declared no conflict of interest

			Renal dysfunction Ref <1 month 1-6 months OR=1.7 (95% CI 0.2, 9.8) 7-12 months OR=5.8 (95% CI 1, 33.4) >12 months OR=5.5 (95% CI 1.1, 26.6) N.B: wide 95% CI that either containing or touching 1	
Niu et al.[28] 2010 Taiwan National newborn metabolic screening program in 3 centers in Taiwan Retrospective observational study	n=1,495,132 for HCY n=592,717 for TYRI *Period*: March 2000-June 2009 *Condition*: TYRI, HCY and others (e.g. MSUD, MMA, MCAD)	*Time of sample collection:* after the first feeding (24-72 hours) *Platform:* Micromass Quatro micro API Mass Spectrometer 2000 and 3000; PerkinElmer MS/MS 1445 *Target:* methionine for HCY; tyrosine for TYRI *Cut-off:* >120 µmol/L for methionine; >500 µmol/L for tyrosine *Confirmatory test:* Repeat screening, plasma amino acids, and total homocysteine for HCY and urine SA for TYRI	*Diagnostic accuracy:* 11 cases of HCY were suspected, all of them were FP 13 cases of TYRI were expected, 11 among them were FP, and the remaining 2 were TYRII	The authors report 2nd sample collection after 1 month of birth for pre-terms Methionine values 60-120 µmol/L and tyrosine values 250-500 µmol/L were considered borderline At the start of the program, cut-offs were initially set at 4X the SD above the mean, then slightly modified overtime
Rhode et al.[36] 1998 Germany Laboratory Case-control	n= 2 cases and 96 controls comparing the test to Bohringer GALT test and 7 cases versus 686 controls comparing the test to Isolab GALT test *Period:* NR *Condition:* GALT	*Time of sample collection:* NR *Platform:* NR Optimized Beutler test for GALT fluorescence assay *Target:* GALT *Cut-off:* 10% of the mean of the GALT fluorescence assay obtained from all newborn samples of a plate *Confirmatory test*: GALT activity using Beutler test from Bohringer and Isolab	*Diagnostic accuracy:* Sn: 100% Sp: 100% Versus both Bohringer and Isolab tests	The authors note that variation in the results is expected among different seasons, because temperature causes denaturation of the enzyme protein

Sander et al.[19] 2006 Germany Multicenter newborn metabolic screening program Retrospective observational study	n=61,344 + 2 confirmed hepatorenal tyrosinemia children *Ethnicity:* The 4 tyrosinemia patients were of Albanian, Turkish, and Kurdish descent *Prematurity:* 1 newborn diagnosed by screening was born 2 weeks earlier *Period:* NR *Condition:* TYRI	*Time of sample collection:* 36-72 hours according to German regulations *Platform:* 2 MS/MS were used in the study MS/MS micro™, and Quatro LC™ Waters/ Micromass Inc. *Target:* SA *Cut-off:* 10 µmol/L	*Diagnostic accuracy:* The authors reports that there were no FP or FN results discovered clinically. Translating the diagnostic accuracy parameters into 100% Sn and Sp, respectively. *Treatment receipt and outcomes:* The clinically diagnosed patients started their NTBC treatment 2 and 4 months respectively. Both patients showed steady and prompt recovery of the liver functions The two patients that were diagnosed via screening started their NTBC treatment at 7 and 17 days after birth respectively. One of them did not have any biochemical abnormality. 9 weeks of follow-up reveled normal development of the patients. The second patient showed normalization of his coagulation tests within 5 days	The study presents a newly described method using SA quantification in routine metabolic screening The authors report that they were able to process 1,000 thousand samples/day, reflecting high throughput capacity of the platforms used, with very minimal additional manual work requirement Besides the 2 MS/MS platforms that were used, the authors reported the further 2 were available as back up and for other scientific purposes Source of funding and conflict of interests were not reported
Tu et al.[15] 2012 China Newborn screening in neonatal ICU Retrospective observational study	n=724 *Period:* NR *Condition:* TYRI and others (e.g. MMA, PA, MCADD, and MSUD)	*Time of sample collection:* 1-3 days *Platform:* LC MS/MS, API 3000 MS/MS, Applied Biosystems *Target:* tyrosine *Cut-off:* 250 µmol/L *Confirmatory test*: -Platform: GC MS/MS -Target: SA -Cut-off: 6 µmol/L	*Diagnostic accuracy:* PPV: 8/22=36% (calculated) for the overall performance of the program (TYRI and the other conditions)	Cases were confirmed by clinical symptoms, gas chromatography MS/MS, and genetic testing The study also reports an incidence of 1.1% for all IEM in the neonatal ICU setting The authors also report no FN results without further details Source of funding: academic The authors declared that there was no conflict of interest

Waisbern et al.[38] 2002 USA, New England Cross-sectional	28 IEM patients diagnosed via screening and 17 diagnosed clinically *Age at screening*: <2 weeks *Age at diagnosis:* 0-14 days for the screened group *Median:* 2 weeks, range up to 6 years for those diagnosed clinically P<0.001 *Period:* NR *Conditions:* GALT, HCY, and others (MSUD and biotinidase) The NBS group had 1 HCY and 17 galactosemia patients The clinically diagnosed group had 2 HCY and 9 galactosemia patients	NR	*Developmental and parental stress outcomes:* Patients diagnosed clinically showed higher incidence of mental retardation, their parents experienced greater stress and found greater difficulty meeting their child's needs Mental retardation: 47% in clinically diagnosed group 14% NBS p=0.03 Parents having difficulty meeting their children needs: 29% in clinically diagnosed group 7% NBS p=0.09 Hospitalization rate: Albeit higher in the clinically diagnosed group, there was no statistically significant difference 77% in clinically diagnosed group 64% in NBS p=0.51 >20% in each group required more than 3 times hospitalization, p=0.7	Parent questionnaires were used to compare the hospital admission rates and parental stress The study originally had 263 patients (139 diagnosed by screening, 124 diagnosed clinically) NBS group showed better outcome results
Mulvihill et al.[40] 2001 Ireland Retrospective cohort	Late diagnosed patients (after 6 weeks) n=14 (vitamin B6-responsive 4, and vitamin B6-nonresponsive 10) *Median age:* 24 years Early diagnosed by NBS n=21 all were vitamin B6-nonresponsive Early diagnosed patients were	No details on the screened group reported	*Early treatment outcomes:* All 14 late diagnosed patients had either lens sublaxation or dislocation at diagnosis Refractive errors and visual acuity in late diagnosed patients was significantly worse than the poorly controlled group, p=0.03 and	

	further classified to poor control and well controlled in based on monitoring and drug compliance Poor control: n=6 median age 21 years Well controlled: n=15 median age 18 years		the well-controlled group, p=0.0002. The poorly controlled group also had worse visual acuity and refractive errors than the well-controlled group, p< 0.01	
Yap et al.[41] 1998 Ireland Case-control	n=23 NBS=19, among them 13 were compliant (mean age 14.4 years), and 6 were poorly compliant (mean age 19.9 years) Non treated n=2 Treated late n=2 Control group unaffected siblings n=10 (mean age 19.4 years)	No details reported for the screened group	*Early treatment outcomes:* The groups were compared in terms of full scale IQ FIQ The compliant NBS group had FIQ of 105.8 The poorly compliant NBS group had FIQ of 80.8 The control group had FIQ of 102 The 2 late detected patients had FIQ of 80 and 102, respectively The 2 untreated patients had FIQ of 52 and 53, respectively	Treatment compliance was defined as life time plasma homocysteine <11µmol/L

AFP: alpha-fetoprotein; ALT: alanine transferase; CFLPA: cleavage fragment length polymorphism analysis; CI: confidence interval; DGGE: denaturing gradient gel electrophoresis; FIQ: full scale intellectual quotient; FN: false negative; FNR: false negative rate; FPR: false positive rate; gal-1-p: galactose-1-phosphate; GALE: galactose epimerase; GALK: galactokinase; GALT: classic galactosemia; HCC: hepatocellular carcinoma; HCY: homocystinuria; IEM: inborn errors of metabolism; INR: international normalized ratio; IQ: intellectual quotient; MCAAD: medium chain acyl coA dehydrogenase deficiency; MMA: methylmalonic academia; MS/MS: tandem mass spectrometry or spectrometer; MSUD: maple syrup urine disease; NBS: newborn screening; NPV: negative predictive value; NR: not reported; NTBC: (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione); PA: propionic academia; PCR: polymerase chain reaction; PKU: phenylketonuria; PPV: positive predictive value; OR: odds ratio; SA: succinylacetone; Sn: sensitivity; Sp: specificity; T galactose: total galactose; TP: true positive; TYR: tyrosinemia

Appendix T.D: Results for SCD Studies

Table T.D.1: Characteristics of the included systematic reviews/HTAs for SCD

Study Author/year/objective	Search strategy	Study selection	Quality assessment/Data extraction/ Data analysis and synthesis
Blancquaert[50] 2010 To determine whether the information needed to evaluate the "appropriateness of including sickle cell anemia in the Quebec Newborn Screening Program" is available and of good quality.	**Databases:** PubMed, Embase, and Cochrane Library **Publication period:** PubMed 1996-April 2009; Embase 1996-2007 **Other sources:** 1. Grey lit: internet search (conducted in June 2009) for CPG/position papers, SRs and HTAs. 2. Review of reference list: yes (to trace the oldest key articles) 3. Contact experts: yes **Language limit:** English or French	**Inclusion criteria** (not specified in method section)**:** • Any type of study (SRs, experimental, cohort, longitudinal, and trend study) • Studies on screening test performance that compared two techniques • English and French-language publications **Exclusion criteria:** • Articles dealing exclusively with thalassemia or not relevant to hemoglobinopathies	**Quality assessment:** Tool(s) used: NR Quality assessment by two reviewers: NR **Data extraction:** Use of standardized form: NR Data extraction by two reviewers: no (by single researcher) **Data analysis and synthesis** Qualitative systematic review, but the results from existing meta-analysis are discussed
Davies et al.[48] 2000 To review the literature on hemoglobinopathy (thalassemia and SCD) screening.	**Databases:** Medline **Publication period:** 1985-1996 **Other sources:** 1. Hand search: Davies' publications over 20 years 2. Review of reference list: yes 3. Contact experts: personal contacts with experts in the field in England and North America. **Language limit:** English or French	**Inclusion criteria:** • Published between 1985 and 1996 • Articles in English or French • Peer-reviewed journals **Exclusion criteria:** NR	**Quality assessment:** Tool(s) used: NR Quality assessment by two reviewers: NR **Data extraction:** Use of standardized form: NR Data extraction by two reviewers: NR **Data analysis and synthesis** Qualitative systematic review

Lees[49] 2000 Cochrane review To assess whether there is evidence that neonatal screening for sickle cell disease rather than symptomatic diagnosis reduces adverse short- and long-term outcomes for those in whom the disease is detected, without adverse outcomes in the population screened.	**Databases:** the Cochrane Cystic Fibrosis and Genetic Group Haemoglobinopathies Trials Register (Mainly Cochrane Central Register of Controlled Trials and Medline) **Publication period:** NR (last search date 9 April 2010) **Other sources:** 1. Review of reference list: yes 2. Conference proceedings **Language limit:** NR	**Inclusion criteria:** • Study design: RCT or quasi-RCT • Population: all children screened for SCD and all children diagnosed with SCD • Intervention: all neonatal screening programs enabling early pre-symptomatic diagnosis of SCD. Comparator: placebo, no treatment, or a comparator drug • Outcome: mortality, morbidity, all adverse effects resulting from diagnosis or delayed diagnosis of SCD **Exclusion criteria:** • Studies with historical or concurrent controls • Studies that reported the performance of diagnostic tests	**Quality assessment:** Tool(s) used: not applicable Quality assessment by two reviewers: not applicable **Data extraction:** Use of standardized form: not applicable Data extraction by two reviewers: not applicable **Data analysis and synthesis:** not applicable
Hirst & Owusu-Ofori[51] 2012 Cochrane review To assess the effects of prophylactic antibiotic regimens for preventing pneumococcal infection in children with sickle cell disease.	**Databases:** the Cochrane Cystic Fibrosis and Genetic Group Haemoglobinopathies Trials Register (Mainly Cochrane Central Register of Controlled Trials and Medline) **Publication period:** NR (last search date 28 March 2012) **Other sources:** 1. Review of reference list: yes 2. conference proceedings **Language limit:** NR	**Inclusion criteria:** • Study design: RCT or quasi-RCT, cluster RCT • Population: children under 16 years old with SS, S/ß thal, SC • Intervention: prophylactic antibiotics • Comparator: placebo, no treatment, or a comparator drug • Outcome: prevention of pneumococcal infection **Exclusion criteria:** NR	**Quality assessment:** Tool(s) used: risk of bias tool Quality assessment by two reviewers: yes **Data extraction:** Use of standardized form: yes Data extraction by two reviewers: yes **Data analysis and synthesis:** Qualitative systematic review

CPG: clinical practice guidelines; HTA: health technology assessment; NR: not reported; RCT: randomized control trial; SCD: sickle cell disease; SR: systematic review

Table T.D.2: Quality assessment results for the included systematic review/HTA for SCD, using the AMSTAR tool

AMSTAR questions	QA rating			
	Blancquaert 2010[50]	Davies et al. 2000[48]	Lees et al. 2000[49]	Hirst et al. 2012[51]
1. Was a priori design provided?	No	Yes	Yes	Yes
2. Was there duplicate study selection and data extraction?	No	Cannot answer	Not applicable	Yes
3. Was a comprehensive literature search performed?	Yes	No	Yes	Yes
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion)?	Yes	Cannot answer	Yes	No
5. Was a list of studies (included and excluded) provided?	No	No	Not applicable	Yes
6. Were the characteristics of the included studies provided?	No	No	Not applicable	Yes
7. Was the scientific quality of included studies assessed and documented?	No	No	Not applicable	Yes
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	No	No	Not applicable	No
9. Were the methods used to combine the findings of the studies appropriate?	Yes	Cannot answer	Not applicable	Yes
10. Was the likelihood of publication bias assessed?	Cannot answer	No	Not applicable	No
11. Was the conflict of interest included?	No	No	Not applicable	No
Total score (out of 11)	**3**	**1**	**3**	**7**
Rating (high/medium/low)	**Low**	**Low**	**Low**	**Medium**

Table T.D.3: Quality assessment results for SCD studies reporting on screening accuracy parameters, using the QUADAS-2 tool

Domain	Questions for risk of bias	Lobitz 2014[52]	Moat 2014[47]	McGann 2013[55]	Panigraphi 2012[56]	Boemer et al. 2011[46]
Patient selection	Was a consecutive or random sample of patients enrolled?	No	Yes	No	Yes	Unclear
	Was a case-control design avoided?	Yes	Yes	Yes	Yes	Yes
	Did the study avoid inappropriate exclusions?	Yes	Yes	Unclear	Yes	Unclear
	Risk of bias	High	Low	High	Low	Unclear
Index test	Were the index test results interpreted without knowledge of the results of the reference standard?	Yes	Unclear	Yes	Yes	Yes
	If a threshold was used, was it pre-specified?	Unclear	Yes	Unclear	Unclear	Unclear
	Risk of bias	High	Unclear	Unclear	Unclear	Unclear
Reference standard	Is the reference standard likely to correctly classify the target condition?	No	Yes	No	Yes	Unclear
	Were the reference standard results interpreted without knowledge of the results of the index test?	No	Unclear	No	No	No
	Risk of bias	High	Unclear	High	High	Unclear
Patient flow and timing	Was there an appropriate interval between index test(s) and reference standard?	Unclear	Unclear	Unclear	Unclear	Unclear
	Did all patients receive a reference standard?	No	Yes	No	No	No
	Did all patients receive the same reference standard?	No	Yes	No	No	No
	Were all patients included in the analysis?	No	Yes	No	No	No
	Risk of bias	High	Unclear	High	High	High
Overall risk of bias		High	Unclear	High	High	High

Table T.D.4: Quality assessment results for SCD studies reporting on effectiveness, using the EPHPP tool

Study	Selection bias	Study design	Confounders	Blinding	Data collection	Withdrawals and drop-outs	Global rating
Lobo et al. 2014[53]	Weak	Weak	Weak	Weak	Weak	Strong	Weak
Saint-Martin et al. 2013[54]	Moderate	Moderate	Weak	Moderate	Weak	NA	Weak
McGann et al. 2013[55]	Moderate	Weak	Weak	Moderate	Weak	Strong	Weak
Panigraphi et al. 2012[56]	Moderate	Weak	Weak	Moderate	Weak	Strong	Weak
Quinn et al. 2010[57]	Moderate	Weak	Weak	Weak	Strong	Weak	Weak

Table T.D.5: Evidence on screening test performance for SCD screening

Study Author/year/country/ design/objective	Newborn population screened	Screening/ confirmatory tests	Outcomes	Authors' conclusions
Lobitz et al.[52] 2014 Germany Retrospective observational study This pilot study was conducted to determine for the first time the incidence of SCD in an unselected cohort of neonates born in Germany.	**No. of infants screened:** 34,084 (86.84% of all eligible newborns) **Ethnicity:** NR **Pre-term neonates:** NR **Period:** September 2011-November 2012	**Screening test:** Time of sample collection: 1.5-3 days Platform: HPLC (Bio-Rad Laboratories, Munich, Germany) Cut-off: not applicable **Confirmatory test**: CE - Sebia CAPILLARYS 2 CE system (Sebia, Fulda, Germany). **Primary target condition(s):** Hb SS, SC, SD-Punjab, SE, S/Lepore, SO-Arab, S/ß-thal, S/δß-thal, S/HPFH	**Incidence:** SCD 14/34,084 (4.11/10,000 or 1:2,435) **Harms:** NR **Accuracy of screening test:** all 14 SCD identified by HPLC were confirmed by CE FP: 0 FN: NR **Carrier:** 265 sickle cell carrier (0.77%), results not reported to the family	This study indicated a 95% probability that the incidence of SCD in Berlin is at least 2.5/10,000. Data from other areas are required to decide if Germany as a whole is in need of a national screening program for SCD, and if yes, who, when, and how to test.
Moat et al.[47] 2014 UK Retrospective observational study To establish cut-offs for the specific variant to wide-type Hb peptide ratios using MS/MS and to develop a protocol to identify only the disease states of SCD in a routine newborn screening laboratory.	**No. of infants screened:** 13,249 **Ethnicity:** NR **Pre-term neonates:** NR **Period:** during October 2012	**Screening test:** Time of sample collection: NR Platform: MS/MS to measure Hb peptides following tryptic digestion of extracts from dried blood spots Cut-off for SCD disease state: S:A ratio ≥2.1 (based on the 99th percentile of 387 Hb S carriers) Cut-off for carrier state: 0.10<S:A<2.1 **Confirmatory test**: HPLC (VARIANT™nbs; Bio-Rad Laboratories) **Primary target condition(s):** SCD	**Incidence:** SCD 7/13,249 **Harms:** NR **Accuracy of screening test:** MS/MS detected all 7 SCD identified by HPLC FP: 8 (6 Hb SC, 2 Hb SS) FN: 0 **Carrier**: 328 carrier infants were excluded by this protocol	The screening protocol developed correctly identified infants with the disease states of SCD. This protocol was found to be robust for routine screening and reduces the cost of the screening program by preventing large numbers of sickle cell carrier infants from being identified and avoiding unnecessary follow-up testing and referral for genetic counselling.

Boemer et al.[46] 2011 Belgium Retrospective observational study To report the results of a 3-year experience using MS/MS for the detection of the major Hb variants in East-Belgian newborns.	**No. of infants screened:** 43,736 **Ethnicity:** NR **Pre-term neonates:** NR **Period:** 3 years	**Screening test:** Time of sample collection: 3-5 days of life Platform: MS/MS (Quattro Premier triple quadrupole mass spectrometer source, Waters, Manchester, UK) Cut-off: NR **Confirmatory test**: genotyping the whole ß globin gene **Primary target condition(s):** Hb SS, S/ß-thal, SC	**Screening positive:** 444 (1.0%) **Incidence:** SCD 14 (0.03% or 1:3,124): Hb SS 12, Hb S/ß-thal 1, Hb SC 1 **Harms:** NR **Accuracy of screening test:** no discrepancy between screening and confirmatory tests FP: 0 FN: NR **Carrier**: 351 sickle cell trait (these results were communicated with clinicians)	Mass spectrometry provides an efficient alternative approach for Laboratories performing neonatal screening of hemoglobin disorders.

CE: capillary electrophoresis; FN: false negative; FP: false positive; Hb: hemoglobin; HPLC: high performance liquid chromatography; MoM: Multiple of median value; MS/MS: tandem mass spectrometry or spectrometer; NBS: newborn screening; NR: not reported; S:A: ratio of S to A; SCD: sickle cell disease

Table T.D.6: Effectiveness of newborn screening program for SCD

Study Author/year/country/ design/objective	NBS program	Outcomes	Authors' conclusions
Lobo et al.[53] 2014 Brazil Retrospective observational study To evaluate the results obtained during 10 years of the Newborn Hemoglobinopathy Screening Program in the State of Rio de Janeiro.	**No. of infants screened:** 1,217,833 **Primary target condition(s):** SCD **Screening test:** HPLC using automated VARIANT I® system (Bio-Rad Laboratories, Hercules, CA) and the sickle cell® kit **Confirmatory test:** HPLC, complete blood count, and extended typing of blood group phenotypes **Treatment intervention:** prophylactic penicillin (monthly injection until 5 years of age) and vaccination **Follow-up:** educating family members and regular monthly follow-up for 6 months. After 6 months, the extended blood group phenotype was repeated and infants were transferred to government clinics, where pediatricians, trained at HEMORIO, care for these children. **Period:** 1 August 2000 to 31 July 2010 (10 years)	**Screening positive rate:** 0.19% **Incidence:** 912 SCD (0.07% or 1: 1335): 639 Hb SS (70%), 201 Hb SC (22%), 26 Hb SD (2.9%), 46 Hb S/ß-thal (5.0%) **Harms:** NR **Effectiveness:** *Mortality:* 3.7% during 10 years *Survival:* The chance of individuals with SCD being alive at 120 months (10 years) is 94% (95% CI: 0.918-0.914); overall chance of death is 1.5 deaths per 100 patients annually. For Hb SS only, the chance of death is 2.1 per 100 patients per year. The probability of survival at the end of period (10 years) was 92.4% (95% CI: 0.894-0.995) *Morbidity:* NR *Carrier:* sickle cell trait 4.06%; not recalled for confirmatory test but education package provided to their parents.	This 10-year study showed that early diagnosis and treatment of newborns was associated with improved survival and quality of life of Brazilian children with SCD.
Saint-Martin et al.[54] 2013 France Retrospective observational study To report the main results from the NBS program from 1984 to 2010 and consider how the establishment of the sickle cell center affected the program.	**No. of infants screened:** 178,428 **Primary target condition(s):** SCD and other hemoglobinopathies **Screening test:** thin layer IEF (PerkinElmer Massachusetts, USA) (from 1984 to 1991) **Confirmatory test:** hemoglobin citrate agar electrophoresis (from 1984 to 1991), HPLC (Bio-Rad Laboratories, Hercules, CA) (after 1991) Various molecular genotyping methods including reverse dot blot, PCR-RFLP, and direct DNA sequencing were used to identify ß-thalassemia mutation, to identify uncharacterized Hb variants, and to resolve ambiguous primary screening results.	**Screening positive:** NR **Incidence:** Disease state SCD: 585 (Hb SS 53%, Hb SC 39.5%) Hb SS 310 (0.17% or 1:575) Hb SC 231 (0.13% or 1:771) Hb S/ß-thal (including Hb E and Hb Lepore) 42 (0.02% or 1:4,243) Carrier state AS 14,126 (7.6% or 1:13) Failure for confirmation: 239 (0.13%) **Harms:** NR **Effectiveness:**	The establishment of the comprehensive sickle cell centre in 1990 had a positive impact on both the percentage of newborn screed, which was higher than 98.5% since 2002, and delay time in the medical management of SCD infants, with a mean medical management delay time of less than 2 months. The universal NBS program conducted in Guadeloupe has enabled the estimation of the incidence rates for

	Treatment intervention: penicillin prophylaxis, vaccination, parental education **Follow-up:** NR **Period:** January 1984 to December 2010	*Mortality:* NR *Survival:* NR *Morbidity:* NR *Mean medical management delay time* (defined as the delay between the birthdate and the date of the first medical visit, i.e. the age of the infant at first medical visit): significant reduction of the delay time from about 53 months in 1984 to less than 2 months after 1990.	the major SCD as well as the frequencies of the most commonly encountered abnormal variants in in this population. The management of the NBS program by a unique structure improved the program efficiency.
Quinn et al.[57] 2010 USA Prospective observational study To provide a contemporary estimate of 18-year survival for newborns with SCD and document the changes in the causes and timing of death over time, and to explore the temporal association between the improved medical care and survival.	**No. of infants screened:** NR **Primary target condition:** SCD (Hb SS, Hb Sß$^+$, Hb Sß^o, Hb SC) **Screening test:** NR **Confirmatory test**: NR **Treatment intervention:** Penicillin prophylaxis until age 5 years; pneumococcal vaccination at age 2 and 5 years **Follow-up:** mean 9.4 (median 9.2, range 0.08 to 20.6) years; more children with Hb SC and Hb Sß$^+$ were lost to follow-up than children with Hb SS and Hb Sß^o (14.4% vs. 3.2%, P < 0.001) **Period:** 1983- 2007	**Screening positive rate:** NR **Incidence:** SCD: 940 Hb SS and Hb Sß^o: 593 Hb SC and Hb Sß$^+$: 347 **Harms:** NR **Effectiveness:** *Mortality*: 32 (23 related to SCD) *Survival at age 18:* 93.9% (95% CI: 90.3-96.2) in children with Hb SS and Hb Sß^o; 98.4% (95% CI: 94.4-99.5) in children with Hb SC and Hb Sß$^+$. *Morbidity:* NR	The study showed that most children with sickle cell anemia (93.9%) and nearly all children with mild forms of SCD (98.4%) live into adulthood.
Panigraphi et al.[56] 2012 India Retrospective observational study To evaluate feasibility of systematic neonatal screening for SCD in Chhattisgarh, India	**No. of infants screened:** 1,158 **Primary target condition:** sickle cell anemia **Screening test:** HPLC sickle cell short program (Bio-Rad) **Confirmatory test**: HPLC β -thalassemia short program **Treatment intervention:** Penicillin prophylaxis and folic acid **Follow-up:** 6-9 months after initial screening 14 premature were normal on the initial screening, 4 lost to follow-up. Of the remaining 10, 2 had SCD, 1 had sickle cell trait, 7 were normal. FU after 6 months in 68 carrier infants: 7 lost FU; of the remaining 61, 1 had SCD, and the other 60 were sickle cell trait.	**Screening positive rate:** NR **Incidence:** SCD Initial screening: 3 cases (0.2%, 95% CI 0.12-0.28) Follow-up: 2 Total: 5 (0.4%) SCD carrier Initial screening: 68 cases (5.8%, 95% CI 4.5-7.5) Follow-up: 61 cases (5.26%) **Accuracy of screening test:** FP: 0 FN: 3 **Harms:** NR **Effectiveness:**	Early detection of Hb SS by neonatal screening will help in early prevention and management of complications in postnatal period.

	Period: February 2008-January 2009	*Mortality*: no death by 2 years of age *Survival:* all alive by 2 years of age *Morbidity:* 2 had anemia, splenomegaly between ages of 1.5 to 2.5 years. The other 3 are asymptomatic by 2 years of age.	
McGann et al.[55] 2013 Angola Prospective observational study To describe the early successful results of the screening and treatment program in this limited-resource setting.	**No. of infants screened:** 36,453 (50-70% of eligible infants) **Primary target condition:** SCA **Screening test:** IEF - RESOLVE® neonatal hemoglobin system, PerkinElmer, Inc. **Confirmatory test**: CE - CAPILLARYS 2 NEONAT FAST® system, Sebia, Inc. **Treatment intervention:** Penicillin prophylaxis (125 mg, oral, twice daily), parent education, pneumococcal immunization, and insecticide-treated bed nets (for malaria prophylaxis) **Follow-up:** every 2 months for the first 6 months, and every 3 months thereafter. 132/244 were followed up ≥1 year **Period:** June 2011-June 2013	**Screening positive:** NR **Incidence:** SCD Hb SS: 550 (1.51%) (10 of them repeated at 6-8 weeks to be FAS) Hb SC: 7 (0.02%) SCD carrier FAS 7,666 (21.03%) FAC 21 (0.06%) **Accuracy of screening test:** Concordance between IEF and CE 99.8%, discordance in 9 samples FP: NR FN: NR **Harms:** NR **Effectiveness:** n=244 SCA (227 compliant with follow-up, 9 died during follow-up, 8 lost to follow-up) *Mortality:* 9/244 (3.6%); calculated 1st-year mortality 6.8% vs. national infant mortality rate 9.8% *Survival:* NR *Morbidity:* NR	This pilot study provides compelling data regarding the diagnosis and treatment of infants with SCA in Luanda, warranting expansion of the program to additional provinces, with an eventful national strategy for the diagnosis, care, and treatment of children with SCA throughout the entire country.

CE: capillary electrophoresis; CI: confidence interval; FN: false negative; FP: false positive; Hb: hemoglobin; HPLC: high performance liquid chromatography; IEF: isoelectric focusing; NBS: newborn screening; NR: not reported; SCA: sickle cell anemia; SCD: sickle cell disease; TLIF: thin-layer isoelectric focusing

Appendix T.E: Results for SCID Studies

Table T.E.1: Characteristics of the included systematic review for SCID

Study Author/year/objective	Search strategy	Study selection	Quality assessment and data extraction	Data analysis and synthesis
Lipstein et al.[87] 2010 To conduct a systematic review of the evidence for newborn screening for SCID, including test characteristics, treatment efficacy, and cost-effectiveness.	**Databases:** Medline, OVID In-Process, other non-indexed citation databases (no details) **Publication period:** January 1988-October 2008 **Other sources:** 1. hand search: NR 2. reference list: Yes 3. contact experts: Structured interviews with clinical experts in the field of SCID) **Language limit:** English only	**Inclusion criteria:** no clearly defined set of criteria was presented **Exclusion criteria:** 1 Reviews, editorials, opinion paper; 2. Case series of <4 patients; 3. Contained only adult subjects; and 4. Non-human data **Abstract screening:** by two reviewers	**Quality assessment:** Quality assessment tool: Pandor 2004,[12] Pollitt 1997[107] Quality assessment by two reviewers: yes **Data extraction:** Use of standardized form: yes Data extraction by two reviewers: for 13% of the included studies	Qualitative systematic review; no meta-analysis was performed due to the variations in types of included studies, HSCT methodologies used, and the discrepant comparison groups.

HSCT: hematopoietic stem cell transplantation; NR: not reported; SCID: severe combined immunodeficiency

Table T.E.2: Quality assessment results for the included systematic review for SCID, using the AMSTAR tool

AMSTAR questions	Rating for Lipstein et al.[87]
1. Was a priori design provided?	Yes
2. Was there duplicate study selection and data extraction?	No
3. Was a comprehensive literature search performed?	Yes
4. Was the status of publication (i.e. grey literature) used as an inclusion criteria)?	Yes
5. Was a list of studies (included and excluded) provided?	No
6. Were the characteristics of the included studies provided?	Yes
7. Was the scientific quality of included studies assessed and documented?	Yes
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	No
9. Were the methods used to combine the findings of the studies appropriate?	Yes
10. Was the likelihood of publication bias assessed?	Cannot answer
11. Was the conflict of interest included?	No
Total score (out of 11)	**6**
Rating (high/ medium/low)	**Medium**

Table T.E.3: Quality assessment results for SCID studies reporting on test accuracy parameters, using the QUADAS-2 tool

Domain	Questions for risk of bias	Kwan 2014[80]	Jilkina 2014[96]	Vogel 2014[84]	Adams 2014[97]
Patient selection	Was a consecutive or random sample of patients enrolled?	Unclear	No	Unclear	Unclear
	Was a case-control design avoided?	Yes	No	Yes	Unclear
	Did the study avoid inappropriate exclusions?	Unclear	Unclear	Unclear	Unclear
	Risk of bias	Unclear	High	Unclear	Unclear
Index test	Were the index test results interpreted without knowledge of the results of the reference standard?	Yes	Yes	Yes	No
	If a threshold was used, was it pre-specified?	Unclear	Yes	Yes	No
	Risk of bias	Unclear	Low	Low	High
Reference standard	Is the reference standard likely to correctly classify the target condition?	Yes	Unclear	Yes	Unclear
	Were the reference standard results interpreted without knowledge of the results of the index test?	No	Yes	No	Yes
	Risk of bias	High	Unclear	High	Unclear
Patient flow and timing	Was there an appropriate interval between index test(s) and reference standard?	Yes	No	Unclear	No
	Did all patients receive a reference standard?	No	Yes	No	Yes
	Did all patients receive the same reference standard?	No	Unclear	No	Unclear
	Were all patients included in the analysis?	No	No	No	No
	Risk of bias	High	High	High	High
Overall risk of bias		High	High	High	High

Table T. E. 3: Quality assessment results for SCID studies reporting on test accuracy parameters, using the QUADAS-2 tool (cont'd)

Domain	Questions for risk of bias	Kwan et al. 2013[105]	Verbsky et al. 2012[81]	la Marca et al. 2013[89]	la Marca et al. 2013[90]
Patient selection	Was a consecutive or random sample of patients enrolled?	No	Yes	No	No
	Was a case-control design avoided?	Yes	Yes	Unclear	No
	Did the study avoid inappropriate exclusions?	Yes	Yes	Unclear	Unclear
	Risk of bias	High	Low	High	High
Index test	Were the index test results interpreted without knowledge of the results of the reference standard?	Yes	Yes	No	No
	If a threshold was used, was it pre-specified?	Yes	Yes	No	Yes
	Risk of bias	Low	Low	High	High
Reference standard	Is the reference standard likely to correctly classify the target condition?	Yes	Yes	Unclear	Unclear
	Were the reference standard results interpreted without knowledge of the results of the index test?	No	Unclear	Yes	Yes
	Risk of bias	High	Unclear	Unclear	Unclear
Patient flow and timing	Was there an appropriate interval between index test(s) and reference standard?	Yes	Yes	No	No
	Did all patients receive a reference standard?	No	No	Yes	Unclear
	Did all patients receive the same reference standard?	No	No	Unclear	Unclear
	Were all patients included in the analysis?	No	No	No	No
	Risk of bias	High	High	High	High
Overall risk of bias		High	High	High	High

Table T.E.4: Quality assessment results for SCID studies reporting on effectiveness, using the EPHPP tool

Study	Selection bias	Study design	Confounders	Blinding	Data collection	Withdrawals and drop-outs	Global rating
Kwan et al. 2014[80]	Moderate	Weak	Weak	Moderate	Weak	Strong	Weak
Pai et al. 2014[98]	Moderate	Weak	Weak	Weak	Weak	Strong	Weak
Kwan et al. 2013[105]	Strong	Weak	Weak	Moderate	Weak	Strong	Weak
Dvorak et al. 2013[104]	Strong	Weak	Weak	Moderate	Weak	Strong	Weak
Brown et al 2011[99]	Moderate	Moderate	Weak	Weak	Weak	Weak	Weak
Chan et al. 2011[100]	Moderate	Moderate	Weak	Weak	Weak	Strong	Weak
Buckley et al 2011[102]	Moderate	Weak	Weak	Weak	Strong	Strong	Weak
Gennery et al. 2010[101]	Moderate	Moderate	Weak	Moderate	Strong	Strong	Moderate
Neven et al. 2008[103]	Moderate	Weak	Weak	Moderate	Weak	Strong	Weak

Table T.E.5: Results reported in the systematic review for SCID

Included studies	Accuracy of screening test	Harms/Effectiveness	Authors' conclusions
No. of included studies: 26 **Types (no.) of studies:** cohort (5); case-control (3); large case series (> 50 patients) (5); small case series (12); cost-effectiveness analysis (1) (results were not reported here) **Publication period:** 1989-2007 (3 studies published after 2006) **Target conditions:** SCID **Screening test(s):** • Lymphocyte counts • Quantitative PCR for TRECs • ELISA for IL-7	**Quantitative PCR for TRECs on DBS** (1 case control study: n=23 with SCID, 2 with non-SCID immunodeficiencies, 242 without SCID) At cut-off <30 copies/µl: Sn 100%, Sp: 97% At cut-off of undetectable TRECs: FP rate 1.5% among children discharged from routine nurseries and 5% among children from special-care nurseries. **Two-tier (IL-7+TRECs) on DBS** (1 case control study: n=13 with SCID, 183 without SCID) ELISA for IL-7: Sn 85% (CI 55%-98%), Sp 96.1% TRECs: Sn 100%, Sp 92.3% **Lymphocyte counts on whole blood** (2 studies) 1 case control study (n=45 with SCID, 90 without SCID): At cut-off <$2.8x10^9$/L: Sn 86%, Sp 94% 1 cohort study (n=18 with SCID, 18 without SCID): At cut-off <$5.0x10^9$/L: Sn 56%, Sp 100% PPV: NR NPV: NR	**Harms:** found no study addressed harms associated with newborn screening for SCID. **Treatment effectiveness** ***Intervention:*** early HSCT (4 studies, one cohort and 3 case series). Results from other studies on various HSCT protocols were not reported here. ***Mortality/Survival:*** Survival rate: 3 studies showed substantially better survival rates with early HSCT (before 28 days or before 3.5 months). Survival duration (1 case series of 13 patients who received HSCT 7-68 days): all alive 0.5-11.5 years after HSCT (median follow-up: 3 years) **Feasibility:** found no study for feasibility of population-based newborn screening for SCID. **Acceptability:** NR	**Screening test performance:** The published newborn screening articles are primarily proofs of concept. The results of several small studies suggested that screening for SCID is possible. The development of methods to screen for SCID by using dried blood spots, in contrast to using lymphocyte counts, which require whole blood, improved the feasibility of instituting SCID screening. **Treatment effectiveness:** Evidence from large case series indicates that children who received early HSCT consistently demonstrated improved survival outcome compared to children who were treated later, with recipients from matched related donors having the best survival rate.

CI: confidence interval; DBS: dried blood spot; FP: false positive; HSCT: hematopoietic stem cell transplantation; NPV: negative predictive value; NR: not reported; PCR: polymerase chain reaction; PPV: positive predictive value; SCID: severe combined immunodeficiency; Sn: sensitivity; Sp: specificity; TREC: T-cell receptor excision circles

Table T.E.6: Evidence on newborn screening test performance for SCID

Study Author/year/country/ design/objective	Newborn population screened	Screening/confirmatory tests	Outcomes	Authors' conclusions
Adams et al.[97] 2014 UK Case control To test a newly available commercial duplex assay to measure TRECs to establish if this would be suitable for NBS for SCID in the UK.	**No. of infants screened:** 5,081 DBS + 18 confirmed SCID (4 ADA SCID, 2 Gamma-Chain ACID, 2 Omenn's SCID, 2 RAG deficient SCID, 1 PNP SCID 7 undefined SCID) **Ethnicity:** NA **Pre-term neonates:** <32 weeks - 1.7% 32-36 weeks - 4.5% >36 weeks - 93.8% **Period:** NR	**Screening test:** Time of sample collection: NR Platform: Duplex TREC (EnLite™ Neonatal TREC kit, Wallace Oy) Cut-off: TRECs >20, 25, 30, 35, or 40 copies/ µL **Confirmatory test**: flow cytometry test was not performed on presumptive positive samples from the 5,081 anonymized DBS **Primary target condition(s):** SCID	**Overall referral rate:** 1% (i.e. 7,000 samples/year) at cut-off at <40 copies/µL; 0.04% at cut-off <20 copies/µL (i.e. 208 samples/year) **Incidence of SCID:** NR **Harms:** Over-diagnosis: NR Psychosocial harm: NR **Screening test performance:** Sn: NR Sp: NR FN: 0 at cut-off <20 copies/µL (all 18 SCID cases were detected)	The study demonstrated that this duplex assay kit will identify all newborns with SCID as presumptive positives. The data also shows that with suitable TREC cut-off settings the number of presumptive positives from non-SCID newborns will be manageable in the context of a national screening service.
Jilkina et al.[96] 2014 Canada, Manitoba Case control To retrospectively test confirmed SCID infants using archived Guthrie cards to determine the applicability of the TREC assay in the ethnically unique population with a higher proportion of infants affected by atypical forms of SCID.	**No. of infants screened:** 13 13 SCID and 5 PID (diagnosed between 1992 and 2010; all received BMT); normal control: 982 **Ethnicity:** Manitoba population **Pre-term neonates:** NR **Period:** 1992-2010	**Screening test:** Time of sample collection: NR Platform: multiplexed TREC qPCR assay Cut off: all 4 TREC results <78 and at least 3 results <39 copies/reaction mixture (corresponding to 501 and 252 TREC copies/µl of whole blood, respectively **Confirmatory test**: unclear **Primary target condition(s):** T positive SCID	**Overall referral rate:** NR **Incidence:** NR **Harms:** Over-diagnosis: NR Psychosocial harm: NR **Screening test performance:** Sn: NR Sp: NR FN: 6/13 confirmed SCID patients	TREC will identify T-negative SCID and T-negative PID. To identify other SCID infants, newborn screening in Manitoba must include supplemental targeted screening for ethnic-specific mutations.

La Marca et al.[89] 2013 Italy Case control To provide an analytical method that could allow the quantitation of ADA SCID metabolites at the same time with the other metabolites that are commonly determined in expanded NBS programs.	**No. of infants screened:** 9 ADA-SCID patients (4 early onset and 5 delayed onset) + 50,000 DBS (collected between January 2011 and June 2012) **Ethnicity:** NR **Pre-term neonates:** NR **Period:** January 2011-June 2012	**Screening test:** Time of sample collection: 2-3 days Platform: MS/MS to measure adenosine and 2' deoxyadenosine on DBS Cut-off: 2' deoxyadenosine (dAdo) ≥0.09 µmol/L, adenosine (Ado) ≥1.61 µmol/L **Confirmatory test**: unclear **Primary target condition(s):** ADA SCID	**Overall referral rate:** 0.02% after 1st test; 90% of them were normal at 2nd-tier test **Incidence:** NR **Harms:** Over-diagnosis: NR Psychosocial harm: NR **Test performance:** Sn: NR Sp: NR FN: 0	The results show that the method having great simplicity, low cost, and low process preparations can be fully applicable to a mass screening program.
La Marca et al.[90] 2013 Italy Case control To investigate whether analysis of DBSs collected during NBS procedures can identify patients with delayed or late-onset ADA SCID by using TREC analysis, MS/MS, or both.	**No. of infants screened:** 4 infants with delayed onset ADA SCID, Control: 2 newborns who carries ADA variants associated with SCID, and 3 with ADA SCID **Ethnicity:** NR **Pre-term neonates:** NR **Period:** NR	**Screening test:** Time of sample collection: NR Platform: 1) MS/MS to measure Ado and dAdo on DBS; and 2) qPCR to measure TRECs Cut-off: MS/MS: dAdo >0.07 µmol/L, Ado ≥1.5 µmol/L TREC < 25/ µL **Confirmatory test**: lymphocyte subset and proliferation measured by cytofluorometric methods **Primary target condition(s):** Delayed onset ADA SCID	**Overall referral rate:** NR **Incidence:** NR **Harms:** Over-diagnosis: NR Psychosocial harm: NR **Test performance:** NR Sn: NR Sp: NR FN: all 3 patients with delayed onset ADA SCID with TREC assay but none with MS/MS	MS/MS analysis of adenosine and 2' deoxyadenosine but not TREC quantifications identifies newborns with delayed or late-onset ADA SCID.

ADA SCID: adenosine deaminase deficiency; Ado: adenosine; BMT: bone marrow transplantation; CI: confidence interval; CS: case series; dAdo: 2' deoxyadenosine; DBS: dried blood spot; FN: false negative; FP: false positive; HSCT: hematopoietic stem cell transplantation; MS/MS: tandem mass spectrometry or spectrometer; NBS: newborn screening; NPV: negative predictive value; NR: not reported; PCR: polymerase chain reaction; PID: primary immunodeficiency; PPV: positive predictive value; quantitative (or real-time) polymerase chain reaction; SCID: severe combined immunodeficiency; Sn: sensitivity; Sp: specificity; SR: systematic review; TCL: T-cell lymphopenia; TREC: T-cell receptor excision circles

Table T.E.7: Effectiveness of treatment and management for SCID

Study Author/year/country/ design/objective	Population	Intervention	Treatment effectiveness	Authors' conclusions
Pai et al.[98] 2014 USA Retrospective case series (multi-centres) To report a retrospective analysis of data from 240 infants with classic SCID who received HSCT at 25 PIDTC institutions during a 10-year period.	**No. of patients:** 240 classic SCID **Age at HSCT:** 68 (28%) ≤3.5 months, 172 (72%) >3.5 months **Gender:** 72% males, 28% females **Ethnicity:** Non-Hispanic white 49%, Black 10%, Hispanic 28%, Asian 4%, Native American 3%, other 5% **Period:** 1 January 2000-31 December 2009	**Treatment intervention:** HSCT n=240 (bone marrow 58%, mobilized peripheral blood 24%, umbilical-cord blood 18%) **Follow-up:** 10 years	**Mortality:** 62 deaths; most deaths occurred during the 1st year after transplantation, and most deaths were due to infections or pulmonary infections. **5-year survival:** overall: 74% (178/240); 94% among infants who received HSCT at ≤3.5 months; 90% among older infants without prior infection; 82% among infants with infection that had resolved; 50% among children were >3.5 months and had active action at the time of HSCT.	The data from this study indicate that children with classic SCID diagnosed at birth or before the onset of infection who receive transplants from mismatched related donors, transplants from unrelated donors, or cord-blood transplants soon after diagnosis have more than a 90% probability of survival with T-cell and variable B-cell immune reconstitution.
Dvorak et al.[104] 2013 USA Prospective trial (multi-centres) To report baseline clinical, immunologic, and genetic features, and the initial therapy received, for the first 50 SCID and SCID atypical patients enrolled in a prospective study over a 21-month timeframe.	**No. of patients:** 50 (37 typical, 13 atypical SCID including leaky SCID, Omenn syndrome, reticular dysgenesis) **Age at HSCT:** median 67 days for those diagnosed by family history or NBS (74% at <3.5 months) vs. 214 days for those diagnosed by clinical signs (17% at <3.5 months) (p<0.001) **Gender:** 76**%** male and 24% female in typical SCID (n=37) **Ethnicity:** White/Non-Hispanic 32%, White/Hispanic 24%, African American 14%, Asian or Pacific Islander 8%, Native American 3%, other 19% in typical SCID (n=37) **Period:** August 2010-May 2012	**Treatment intervention:** HSCT: n=46 Gene therapy n=3 Enzyme replacement n=1 **Follow-up:** median 9.2 (range 3.3-17.5) months for HSCT; 15.7 (range 10.6-28.5) months for gene therapy or enzyme replacement therapy	**Mortality:** 6/46 who received HSCT died **1-year survival:** overall 86% (95% CI 72-94%) among children who received HSCT; Difference in survival between infants who received HSCT early vs. late were not available due to insufficient follow-up time. All 4 patients treated with enzyme replacement or gene therapy were alive during the follow-up.	This study demonstrated that in the states where NBS is available, patients with SCID were typically diagnosed at less than a month of age, and the majority (74%) proceeded to transplant by 3.5 months of age, a time point that has been shown to be associated with superior outcomes.

Buckley et al.[102] 2011 USA Case series (single centre) To summarize the long-term outcome, according to molecular type, of 166 consecutive SCID infants given non-conditioned related donor bone marrow transplants at a single institute over 28.3 years.	**No. of patients:** 166 **Age at HSCT:** 48/166 ≤3.5 months, 118/166 >3.5 months **Gender:** NR **Ethnicity:** NR **Period:** 1982 -2010	**Treatment intervention:** HSCT **Follow-up:** range from 2 months to 29.3 years; median follow-up for surviving patients was 10 years.	**Mortality:** 40 deaths (30 from viral infections) **Survival:** overall 76% (126/166); ≥1 year: 125 patients; ≥5 years: 110 patients; ≥10 years: 83 patients. 94% (45/48) of those received HSCT at <3.5 months vs. 69% (81/118) of those who received HSCT at >3.5 months **Long-term clinical outcomes:** Compared to 70 SCID patients transplanted later, 41 SCID infants transplanted at <3.5 months showed a significant superior survival rate, a lower rate of clinical problems, less need for booster transplants, and fewer with weight <3%.	SCID patients who were transplanted early (<3.5 months) had a superior survival rate, a lower rate of clinical problems, less need for booster transplant, and better nutritional status.
Chan et al.[100] 2011 USA Survey To conduct a survey of parents of children with SCID to evaluate the impact of SCID on families.	**No. of patients:** 158 SCID or Omenn Syndrome **Age at HSCT or enzyme replacement:** mean 34 (median 28) weeks (n=98) **Gender:** NR **Ethnicity:** NR **Period:** January 2009	**Treatment intervention:** HSCT or enzyme replacement therapy **Follow-up:** NA	**Mortality:** 61/158 died (51% after HSCT or enzyme replacement treatment) **Survival:** Overall 61% (95% CI 54-69%) Overall survival rate of treated patients: 81.4% 85% of those who were tested early vs. 58% of those who were not tested early (p=0.026) Mean age at treatment 29 weeks for those treated and survived (n=78) vs. 57 weeks for those treated and died (n=20) (p=0.038)	Affected infants diagnosed with SCID as neonates had better survival, demonstrating the potential benefit of universal newborn screening.

Brown et al.[99] 2011 USA Comparative study To compare the outcomes of a cohort of SCID patients who were diagnosed earlier with their siblings who were diagnosed later.	**No. of patients:** N=108 G1=60 (early diagnosis group, at median 0; range 0-29 days) G2=48 (late diagnosis group, at median 143; range 1-455 days) **Age at treatment:** NR **Gender:** NR **Ethnicity:** NR **Period:** 1982-2010 for early diagnosis group; 1979-2009 for late diagnosis group	**Treatment intervention :** HSCT or gene therapy **Follow-up:** over 20 years	**Mortality:** 10% in the early diagnosis group vs. 60% in the late diagnosis group. **Survival:** overall 90% in the early diagnosis group vs. 40% in the late diagnosis group. Among patients treated with HSCT, 91.5% in the early diagnosis group vs. 61.3% in the late diagnosis group (P<0.01)	The data showed that the improved survival both before and after HSCT and after HSCT is seen irrespective of donor choice, conditioning regimen used, or underlying diagnosis. It is highly probable that the improved survival relates to the ability to make an early diagnosis, thereby protecting infants from infection and secondary organ damage and improving nutritional status, and therefore allowing an improved ability to withstand HSCT.
Neven et al.[103] 2009 France Retrospective case series To perform a retrospective, in-depth analysis of the clinical and immunologic follow-up of all patients with SCID having survived for more than 2 years after HSCT.	**No. of patients:** 149 SCID treated with HSCT; 94 survived 2 years after HSCT, in 90 of them long-term follow-up available. **Age:** At diagnosis: median 4 months; 39% ≤35 months and 61% >35 months At HSCT: 25% ≤35 months and 75% >35 months **Gender:** 61% male, 39% female **Ethnicity:** NR **Period:** 1972-2004	**Treatment intervention:** HSCT **Follow-up:** median 14 (range from 2 and 34) years; <5 years in 12 patients, 5-15 years in 40 patients, >15 years in 38 patients	**Mortality:** 8 patients (9%) died 2.5 to 11 years after HSCT due to poor immune reconstitution, chronic GvHD, and related complications such as autoimmune/inflammatory events. **Survival:** 2 year survival 63% (94 of 149), long-term survival 91% (82/90) **Morbidity:** 48/90 (43%) experienced clinical events (persistent chronic GVHD, autoimmune/inflammatory events, severe or recurrent infection, chronic HPV infection, growth insufficiency and need of nutritional support, etc.) 58 (71%) Of the 82 living patients did not require any form of treatment. No significant association between age at HSCT and clinical events	The analysis showed that the occurrence of clinical events correlated with non-geno-identical donors, diagnosis of Artemis SCID, and quality of immune reconstitution. In most cases, HSCT enables long-term survival with infrequent sequelae. However, the occurrence of relatively late-onset complications is a concern that requires specific means of prevention and justifies careful patient follow-up.

Gennery et al.[101] 2010 Europe Case series (multi-centres) To analyze the long-term outcome of patients with SCID and non-SCID PID from European centers treated between 1968 and 2005.	**No. of patients:** 699 SCID, 783 with non-SCID PID (data not reported here) **Age at HSCT:** 42% <6 months; 37% 6-11 months; 21% >12 months **Gender:** NR **Ethnicity:** NR **Period:** 1968-December 2005	**Treatment intervention:** HSCT **Follow-up:** 26% ≤5 years, 22% 5-10 years; 52% >10 years	**Mortality:** 27% in <6 months; 36% in 6-11 months; 42% in >12 months **Survival:** 10-year survival 68% (95% CI 62-74%) in patients <6 months at HSCT vs. 59% (95%CI 53-67%) in patients 6-11 months at HSCT vs. 51% (95% CI 42-61%) in patients >12 months at HSCT (p=0.0008) 90% for patients receiving HSCT with geno-identical donors performed from 2000 to 2005; 66% with mismatched relatives; 69% with unrelated donors	This is the largest cohort study of SCID and non-SCID PID patients with the longest follow-up. The data clearly demonstrate an improved outcome when patients are transplanted before 6 months of age. As survival has continued to improve, the long-term quality of immune-reconstitution and other life quality issues become important.

CI: confidence interval; GvHD: graft-versus-host disease; HSCT: hematopoietic stem cell transplantation; NBS: newborn screening; NR: not reported; PID: primary immunodeficiency; PIDTC: Primary Immune Deficiency Treatment Consortium; SCID: severe combined immunodeficiency; SS: sickle cell anemia

Table T.E.8: Effectiveness of newborn screening program for SCID

Study Author/year/country/ design/study period/objective	NBS program	Outcomes	Authors' conclusions
Kwan et al.[80] 2014 USA (11 states) Epidemiological and retrospective observational study To present data from a spectrum of SCID newborn screening programs, establish population-based incidence for SCID and other conditions with T-cell lymphopenia, and document early institution of effective treatments.	**No. of infants screened:** 3,030,083 **Primary targets:** typical SCID, leaky SCID, Omenn Syndrome that required immune-restoring treatment **Secondary targets:** additional diagnoses detected **Screening test:** TREC by qPCR **TREC cut-offs (TREC/μL):** varied across NBS programs (≤25 in 3 states, <30 in 2 states, and ≤7, <27, <40, <125, ≤150, <252 in 1 state each) **Confirmatory test**: flow cytometry; HIV PCR or maternal serodiagnosis, and further evaluation (gene and syndrome diagnosis **Flow cytometry cut-offs for TCL (T cells/μL):** varied across NBS programs (<1,500 in 6 states, <2,500 in 3 states, <3,505 in 1 state, not defined in 1 state) **Treatment intervention:** HSCT, enzyme replacement, and/or gene therapy **Follow-up:** Regular reviews between public health personnel and clinical experts to uncover any missed (false negative) cases and monitor screening test performance and follow-up **Period:** 2008-2013	**Overall flow cytometry referral rate:** 0.042% (41.8/100,000) **No. of identified cases:** 52 SCID (42 typical, 9 leaky SCID, and 1 Omenn syndrome); 411 non-SCID TCL **Incidence of SCID:** 1:58,000 (95% CI: 1:46,000-1:80,000) **Harms:** Over-diagnosis: none Psychosocial: NR **Accuracy of screening test:** FN: none **Effectiveness:** 49/52 received immunity restoring treatment (44 had HSCT, 4 had gene therapy, 2 had ADA enzyme therapy) **Mortality:** 7 deaths (4 after HSCT) **Survival:** overall 87% (45/52); 92% (45/49) among treated **Acceptability:** NR	This multi-state experience has demonstrated the feasibility of TREC assay, a biomarker for naïve T-cell lymphopoiesis, followed by confirmed flow cytometry, as a means to identify SCID. Newborn screening in 11 programs in the United States identified SCID in 1 in 58,000 infants, with high survival. The usefulness of detection of non-SCID T-cell lymphopenia by the same screening remains to be determined.
Kwan et al.[105] 2013 USA, California	**No. of infants screened:** 993,724 (9% from NICU) **Primary targets:** typical SCID, leaky SCID, Omenn syndrome, and complete DiGeorge syndrome that	**Overall flow cytometry referral rate:** 0.016% (66% from NICU) **No. of identified cases:** 50 significant TCL, 15/50 primary targets requiring HSCT (11 typical SCID, 3 leaky SCID	The NBS program with TREC assay in California has achieved early diagnosis of SCID and other conditions with T-cell lymphopenia, facilitating management and

Retrospective observational study To report results of the first 2 years of TREC NBS in California	required immune-restoring treatment **Secondary targets:** clinically significant TCL with <1,500 autologous T-cells/μL **Screening test:** TREC by PCR **TREC cut-offs (TREC/μL)**: initial test ≤40, 2nd test ≤25 **Confirmatory test**: complete and differential blood count and lymphocyte subset analysis by flow cytometry **Flow cytometry cut-offs for TCL (T-cells/μL):** <1,500 **Treatment intervention:** HSCT, gene therapy, or thymus transplantation **Follow-up:** ranged from 4 to 27 months after their most recent treatments **Period:** 2010-2012	or Omenn syndrome, 1 complete DiGeorge syndrome) **Incidence of SCID:** 1: 66,250; 1:19,900 (0.005%) for significant TCL (T-cells <1,500/μl) **Harms:** Over-diagnosis: NR Psychosocial: NR **Accuracy of screening test:** FP rate: 0.01% PPV: high **Effectiveness:** 15 patients received HSCT (13 patients), gene therapy, or thymus transplantation **Mortality:** 1 death in 15 treated patients **Survival:** 93% in 15 treated patients **Acceptability:** NR	optimizing outcomes.
Vogel et al.[84] 2014 USA, New York Retrospective observation study To describe the process and assess outcomes for the first 2 years of NBS for SCID in New York State.	**No. of infants screened:** 485,912 **Primary targets:** Typical and leaky SCID **Secondary targets:** significant TCL **Screening test:** multiplex qPCR reaction to detect TREC copy number **TREC cut-off:** originally ≤200 TRECs; since 2011 the borderline category was expanded to 125-200 TRECs (repeat TREC test) **Confirmatory test**: complete blood count and flow cytometry; if clinically indicated, T-cell activation with mitogens, chromosome analysis and genetic testing were performed as appropriate. At the time of referral, a repeat TREC assay was performed. **Flow cytometry cut-offs:** not defined (diagnoses of SCID or leaky SCID were based on the clinical expertise of the specialists)	**Overall flow cytometry referral rate:** 0.1% (531 infants) **No. of identified cases:** 10 SCID; 87 sTCL **Incidence of SCID:** 1:48,500 live birth **Harms:** Over-diagnosis: NR Psychosocial harm: NR **Accuracy of screening test:** PPV: 2.1% for typical and leaky SCID, 20.3% for sTCL NPV: 100% (no known FN) **Premature neonates:** 561 infants with screen test positive requiring repeat TREC **Effectiveness** 7/10 patients received HSCT, 2 received enzyme replacement therapy ***Mortality:*** 1 death ***Survival:*** overall 90%	Newborn screening for severe T-cell deficiencies in New York State identified 10 infants with SCID. 9 of the 10 patients (90%) received HSCT or enzyme replacement therapy and are doing well demonstrating that NBS for SCID is beneficial.

	Treatment intervention: HSCT or enzyme replacement **Follow-up:** no details about the review/ monitoring system for identifying any false negatives **Period:** September 2010-September 2012	**Acceptability:** NA	
Verbsky et al.[81] 2012 USA, Wisconsin Retrospective observational study To summarize the results of 3 years of NBS in Wisconsin for SCID using the TREC assay.	**No. of infants screened:** 207,696 (9.13% pre-term infants) **Primary targets:** SCID/ TCL **Secondary targets:** NA **Screening test:** TRECs by RT-qPCR **TREC cut-off:** originally <25 TRECs/μL, increased to <40 TRECs/μL since 2009 **Confirmatory test**: Lymphocyte subset analysis by flow cytometry (FACSCalibur™ flow cytometer, Becton Dickinson) **Flow cytometry cut-offs:** NR **Treatment intervention:** HSCT or enzyme replacement **Follow-up:** no details about the review/ monitoring system for identifying any false negatives **Period:** January 2008- December 2010	**Overall flow cytometry referral rate:** 0.034% (72/207,696); 0.03% for full term, 0.045% for pre-term infants **No. of identified cases:** 5 SCID; 28 non-SCID TCL **Incidence of SCID:** 1:41,539* (5/207,696) **Harms:** Over-diagnosis: NR Psychosocial harm: NR **Accuracy of screening test:** For SCID: FN: none For TCL of any cause: Sp: 99.98% PPV: 45.83% FP rate: 0.018% **Effectiveness** 3 of 5 SCID infants received HSCT, one received enzyme replacement therapy, 1 waiting for HSCT **Mortality:** no death **Survival:** all 5 SCID infants alive	The 3-year NBS for SCID has shown that the TREC assay is sensitive, specific, and detects known and unknown causes of SCID/TCL that leads to life-saving therapies such as HSCT prior to the acquisition of severe infections.

ADA: adenosine deaminase deficiency; CI: confidence interval; FN: false negative; FP: false positive; HSCT: hematopoietic stem cell transplantation; NBS: newborn screening; NR: not reported; PPV: positive predictive value; qPCR: quantitative (or real-time) polymerase chain reaction; SCID: severe combined immunodeficiency; TCL: T-cell lymphopenia; TREC: T-cell receptor excision circles

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SECTION FOUR: ECONOMIC ANALYSIS

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4.1 Introduction

Objectives and Research Questions

The *Economic Analysis* (**E**) section of this STE report will assess the cost-effectiveness and budget impact of adding one or a combination of the aforementioned seven conditions to the Alberta NMS Program. The specific research questions addressed are as follows:

1. Taking into consideration the positive predictive value and the population of Alberta, what would be the annual number of positive screens that would be referred for follow-up assessment if this condition were screened for in Alberta?
2. What are the costs associated with the treatment for the condition? Who would be responsible for these costs?
3. How do outcomes vary between cases being managed after early diagnosis and later diagnosis?
4. For each condition, determine whether screening is cost-effective. Note that cost-effectiveness is informed by questions 1, 2, and 3 above, as well as by information contained in the S and T sections of the report.
    i. What is the unit cost of the screening tests and the unit cost of delivering the associated service (including equipment, facility, healthcare provider fees, etc.)?
    ii. What is the marginal cost of providing newborn blood spot screening to the population for which it is indicated using effective screening tests?
5. What is the associated budget impact of adding conditions to the current Alberta newborn blood spot panel?
    i. What costs would be associated with such additions to the current NMS panel, including but not limited to screening and all required follow-up services?
    ii. Would the cost be expected to rise in the future? If so, why, and by how much?

Methods

Economic Evaluation

For each condition, an economic model was developed to conduct a cost-effectiveness analysis (CEA) of screening compared to not screening. Specifically, using the care pathway associated with each condition, a Markov simulation model was used to compare the associated costs and health benefits of screening compared to not screening. Details of each care pathway are presented in the S section of this report (see Appendix S.B).

The CEA adopted a payer perspective and considered direct medical service costs to the Alberta health system, including costs of physician management, dieticians, genetic counselling, medication, hospitalization, and laboratory services. The analysis also included the cost of education and social services for mental or developmental sequelae. The time horizon for the analysis considered lifelong

costs and benefits, starting from birth to 80 years of age. The cycle length for the Markov model is three months.

Targeted Conditions

Due to their similarity in treatment and resource uses, the three hemoglobin conditions (Hb SS, Hb S/β-thal, and Hb SC) are grouped together in the model for parsimony, and are referred to as SCD in the model. The other conditions (GALT, TYRI, HCY, and SCID) are modelled separately.

In addition to the primary targeted conditions, the newborn screening tests can detect associated secondary conditions or variants. While these are usually deemed to be milder conditions, it is important to inform the number of secondary conditions/variants detected with the screening tests. Secondary conditions were included to capture the associated costs and resources of monitoring those conditions that would otherwise not have been identified in the absence of screening. The analysis therefore included the secondary conditions/variants as defined from the literature in the analytic model.[1-4] However, as described in the T section, screening for TYRI with succinylacetone is specific for TYRI and the incidence of secondary conditions/variants (TYRII or TYRIII) are extremely rare,[5] and they are therefore excluded in the analysis. The secondary conditions/variants considered in the analysis are as follows:

- GALT: Duarte 2 variant[1]
- HCY: MAT I/III variant[3]
- SCD: α-thalassemia syndromes and other mutations (for example, Hb EE, Hb CC, Hb C-HPFH, and heterozygote variants)[4]
- SCID: syndromes with T-cell impairment, secondary T-cell impairment, and variant SCID[2]

Markov Model

Appendix E.A, Figure E.A.1 shows the simulation process of screening for SCD in the context of a true positive (TP). As described in the S and T sections of this report, the benefit of screening and early intervention is preventing sepsis-related mortality with early prophylaxis therapy within the first five years of life. Given that the prevention of sepsis is the main benefit of screening, the risk of other sequelae and etiology of the condition is not considered to be significantly different for infants who are screened or not screened, and these other impacts are excluded in the model, as they would cancel each other out. There is also evidence that suggests that the effectiveness of prophylaxis therapy beyond 5 years of age was not significant.[6] The analysis therefore only includes the benefits of prophylaxis therapy for five years.

The model separates SCD (the primary target) from other secondary conditions that would also be identified through screening. Monitoring costs are factored for one year. For SCD, there are four main states in the model: 1) infants without sepsis; 2) sepsis; 3) death due to sepsis; 4) and all-cause death. Note that all-cause death was included to account for death due to other causes not related to the condition throughout an individual's lifetime. All infants start with having no sepsis and are screened. Those with positive screen results go on to confirmatory testing. If confirmatory testing is positive, they receive treatment. If treatment is successful, the infant does not develop sepsis. Otherwise, they are at risk of developing sepsis and experiencing sepsis-related mortality.

Appendix E.A, Figure E.A.2 shows the simulation process of screening for SCD in the context of a false negative (FN). The model structure is identical to Appendix E.A, Figure E.A.1, with the

exception that an 'undiagnosed' state is added to the model. This state captures the time and resources used to diagnose the condition based on clinical symptomology (that is, the diagnostic odyssey), which can be more difficult and less timely, resulting in higher overall risk of developing sepsis-related morbidity and mortality. The simulation process for a true negative (TN) simply calculates the costs of screening and confirmatory diagnosis for those infants. The simulation process for a false positive (FP) simply calculates the cost of screening and confirmatory diagnosis.

The simulation process for no screening is identical to screening, with the exception that the model structures for TN and FN have the added undiagnosed state to capture the resources associated with the diagnostic odyssey and higher overall risk of developing sepsis-related morbidity and mortality. Costs associated with screening and confirmatory testing are also excluded.

The Markov process for the other conditions uses similar logic to modeling SCD (Appendix E.A, Figures E.A.3 to E.A.10), with the exception that the sequelae that are to be prevented or ameliorated differ between the conditions (refer to the S section for further details). For instance, for HCY, the associated sequelae include lens dislocation, development delay, and spinal osteoporosis, whereas for TYRI they include liver transplantation, liver disease, renal dysfunction, and learning/language difficulties.

Note that for HCY, infants were separated into whether they were vitamin B6-responsive or -unresponsive, which is known to impact clinical management and health outcomes. Furthermore, for TYRI, since a small proportion of TYRI may be detected when screening for PKU (described in the S section), the model subtracts these cases to capture the number of cases attributable to TYRI screening only.

All analyses were conducted using Microsoft Excel 2003 and TreeAge Pro Suite (TreeAge Software Inc.; Williamstown, MA). All proportions of condition-related events and mortality rates were adjusted to 3-month probabilities (see Table E.1).

Model inputs

Model inputs included: the incidence of the condition and associated secondary conditions/variants that would be identified via screening; the test performance of screening and confirmatory testing (that is, sensitivity and specificity); the effectiveness of screening and early detection on the development of condition-associated sequelae; and associated health system costs and resources. This required data was derived from the published literature (see Table E.1). The mortality rate of SCID was based on Alberta program data. The all-cause mortality rate is based on the Alberta life table (see Appendix E.B for details). Cost data included physician, dietician, genetic counselling, medication, hospitalization, and laboratory services that are associated with screening and confirmatory tests, condition management, and the treatment of sequelae. The costs of educational and social services for people with mental disability were also included. The cost data were primarily obtained from Alberta sources; when Alberta data were not available, the costs were derived from the literature. Table E.2 presents the cost inputs and their sources. The hospitalization cost of treatment for spinal osteoporosis was derived from a United States study.[7] The US dollars reported in the study were converted to Canadian dollars using purchasing power parity collected from Statistics Canada. All costs were converted to 2015 Canadian dollars using the Alberta Consumer Price Index (CPI).

Table E.1: Model inputs

Condition	Parameter	Value	Low limit	High limit	Source
Sensitivity and specificity of screening and confirmatory tests					
All conditions	Sensitivity of screening for HCY	38.7%	30.96%	46.44%	Cipriano 2007[8]
	Sensitivity of screening tests	99.0%			T section of this report
	Specificity of screening tests	99.0%			
	Sensitivity of confirmatory tests	99.0%			
	Specificity of confirmatory tests	99.0%			
Incidence of primary targeted conditions					
GALT	Classic galactosemia†	1:60,000	1:80,000	1:14,000	Ontario fact sheet,[9] Applegarth 2000[10]
TYRI	Tyrosinemia type I†	1:100,000	1:120,000	1:80,000	Cipriano 2007[8]
HCY	Homocystinuria	1:250,000	1:300,000	1:200,000	
SCD	Sickle cell disease†	1:5,650	1:6,780	1:4,520	Lieberman 2009[11]
SCID	Severe combined immunodeficiencies	1:58,000	1:100,000	2:58,000	Kwan 2014[2]; EAG members, personal communication, 2015
Incidence of secondary targeted conditions/variants					
GALT	Duarte 2 variant	1:4,000			Carney 2009[1]
TYRI	TYRII	1:250,000			Bouyacoub 2013[5]
HCY	MAT I/III variant	1:27,400			Marcão 2015[3]
SCD	α-thalassemia syndromes	1:9,009			Michlitsch 2009[4]
	Other mutations	1:5,235			
SCID	Syndromes with T-cell impairment, secondary T-cell impairment, and variant SCID	1:11,434			Kwan 2014[2]
Proportion of condition-related events and mortality rate					
GALT	***With screening and early detection***				
	Developmental delay†	35.00%	28.00%	42.00%	Waggoner 1990[12]
	Liver disease	0.00%			
	Cataract	0.00%			EAG members, personal communication, 2015
	Mortality rate, sequelae†	4.76%	3.81%	5.71%	Carroll 2006[13]
	Without screening and delayed detection				
	Developmental delay†	67.00%	53.60%	80.40%	Waggoner 1990[12]
	Liver disease†	41.00%	32.50%	48.75%	Jumbo-Lucioni 2012[14]
	Cataract	100.00%			Assumption, based on EAG members, personal communication, 2015

	Mortality rate, sequelae†	14.00%	11.20%	16.80%	Badawi 1995[15]
	Time elapsed before diagnosed	1 week if presenting <14 days, 4 weeks if >15 days			Jumbo-Lucioni 2012[14]
TYRI	***With screening and early detection***				
	Liver transplantation†	10.00%	8.00%	12.00%	EAG members, personal communication, 2015; Mayorandan 2014[16]
	Liver disease†	0.00%			Mayorandan 2014[16]
	Renal dysfunction†	3.57%	2.86%	4.29%	
	Learning/language difficulties†	35.00%	28.00%	42.00%	Assumption, based on EAG members, personal communication, 2015
	Mortality rate	0.00%			Larochelle 2012[17]
	Without screening and delayed detection				
	Liver transplantation†	26.92%	21.54%	32.31%	Larochelle 2012[17]
	Liver disease†	35.00%	28.00%	42.00%	Mayorandan 2014[16]
	Renal dysfunction†	25.00%	20.00%	30.00%	
	Learning/language difficulties†	72.92%	58.33%	87.50%	
	Combination of symptoms†	44.05%	35.24%	52.86%	
	Mortality rate†	7.69%	6.15%	9.23%	Larochelle 2012[17]
	Time elapsed before diagnosed	3 months			Assumption, based on EAG members, personal communication, 2015
HCY	***With screening and early detection***				
	Developmental delay	9.00%	0.00%	18.00%	Tiwana 2012[7]
	Spinal osteoporosis	5.00%	0.00%	10.00%	
	Lens dislocation	5.00%	0.00%	10.00%	
	Mortality rate	10.00%	0.00%	20.00%	
	Without screening and delayed detection				
	Developmental delay	89.00%	79.00%	99.00%	Tiwana 2012[7]
	Spinal osteoporosis	50.00%	35.00%	67.00%	
	Lens dislocation	100.00%			Assumption, based on EAG members, personal communication, 2015
	Mortality rate	14.00%	4.00%	24.00%	Tiwana 2012[7]
	Vitamin B6-responsive rate	91.00%			Cruysberg 1996[18]
	Lens dislocation leading to HCY	85.60%			Mudd 1985[19]

	Time elapsed before diagnosed	12 months			Assumption, based on EAG members, personal communication, 2015
SCD	***With screening and early detection***				
	Incidence of sepsis†	1.59%	1.27%	1.91%	Panepinto 2000[20]
	Mortality rate, sepsis†	18.30%	14.64%	21.96%	
	Without screening and delayed detection				
	Incidence of sepsis†	9.89%	7.91%	11.87%	Panepinto 2000[20]
	Mortality rate, sepsis†	27.00%	21.60%	32.40%	
	Time elapsed before diagnosed	24 months			Assumption, based on EAG members, personal communication, 2015
SCID	***With screening and early detection***				
	Sequelae	0.00%			Assumption, based on EAG members, personal communication, 2015
	Mortality rate†	8.16%	6.53%	9.80%	Kwan 2014[2]
	Without screening and delayed detection				
	Sequelae	100.00%			Assumption, based on EAG members, personal communication, 2015
	Mortality rate†	72.73%	58.18%	87.27%	Alberta data, EAG members, personal communication, 2015
	Time elapsed before diagnosed	3 months			Assumption, based on EAG members, personal communication, 2015

*Input parameters are assigned beta distributions in sensitivity analysis based on the ranges of variation.
†Range based on an assumption of ±20%.
AHS: Alberta Health Services; EAG: Expert Advisory Group

Table E.2: Cost inputs (in 2015 CAN$)

Disease	Cost description	Cost ($)	Source	Note/Assumption
All conditions	**Screen**			
	Incremental cost of adding GALT to current program	10.18	Laboratory Services (AHS)	Inclusive of equipment, labour and supplies
	Incremental cost of adding TYRI to current program	6.47		
	Incremental cost of adding HCY to current program	0.21		
	Incremental cost of adding SCD to current program	10.13		
	Incremental cost of adding SCID to current program	15.02		

GALT	**Confirmatory testing**			
	Follow-up testing (liver function/GALT quant) and genetic confirmation	1,365.00	Laboratory Services (AHS)	Assume 1% of screen-positive patients receive test
	Treatment for sequelae			
	Hospitalization, development delay, yearly	2,458.39	Thanh 2014[21]	Based on Alberta data
	Physician, development delay, yearly	122.43		
	Costs of education and social services	4,838.40	Thanh 2014, 2015[21,22]	Based on Alberta data
	Hospitalization, lens extraction, one time	5,182.95	IHDA†	
	Physician, lens extraction, one time	273.49	Cipriano 2007[8]	Assume 5% of hospitalization cost
	Hospitalization, liver disease	9,610.97	IHDA	
	Physician, liver disease	439.22	Taylor 2002[23]	Assume 4.57% of hospitalization cost
	Condition management			
	Initial physician consultation, per visit	346.00	Schedule of Medical Benefits	1.5 hours per visit
	Follow-up physician consultation, per visit	123.60	Schedule of Medical Benefits	Every 3 months less than 1 year of age and every 6 months afterwards
	Diet, medication, yearly	12,319.73	Carroll 2006[13]	Based on Hannigan 2007,[29] assume1 year treatment
	Dietitian, yearly	172.76	ALIS, Expert Advisory Group	Assume 4 visits per year for 1 years, 1 hour each visit, wage $43.19 per hour
	Genetic counselling, one time	266.37	ALIS, Expert Advisory Group	5-8 hours, wage $40.98 per hour
	Follow-up evaluation			
	Erythrocyte galactose-1-phosphate concentration	119.82	Laboratory Services (AHS)	
	Brain MRI	99.41	Schedule of Medical Benefits	
	Eye examination	54.97		
TYRI	**Confirmatory testing**			
	Follow-up testing (Tyr/AAQ/FAH enzyme/liver function) and genetic confirmation	2,992.00	Laboratory Services (AHS)	Assume 1% of screen-positive patients receive test
	Treatment for sequelae			
	Hospitalization, liver transplant	83,067.30	IHDA	

	Physician, liver transplant	3,796.18	Taylor 2002[23]	Assume 4.57% of hospitalization cost
	Hospitalization, post-transplant management	14,658.94	Taylor 2002[23]	Costs of post-transplantation account for 15% of overall costs
	Physician, post-transplant management	1,172.71	Letarte 2002[24]	Assume 8% of hospitalization cost
	Hospitalization, liver disease	9,610.97	IHDA	
	Physician, liver disease	439.22	Taylor 2002[23]	Assume 4.57% of hospitalization cost
	Hospitalization, kidney disease	14,253.44	IHDA	
	Physician, kidney disease	651.38	Taylor 2002[23]	Assume 4.57% of hospitalization cost
	Occupational therapist	1,046.58	Thompson 2011[25]	Assume unit cost of $50.29 per hour and 19.36 hours per year
	Physical therapist	514.64		Assume unit cost of $50.29 per hour and 9.52 hours per year
	Condition management			
	Initial physician consultation, per visit	346.00	Schedule of Medical Benefits	1.5 hours per visit
	Follow-up physician consultation, per visit	123.60	Schedule of Medical Benefits	Every 3 months less than 1 year of age and every 6 months afterwards
	Medication, yearly	120,152.20	Cipriano 2007[8]	Based on Hannigan 2007, assume lifelong treatment
	Diet by age (years), yearly		Genetic Lab Services and Alberta Children's Hospital, personal communication, 2015	Based on Hannigan 2007, assumes lifelong treatment
	0 to 1	3,253.33		
	1 to 5	6,190.52		
	6 to 12	8,832.61		
	12 to 18	13,392.26		
	18+	8,869.00		
	Dietitian, yearly	173.00	ALIS	4 visits per year, 1 hour each visit, lifelong
	Genetic counselling, one time	266.37	ALIS, Expert Advisory Group	5-8 hours, wage $40.98 per hour
	Follow-up evaluation			
	Complete blood count (CBC)	17.94	Schedule of Medical Benefits	
	Abdominal imagining CT	80.61		
	X-ray of wrist	54.50		
	Partial thromboplastin time (PTT)	16.54		

	Abdominal ultrasound	255.81		
HCY	**Confirmatory testing**			
	Follow-up testing (plasma homocysteine/CBS enzyme) and genetic confirmation	1,540.00	Laboratory Services (AHS)	Assume 1% of screen-positive patients receive test
	Treatment of sequelae			
	Hospitalization, lens extraction, one time	5,182.95	IHDA	
	Physician, lens extraction, one time	273.49	Cipriano 2007[8]	Assume 5% of hospitalization cost
	Hospitalization, development delay, yearly	2,458.39	Thanh 2014[21]	Alberta data
	Physician, development delay, yearly	122.43		
	Educational and social services	4,838.40	Thanh 2014, 2015[21,22]	Alberta data
	Hospitalization, spinal osteoporosis, yearly	378.74	Tiwana 2012[7]	
	Physician, spinal osteoporosis, yearly	54.33	Brown 2013[26]	Assume 14% of hospitalization cost
	Condition management			
	Initial physician consultation, per visit	346.00	Schedule of Medical Benefits	1.5 hours per visit
	Follow-up physician consultation, per visit	123.60	Schedule of Medical Benefits	Every 3 months less than 1 year of age and every 6 months afterwards
	Diet by age (years), yearly		Genetic Laboratory Services and Alberta Children's Hospital, personal communication, 2015	Based on Hannigan 2007, assume lifelong treatment for vitamin B6-non-responsive and 1 year treatment for vitamin B6-responsive
	0 to 1	4,164.27		
	1 to 5	7,923.86		
	6 to 12	11,305.74		
	12 to 18	17,142.10		
	18+	11,352.32		
	Dietitian, yearly	172.76	ALIS, Expert Advisory Group	Assume 4 visits per year for 5 years, 1 hour each visit, wage $43.19 per hour
	Yearly cost of medications	4,363.42	Tiwana 2012[7]	Assume lifelong treatment for vitamin B6-non-responsive and 1 year for vitamin B6-responsive
	Genetic counselling, one time	266.37	ALIS, Expert Advisory Group	5-8 hours, wage $40.98 per hour

	Follow-up evaluation			
	Total plasma homocysteine on plasma	95.00	Laboratory Services (AHS)	4 times per year for children and 2 times per year for adult
	Enzyme test (measuring CBS)	175.00		
SCD	**Confirmatory testing**			
	Follow-up testing (HPLC + CBC) and genetic confirmation	1,330.00	Laboratory Services (AHS)	Assume 1% of screen-positive patients receive test
	Treatment for sequelae			
	Hospitalization, newborn sepsis, yearly	10,663.30	IHDA	Physician cost is assumed to be same as that of follow-up physician consultation
	Oral amoxicillin, yearly	337.63	Alberta drug benefit	Amoxicillin, DIN/PIN 02036347, 125 mg, unit cost $0.4625, 125 mg twice daily
	Condition management			
	Initial physician consultation, per visit	346.00	Schedule of Medical Benefits	1.5 hours per visit
	Follow-up physician consultation, per visit	123.60	Schedule of Medical Benefits	Every 3 months less than 1 year of age and every 6 months afterwards
	Genetic counselling, one time	163.92	ALIS, Expert Advisory Group	4 hours, wage $40.98 per hour
	Follow-up evaluation			
	Transcranial ultrasound, per test	325.18	Schedule of Medical Benefits	Once per year
	CBC, per test	17.94	Schedule of Medical Benefits	Every 3 months less than 1 year of age and every 6 months afterwards
SCID	**Confirmatory testing**			
	Flow cytometry confirmation, blood count, and genetic confirmation	4,892	Laboratory Services (AHS)	Assume 1% of screen-positive patients receive the test
	Treatment for sequelae (early treatment)**			
	Hospitalization, HSCT, one time	81,818.59	IHDA	
	Physician, HSCT, one time	6,545.49	Letarte 2002[24]	Based on Letarte 2002, assume 8% of hospitalization cost
	Hospitalization, post-transplant management, one time	39,569.83	IHDA, Holbro 2013[27]	Based on Holbro 2013, assume 48% of hospitalization cost
	Physician, post-transplant management, one time	3,165.59	Letarte 2002[24]	Based on Letarte 2002, assume 8% of

				hospitalization cost
	Treatment for sequelae (late treatment)**			
	Hospitalization, HSCT, one time	245,455.77	Chan 2011,[28]	Assume that the cost of HSCT for late treatment is 3 times higher than the early treatment. Physician cost of HSCT is assumed to be the same between early and late treatment
	Physician, HSCT, one time	6,545.49		
	Hospitalization, post-transplant management, one time	118,709.49		
	Physician, post-transplant management, one time	9,496.76		
	Condition management			
	Initial physician consultation, per visit	346.00	Schedule of Medical Benefits	1.5 hours per visit
	Follow-up physician consultation, per visit	123.60	Schedule of Medical Benefits	Every 3 month less than 1 year and 6 months greater than 1 year
	Genetic counselling, one time	266.37	ALIS, Expert Advisory Group	5-8 hours, wage $40.98 per hour

*All costs were in 2015 CAD$

†IHDA is an online data repository that provides health statistics for Alberta

AHS: Alberta Health Services; ALIS: Alberta Learning Information Service; CBC: complete blood count; CBS: cystathionine beta-synthase; FAH: fumarylacetoacetate hydrolase; HPLC: high performance liquid chromatography; HSCT: hematopoietic stem cell transplantation; IHDA: Interactive Health Data Application; PTT: partial thromboplastin time

Model outputs

The outputs generated from the model are as follows:

- Infants with the primary condition with positive test results (true positives)
- Healthy infants (non-condition) with positive screen results (false positives)
- Healthy infants with negative screen results (true negatives)
- Infants with the primary condition with negative test results (false negatives)
- Cases of secondary conditions/variants detected
- Life years
- Total costs per infants screened
- Incremental costs per life year gained

The primary outcome measure to assess value was life years. However, it is acknowledged that this measure of outcome does not account for quality of life. Health-related quality of life data from which a cost utility analysis that accounts for both quantity and quality of life could be drawn is not available.

Criteria for Cost-Effectiveness

The criteria for concluding that an alternative is cost-effective are as follows:

1. Alternatives that are both more costly and less effective compared to other alternatives are dominated and are considered NOT cost-effective. These are eliminated from further consideration.
2. Alternatives that are less costly and more effective compared to other alternatives are dominant and are considered cost-effective. These are included for further consideration.
3. Alternatives that are both more costly and more effective (or less costly and less effective) are not dominant and their cost-effectiveness is uncertain.
    a) Within these alternatives, there can be a situation of extended dominance. That is, among these alternatives there are some alternatives that are more cost-efficient than others. Alternatives that are dominated by extension are not considered cost-effective, and are excluded from further consideration.
    b) For the remaining alternatives that are not dominated by extension, cost-effectiveness is dependent on whether the additional effectiveness is worth the additional costs, which is determined by examining the opportunity cost of adopting the technology.

Sensitivity Analysis

It is important to provide information regarding the degree of variability (that is, the uncertainty) in potential costs and effectiveness to enable decision-makers to evaluate the credible range of potential costs and outcomes. Therefore, a probabilistic sensitivity analysis was conducted using 5000 Monte Carlo simulations on the ranges listed in Tables E.1 and E.2 (based on an assumption of ±20%) to generate the distribution of potential costs and effectiveness associated with each alternative screening option.

A one-way sensitivity analysis was conducted to determine the impact of varying incidence rates on the number of cases detected. A separate one-way sensitivity analysis was also conducted to determine the impact of varying the cost difference between early versus late HSCT for SCID.

Impact of Differential Timing

Given the lifelong time horizon, costs and life years are discounted at an annual rate of 5%.

Cost Attribution Analysis

Each condition has differential resource implications to disparate health sectors, including laboratory, hospitalization, physician, dietician, genetic counselling, medication, diet services, and educational and social services of managing a mental disorder. Differentiating the resource implications of each condition on disparate health sectors from their total system impact is important for elucidating how screening for the condition potentially impacts disparate sectors of the health system that are relevant to the technology in question. Accordingly, a cost attribution analysis was conducted to differentiate the resource implications to disparate health sectors between the conditions.

Budget Impact Analysis

A budget impact analysis (BIA) model was developed to assess the financial impact on the Alberta health system from adding the conditions to the current NMS Program. With seven conditions, there are numerous combinations of screening options, from adding a single condition to adding all the conditions, and all of the possible second-, third- and fourth-order combinations in-between. The BIA will exclude screening options with cost-effectiveness worse than the option of screening for all conditions.

The data sources for the BIA are the same as those used in the CEA, and implementation cost data were from AHS Laboratory Services. The BIA estimated the financial impact over one year after adding the conditions. The time horizon of the BIA was five years, from 2016 to 2020. All costs were presented in 2015 Canadian dollars and with future costs adjusted using the Alberta consumer price index.

4.2 Results

Performance of Screen and Confirmatory Tests

The cases of primary conditions detected (that is, true positives) ranges from 0.02 (of HCY) to 1.75 (of SCD) per 10,000 infants screened (Table E.3). The false positive rate is about 100 per 10,000 infants for all conditions. The cases of secondary targeted conditions/variants are from 0.14 to 2.99 per 10,000 infants screened.

Table E.3: Cases per 10,000 infants

Strategy	TP	FP	TN	FN	Secondary targets/Variants
GALT screening	0.17	100	9,897	0.03	2.48
TYRI screening	0.08	100	9,900	0.001	-
HCY screening	0.02	100	9,900	0.25	0.14
SCD screening	1.75	100	9,895	0.05	2.99
SCID screening	0.17	100	9,899	0.01	0.87

Note: The sensitivity and specificity are 0.99 for all screening tests with one exception. The sensitivity of screening for HCY is 0.387.

Costs-Effectiveness Analysis

Table E.4 shows the cost-effectiveness of adding the conditions to the current NMS Program. When considering only adding single conditions, the estimated added cost of adding each condition ranges from $0.43 to $13.89 per infant screened, with HCY being the least costly, followed by TYRI, GALT, SCD, and SCID. The estimated additional cost per life year gained ranged from $2,621 to $332,360, with screening for SCD providing the greatest value illustrated by the lowest incremental cost-effectiveness ratio (ICER) (that is, the greatest health return for resources invested), followed by HCY, TYRI, GALT, and SCID. The estimated ICER ranged from $5,553 to $244,595 per life year gained when examining second-order combinations, from $3,069 to $170,166 when examining third-order combinations, and from $5,348 to $130,764 when examining fourth-order combinations. If all conditions were screened, the estimated ICER is $8,155 per life year gained.

Table E.5 shows the cost-effectiveness of these combinations, ranked in order of their increasing ICER. Those conditions shaded in dark grey (below the option of screening for all conditions) have an ICER greater than that of screening for all seven conditions. Thus, those options are excluded from further consideration because they provide less value for money than simply screening for all seven conditions.

Table E.4: Cost-effectiveness of screening versus no screening

Strategy	Life long cost per infant screened	Life year	Incr. cost	Life year saved	ICER
HCY No Screening	$0.59	79.06049			
HCY Screening	$1.01	79.06051	$0.43	0.00002	$25,452.50
GALT No Screening	$2.92	79.06054			
GALT Screening	$14.21	79.06063	$11.28	0.00009	$122,749.04
TYRI No Screening	$26.50	79.06061			
TYRI Screening	$28.40	79.06067	$1.90	0.00006	$31,723.53
SCD No Screening	$2.21	79.04959			
SCD Screening	$14.42	79.05425	$12.21	0.00466	$2,620.73
SCID No Screening	$6.36	79.06065			
SCID Screening	$20.26	79.06069	$13.89	0.00004	$332,360.39
Adding combined conditions					
No Screening	$3.51	158.1210			
HCY + GALT Screening	$15.22	158.1211	$11.71	0.00011	$107,755.20
No Screening	$27.09	158.1211			
HCY + TYRI Screening	$29.41	158.1212	$2.33	0.00008	$30,352.81
No Screening	$2.80	158.1101			
HCY + SCD Screening	$15.43	158.1148	$12.64	0.00468	$2,702.49
No Screening	$6.95	158.1211			
HCY + SCID Screening	$21.27	158.1212	$14.32	0.00006	$244,595.49
No Screening	$29.42	158.1211			
GALT + TYRI Screening	$42.60	158.1213	$13.18	0.00015	$86,847.52
No Screening	$5.14	158.1101			
GALT + SCD Screening	$28.63	158.1149	$23.49	0.00475	$4,944.51
No Screening	$9.29	158.1212			
GALT + SCID Screening	$34.46	158.1213	$25.18	0.00013	$188,286.63
No Screening	$28.71	158.1102			
TYRI + SCD Screening	$42.82	158.1149	$14.11	0.00472	$2,989.88
No Screening	$32.86	158.1213			

TYRI + SCID Screening	$48.66	158.1214	$15.79	0.00010	$155,353.46
No Screening	$8.57	158.1102			
SCD + SCID Screening	$34.68	158.1149	$26.11	0.00470	$5,553.17
No Screening	$30.01	237.1816			
HCY + GALT + TYRI Screening	$43.62	237.1818	$13.61	0.00017	$80,747.11
No Screening	$5.72	237.1706			
HCY + GALT + SCD Screening	$29.64	237.1754	$23.92	0.00477	$5,016.53
No Screening	$9.87	237.1817			
HCY + GALT + SCID Screening	$35.47	237.1818	$25.60	0.00015	$170,165.83
No Screening	$33.45	237.1817			
HCY + TYRI + SCID Screening	$49.67	237.1818	$16.22	0.00012	$136,984.88
No Screening	$29.30	237.1706			
HCY + TYRI + SCD Screening	$43.83	237.1754	$14.54	0.00474	$3,069.29
No Screening	$9.16	237.1707			
HCY + SCD + SCID Screening	$35.69	237.1754	$26.53	0.00472	$5,623.80
No Screening	$31.64	237.1707			
GALT + TYRI + SCD Screening	$57.03	237.1755	$25.39	0.00481	$5,277.69
No Screening	$35.79	237.1818			
GALT + TYRI + SCID Screening	$62.86	237.1820	$27.07	0.00019	$139,873.07
No Screening	$35.08	237.1708			
TYRI + SCD + SCID Screening	$63.08	237.1756	$28.00	0.00476	$5,882.21
No Screening	$11.50	237.1707			
GALT + SCD + SCID Screening	$48.89	237.1755	$37.39	0.00479	$7,800.45
No Screening	$32.22	316.2312			
GALT + HCY + TYRI + SCD Screening	$58.04	316.2361	$25.82	0.00483	$5,347.66
No Screening	$36.37	316.2423			
HCY + GALT + TYRI + SCID Screening	$63.87	316.2425	$27.50	0.00021	$130,763.89
No Screening	$38.00	316.2314			
GALT + TYRI + SCD + SCID Screening	$77.28	316.2362	$39.29	0.00485	$8,095.53
No Screening	$35.66	316.2313			
HCY + TYRI + SCD + SCID Screening	$64.09	316.2361	$28.43	0.00478	$5,950.79
No Screening	$12.08	316.2312			
HCY + GALT + SCD + SCID Screening	$49.90	316.2360	$37.81	0.00481	$7,861.90
Total No Screening	**$38.58**	**395.2919**			
Total Screening	**$78.30**	**395.2967**	**$39.71**	**0.00487**	**$8,155.21**

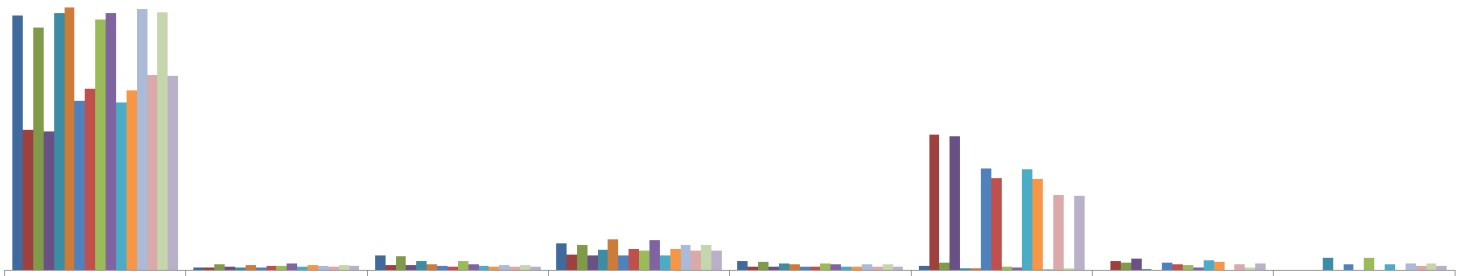
Table E.5: Cost-effectiveness rankings

Strategy	ICER
SCD Screening	$2,620.73
HCY + SCD Screening	$2,702.49
TYRI + SCD Screening	$2,989.88
HCY + TYRI + SCD Screening	$3,069.29
GALT + SCD Screening	$4,944.51
HCY + GALT + SCD Screening	$5,016.53
GALT + TYRI + SCD Screening	$5,277.69
GALT + HCY + TYRI + SCD Screening	$5,347.66
SCD + SCID Screening	$5,553.17
HCY + SCD + SCID Screening	$5,623.80
TYRI + SCD + SCID Screening	$5,882.21
HCY + TYRI + SCD + SCID Screening	$5,950.79
GALT + SCD + SCID Screening	$7,800.45
HCY + GALT + SCD + SCID Screening	$7,861.90
GALT + TYRI + SCD + SCID Screening	$8,095.53
Total Screening	**$8,155.21**
HCY Screening	$25,452.50
HCY + TYRI Screening	$30,352.81
TYRI Screening	$31,723.53
HCY + GALT + TYRI Screening	$80,747.11
GALT + TYRI Screening	$86,847.52
HCY + GALT Screening	$107,755.20
GALT Screening	$122,749.04
HCY + GALT + TYRI + SCID Screening	$130,763.89
HCY + TYRI + SCID Screening	$136,984.88
GALT + TYRI + SCID Screening	$139,873.07
TYRI + SCID Screening	$155,353.46
HCY + GALT + SCID Screening	$170,165.83
GALT + SCID Screening	$188,286.63
HCY + SCID Screening	$244,595.49
SCID Screening	$332,360.39

Cost Attribution Analysis

The cost attribution analysis Figure E.1 shows lifelong cost per infants screened, broken down into service sectors (also see Appendix E.C). The estimated laboratory cost of screening and confirmatory testing comprise the majority of total costs ranging from 46 to 85%, depending on the condition(s) being screened for.

Figure E.1: Distribution of costs across disparate budgetary areas



	Screen	Confirm test	Followup test	Hospital/other AHS pay*	Physician	Medication	Diet	Edu/Soc†
SCD Screening	81.0%	1.0%	4.8%	8.7%	2.9%	1.6%	0.0%	0.0%
TYRI +SCD Screening	44.6%	1.0%	1.8%	5.0%	1.2%	43.3%	3.1%	0.0%
HCY+SCD Screening	77.3%	1.9%	4.6%	8.2%	2.9%	2.5%	2.5%	0.1%
HCY + TYR + SCD	44.2%	1.4%	1.8%	4.9%	1.2%	42.6%	3.9%	0.0%
GALT + SCD Screening	81.7%	1.0%	3.0%	6.5%	2.4%	0.8%	0.6%	4.1%
SCD + SCIDScreening	83.5%	1.8%	2.0%	9.9%	2.1%	0.7%	0.0%	0.0%
GALT + TYRI + SCD Screening	54.0%	1.0%	1.6%	4.8%	1.3%	32.5%	2.6%	2.1%
TYRI + SCD + SCIDScreening	57.7%	1.5%	1.2%	6.8%	1.3%	29.4%	2.1%	0.0%
HCY+GALT + SCD Screening	79.7%	1.5%	3.0%	6.4%	2.3%	1.3%	1.8%	4.0%
HCY +SCD + SCID	81.8%	2.2%	2.0%	9.7%	2.1%	1.1%	1.1%	0.0%
GALT + HCY+ TYRI +SCD Screening	53.5%	1.3%	1.6%	4.8%	1.3%	32.2%	3.2%	2.1%
HCY + TYRI +SCD +SCID Screening	57.2%	1.7%	1.2%	6.8%	1.3%	29.2%	2.7%	0.0%
GALT + SCD + SCID	83.2%	1.6%	1.7%	8.3%	2.0%	0.5%	0.3%	2.4%
GALT + TYRI +SCD +SCID Screening	62.2%	1.4%	1.2%	6.4%	1.4%	24.0%	1.9%	1.5%
HCY + GALT + SCD + SCID	82.0%	1.9%	1.8%	8.2%	2.0%	0.8%	1.1%	2.4%
Total screening	61.7%	1.6%	1.2%	6.3%	1.4%	23.9%	2.4%	1.5%

*These are costs of hospitalization and other services paid by Alberta Health Services including genetic counselling and dieticians.

†Edu/Soc = educational and social services for mental disability

Sensitivity Analysis

Probabilistic Sensitivity Analysis

Figures E.2 to E.6 show the incremental costs-effectiveness scatter plots of screening versus non-screening options for SCD, SCID, TYRI, HCY, and GALT, respectively. The plot shows the scatters of incremental costs and life years generated from the 5000 Monte Carlo simulations. In these figures, 100% of the scatter points are located in the first quadrant for all conditions except for TYRI (Figure E.4) and HCY (Figure E.5), in which 98% and 81% of the points are in the first quadrant, respectively. Scatter points in the first quadrant means that the screening option gains life years but is costly, compared with non-screening. These results indicate that they are consistent and not sensitive to changing model assumptions.

Figure E.2: Incremental cost-effectiveness, SCD screening versus SCD no screening

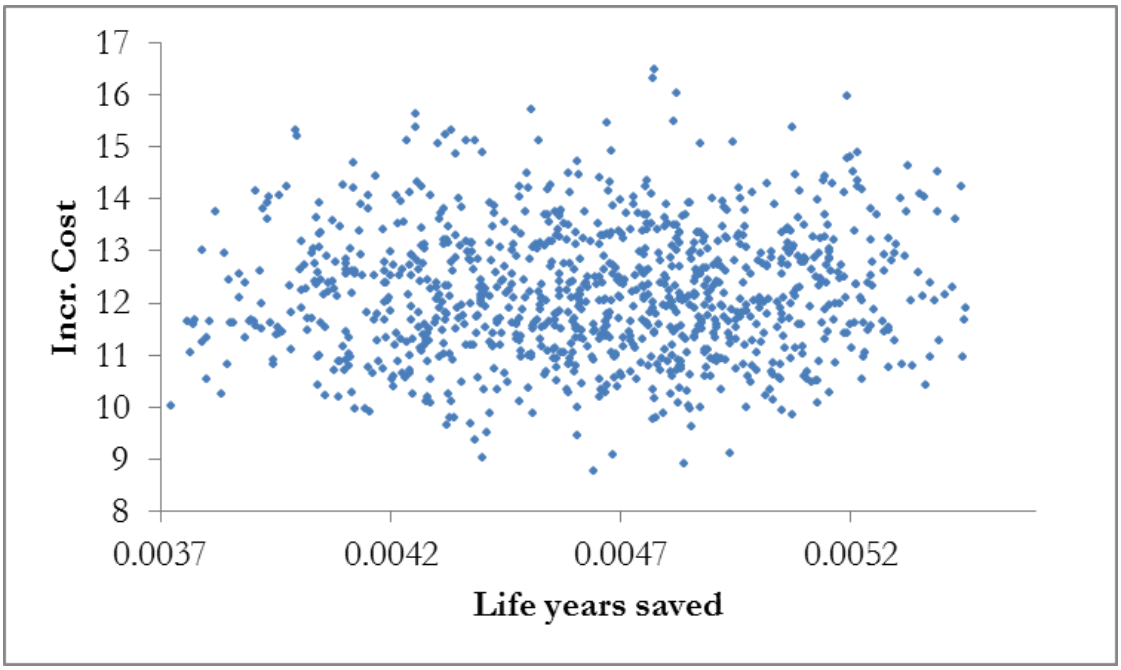


Figure E.3: Incremental cost-effectiveness, SCID screening versus SCID no screening

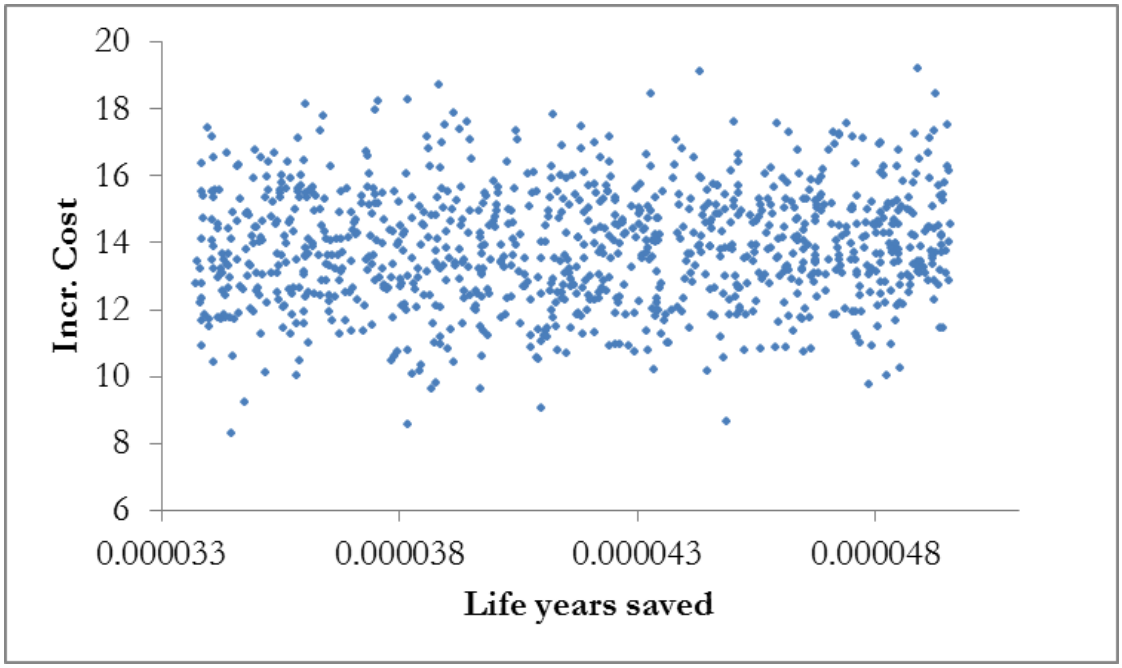


Figure E.4: Incremental cost-effectiveness, TYRI screening versus TYRI no screening

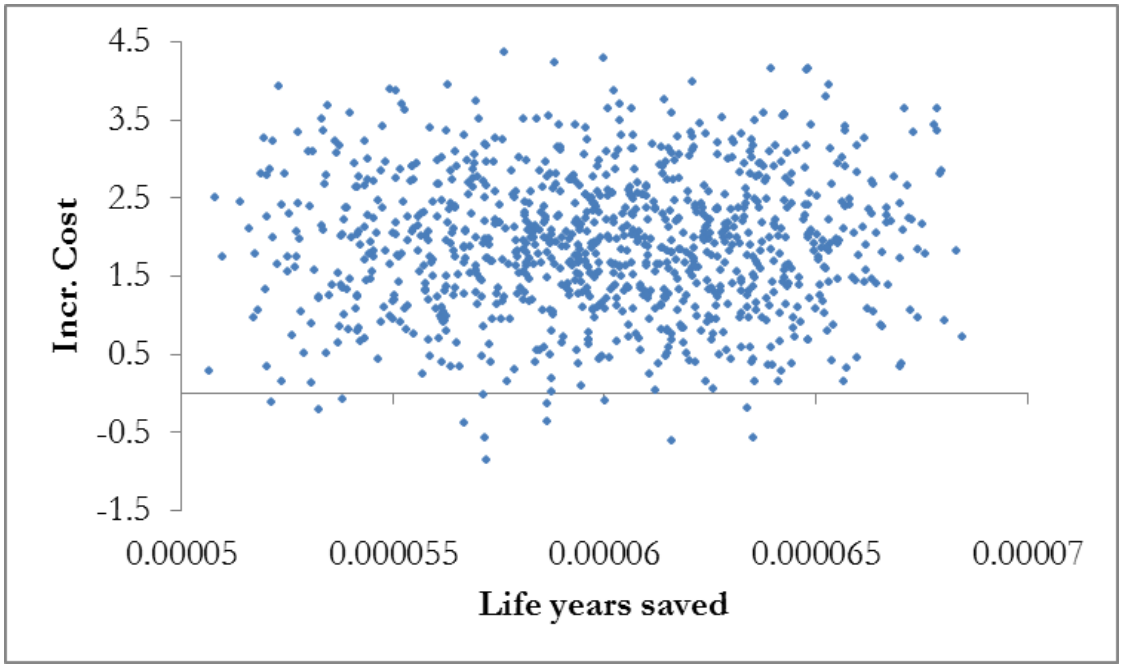


Figure E.5: Incremental cost-effectiveness, HCY screening versus HCY no screening

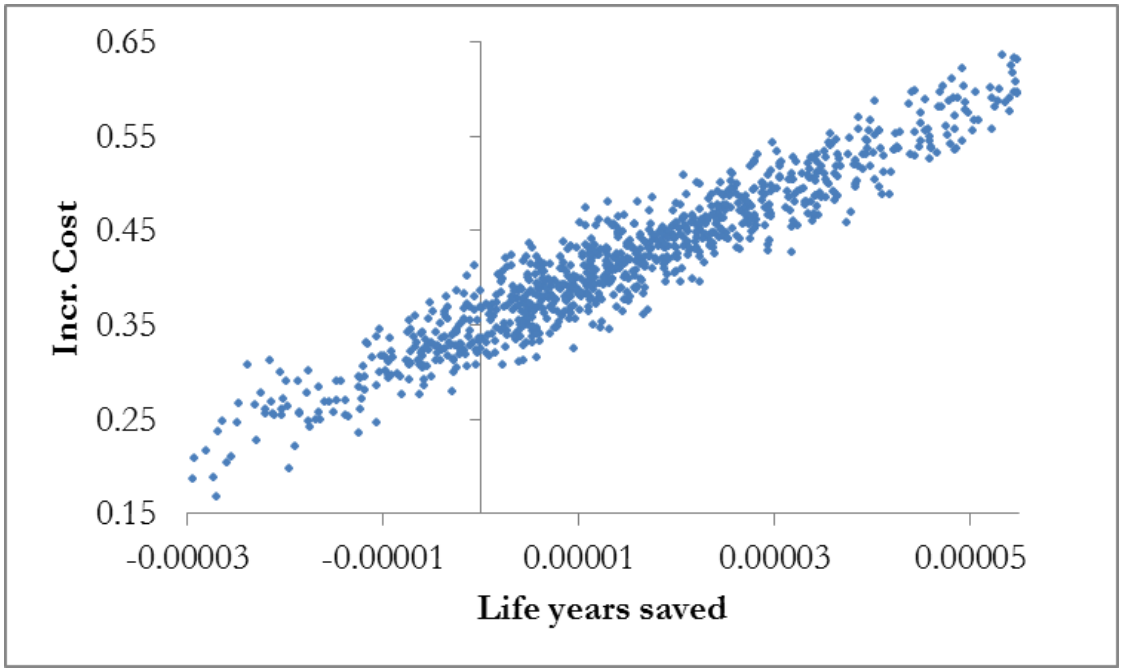
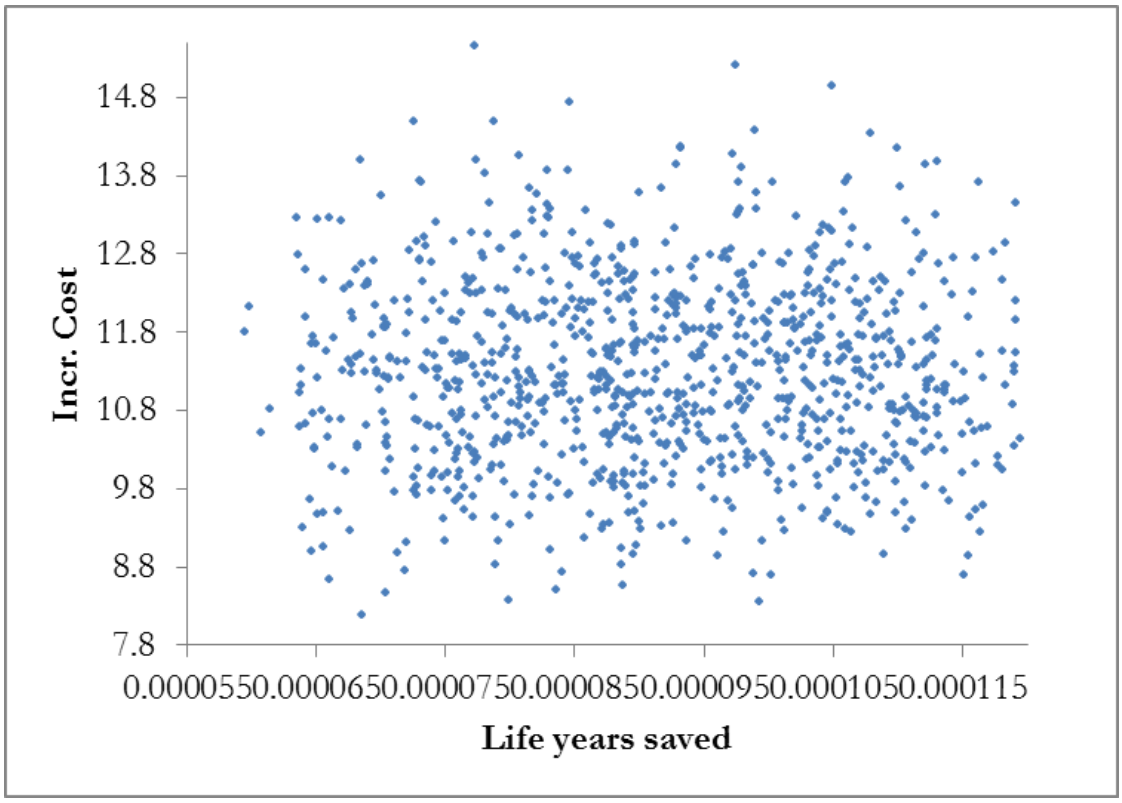


Figure E.6: Incremental cost-effectiveness, GALT screening versus GALT no screening



One-Way Sensitivity Analysis

Table E.6 and E.7 show how varying incidence rates impact the number of cases detected and total average cost per screen. Table E.8 shows how the cost difference between early versus late HSCT transplant can impact total average cost per screen for SCID.

Table E.6: One-way sensitivity analysis of varying incidence rates and its impact on the number of cases detected (per 10,000 infants screened)

Strategy	Incidence rate	TP	FP	TN	FN
Low limit					
GALT Screening	1:80,000	0.12	100	9,897	0.03
SCD Screening	1:6,780	1.46	100	9,896	0.04
HCY Screening	1:300,000	0.01	100	9,900	0.24
SCID Screening	1:100,000	0.10	100	9,899	0.01
TYRI Screening	1:120,000	0.06	100	9,900	0.00
High limit					
GALT Screening	1:14,000	0.71	100	9,897	0.03
SCD Screening	1:4,520	2.19	100	9,895	0.05
HCY Screening	1:200,000	0.02	100	9,900	0.25
SCID Screening	2:58,000	0.34	100	9,899	0.01
TYRI Screening	1:80,000	0.10	100	9,900	0.00

FN: false negative; FP: false positive; TN: true negative; TP: true positive

Table E.7: One-way sensitivity analysis of varying incidence and its impact on total average cost per screen

Condition not added/added*	Base case value	Cost per patient screened	Low limit	Cost per patient screened	High limit	Cost per patient screened
HCY No Screening	1:250,000	$0.59	1:300,000	$0.45	1:200,000	$0.65
HCY Screening		$1.01		$0.85		$1.07
SCD No Screening	1:5,650	$2.21	1:6,780	$1.85	1:4,520	$2.70
SCD Screening		$14.42		$13.94		$15.00
SCID No Screening	1:58,000	$6.36	1:100,000	$3.73	2:58,000	$12.57
SCID Screening		$20.26		$19.23		$22.68

TYRI No Screening	1:100,000	$26.50	1:120,000	$22.06	1:80,000	$33.21
TYRI Screening		$28.40		$23.96		$35.12
GALT No Screening	1:60,000	$2.92	1:80,000	$2.29	1:14,000	$11.26
GALT Screening		$14.21		$13.66		$21.94
Total No Screening		$38.58		$30.38		$60.39
Total Screening		$78.30		$71.64		$95.81

* This table compares the cost per patient of adding a condition to the program versus not adding it, over a range of incidence rates.

Table E.8: One-way sensitivity analysis of varying the cost difference between early versus late HSCT for the treatment of SCID

Inflator	Strategy*	Cost per patient screened	Incr. cost	ICER
1	SCID No Screening	$2.29		
	SCID Screening	$20.17	$17.89	$427,830
1.5	SCID No Screening	$3.30		
	SCID Screening	$20.19	$16.89	$404,051
2	SCID No Screening	$4.32		
	SCID Screening	$20.21	$15.90	$380,272
2.5	SCID No Screening	$5.33		
	SCID Screening	$20.23	$14.90	$356,493
3	SCID No Screening	$6.36		
	SCID Screening	$20.26	$13.89	$332,360

* This table compares the cost of adding SCID to the program versus not adding it, over a range of inflator that measures the cost difference between early and late HSCT.

Budget Impact Analysis

The BIA estimated the financial impact from 2016 to 2020 of adding the screening options that were not excluded in Table E.5. Demand for screening was estimated based on the numbers of live births in Alberta (Table E.9). Based on data from Statistics Canada, the increase rate of live births increased at an average pace of 0.83% from 2011 to 2014 in Alberta. This rate was applied to project future live births from 2016 to 2020, as shown in Table E.9.

Table E.9: Projected live births from 2016 to 2020

	2011	2012	2013	2014	Average
Live birth*	50,853	52,237	54,211	56,582	
Increase rate		2.72%	3.78%	4.37%	
Incremental increase			1.06%	0.59%	0.83%
	2016	**2017**	**2018**	**2019**	**2020**
Projected increase rate	6.03%	6.85%	7.68%	8.50%	9.33%
Projected live births**	63,111	67,435	72,613	78,787	86,138

*Data on the live births were collected from Statistics Canada.

**The live births were projected using the estimated incremental increase in birth rate between 2011 and 2014.

The cost results from the Markov model represent the average lifetime cost per infant, which can be used to estimate the budget impact. However, costs are not evenly distributed over the infant's lifetime, as the major proportion of costs occurred in the first year during screening and confirmatory testing. The cost inputs were therefore adjusted accordingly (see Table E.10). These estimates also exclude the costs associated with education and social services to reflect only the investment costs paid by the public payer system. The incremental costs listed in the table were applied to the estimates in Table E.11 to estimate the BIA. There is a one-time cost to Alberta Health of approximately $26,000 to re-configure the alerting system software (EAG members, personal communication, August 2015).The cost in 2015 was adjusted to future years by using average consumer price index of 1.7%[xxiii] during the past five years in Alberta.

Table E.10: Cost per infant screened by year (2015 $CAD)

Strategy	1st year	2nd year	3rd year	4th year	5th year
HCY No Screening	$0.08	$0.04	$0.05	$0.04	$0.04
HCY Screening	$0.43	$0.05	$0.05	$0.04	$0.04
Incremental*	$0.35	$0.00	$0.00	$0.00	$0.00
GALT No Screening	$0.55	$0.10	$0.10	$0.11	$0.11
GALT Screening	$12.16	$0.09	$0.01	$0.10	$0.04
Incremental	$11.62	-$0.01	-$0.09	$0.00	-$0.07
SCD No Screening	$0.74	$0.45	$0.32	$0.24	$0.16
SCD Screening	$12.16	$0.28	$0.19	$0.20	$0.25
Incremental	$11.42	-$0.17	-$0.13	-$0.04	$0.09
TYRI No Screening	$0.87	$1.19	$1.14	$1.08	$1.08
TYRI Screening	$8.68	$0.89	$0.89	$0.83	$0.82
Incremental	$7.80	-$0.30	-$0.25	-$0.25	-$0.26

[xxiii] Based on data from Statistics Canada, the Alberta annual rate of CPI from 2010 to 2014 was 2.44%, 1.11%, 1.42% and 2.56%, respectively. The average annual rate over the five years was 1.7%.

SCID No Screening	$6.33	$0.02	$0.01	-$0.02	$0.01
SCID Screening	$20.15	$0.07	-$0.04	$0.00	$0.04
Incremental	$13.81	$0.06	-$0.05	$0.03	$0.03

*Incremental cost of screening versus non-screening for each condition.

Table E.11 shows the results of the budget impact analysis. The BIA in a year was the sum total of the cost occurred in the first year plus the subsequent cost for individuals screened in previous years. For instance, the BIA in 2020 is equal to the first year cost for infants screened in 2020 (that is, the current year) plus the second year cost for those born in 2019, the third year cost for those born in 2018, and the fourth year cost for those born in 2017. Note that there are no operational costs incurred in 2016, as these costs are not incurred during the implementation period which is assumed to take place in 2016. Total costs excluding and including potential cost savings by health sector is presented in Appendix E.D.

Table E.11: Budget impact analysis ($ in millions)

Strategy	2016	2017	2018	2019	2020	Cumulative	% in 1st year
Add SCD	$0.43	$0.66	$0.74	$0.84	$0.95	$3.61	11.80%
Add HCY + SCD	$0.48	$0.67	$0.76	$0.86	$0.98	$3.75	12.71%
Add TYRI + SCD	$0.63	$1.19	$1.36	$1.55	$1.77	$6.49	9.66%
Add HCY + TYRI + SCD	$0.68	$1.20	$1.38	$1.57	$1.80	$6.63	10.22%
Add GALT + SCD	$1.16	$1.22	$1.37	$1.54	$1.74	$7.03	16.56%
Add HCY + GALT + SCD	$1.21	$1.24	$1.38	$1.56	$1.77	$7.16	16.94%
Add GALT + TYRI + SCD	$1.36	$1.75	$1.98	$2.25	$2.56	$9.91	13.77%
Add GALT + HCY + TYRI + SCD	$1.41	$1.77	$2.00	$2.27	$2.59	$10.04	14.08%
Add SCD + SCID	$1.26	$1.67	$1.86	$2.08	$2.35	$9.23	13.68%
Add HCY + SCD + SCID	$1.31	$1.69	$1.88	$2.11	$2.38	$9.37	14.02%
Add TYRI + SCD + SCID	$1.46	$2.20	$2.48	$2.80	$3.17	$12.11	12.09%
Add HCY + TYRI + SCD + SCID	$1.51	$2.22	$2.50	$2.82	$3.20	$12.25	12.36%
Add GALT + SCD + SCID	$2.00	$2.24	$2.49	$2.78	$3.14	$12.64	15.82%
Add HCY + GALT + SCD + SCID	$2.05	$2.25	$2.50	$2.81	$3.17	$12.78	16.04%
Add GALT + TYRI + SCD + SCID	$2.18	$2.76	$3.10	$3.50	$3.96	$15.52	14.18%
Add all conditions	**$2.25**	**$2.80**	**$3.15**	**$3.57**	**$4.06**	**$15.82**	**14.22%**

4.3 Discussion

The objective of the analysis was to determine the value for money and budget impact of adding GALT, TYRI, HCY, SCD (Hb SS, Hb SC, and Hb S/β-thal), and SCID to the Alberta NMS Program, singly or in combination. According to the economic analysis, screening for the seven conditions under review would not improve health outcomes at a net cost saving to the health system. Screening for any of the conditions or combination of the conditions would be associated with improvements in health outcomes, but at an additional net cost increase to the health system.

Given that screening does not both improve health outcomes and reduce costs to the health system (which would by definition be considered cost-effective; see criteria for cost-effectiveness, described in the *Methods* section), assessing the value for money requires an examination of how much health would be produced for the dollars invested. When contrasting the additional costs to screen with the quantity of health that is produced (measured in life years gained), adding SCD alone provides the greatest value among all options, at approximately $2,621 to produce an additional year of life.

The option of adding all seven conditions to the screening program was associated with an estimated cost per additional year of life of $8,155. Hence, decision-makers ought to consider only those combinations that had a cost per additional year of life less than $8,155 (Table E.12), because those combinations require less money to produce the same additional life year (that is, less money needed for the same unit of health output). For instance, it would be better to adopt all seven conditions than it would be to adopt HCY alone (approximately $25,453 per additional year of life) or GALT plus TYRI (approximately$86,848 per additional year of life) (see Table E.5). It would be better to adopt all seven conditions, because the same unit of health outcome can be produced for less cost compared to other combinations (that is, higher technical efficiency).

All the options listed in Table E.12 include SCD. This result is not driven by leveraging an existing platform, given that screening for SCD is not conducted on a MS/MS platform. The cost-effectiveness results are driven by the fact that the amount of health gained for the dollars invested is high for SCD, and SCD therefore has the greatest value among all options. Consequently, the lower technical efficiency associated with screening for HCY, TYRI, GALT, or SCID (which each had prohibitively high ICERs, estimated at $25,453 or greater) could be offset by the higher technical efficiency of SCD (that is, higher health gained for the dollars invested). Therefore, when combined, SCD compensates for the other conditions so that their combined result is $8,155 per additional life year gained.

Table E.12: Summary of economic analysis results

Screening option	Measure of efficiency ($ per additional year of life)	Budget impact ($ millions)	
		5 year cumulative	% in year 1
Add SCD	$2,620.73	$3.61	11.80%
Add HCY + SCD	$2,702.49	$3.75	12.71%
Add TYRI + SCD	$2,989.88	$6.49	9.66%
Add HCY + TYRI + SCD	$3,069.29	$6.63	10.22%
Add GALT + SCD	$4,944.51	$7.03	16.56%
Add HCY + GALT + SCD	$5,016.53	$7.16	16.94%
Add GALT + TYRI + SCD	$5,277.69	$9.91	13.77%
Add GALT + HCY + TYRI + SCD	$5,347.66	$10.04	14.08%
Add SCD + SCID	$5,553.17	$9.23	13.68%
Add HCY + SCD + SCID	$5,623.80	$9.37	14.02%
Add TYRI + SCD + SCID	$5,882.21	$12.11	12.09%
Add HCY + TYRI + SCD + SCID	$5,950.79	$12.25	12.36%
Add GALT + SCD + SCID	$7,800.45	$12.64	15.82%

Add HCY + GALT + SCD + SCID	$7,861.90	$12.78	16.04%
Add GALT + TYRI + SCD + SCID	$8,095.53	$15.52	14.18%
Add all conditions	**$8,155.21**	**$15.82**	**14.22%**

It must be recognized, however, that, while SCD was found to have the greatest value for money among the options reviewed, it is unknown whether adopting SCD (or any of the other options listed in Table E.12) is good value for the system. This is because these results only indicate how much it costs to produce a unit of health; they do not indicate whether the amount of health is actually worth the investment. As previously mentioned, none of these options are cost-effective outright (that is, less costly while producing improved health outcomes), and determining whether they are good value for money requires consideration of opportunity costs. In a constrained healthcare system (that is, no new resources), adopting any of these options means that resources must be taken from elsewhere in the system in order to fund its adoption. That action will be associated with health benefits foregone. The amount of health gained by adopting an option must therefore be greater than the amount of health benefit foregone, because, for the same amount of cost, the system is now producing more health on net. When examining which sectors of the health system experience the greatest impact, the laboratory cost of screening and confirmatory testing comprise the majority of total costs.

Caveats

No model can perfectly capture what is or will be observed in reality, and the findings should be evaluated in light of the following caveats:

1. The entire analysis was based on the fact that there is already an existing program in place. The model is structured to capture the incremental cost associated with adding each condition to an existing screening program. The cost inputs also reflect incremental costs, not absolute costs.
2. Clinical and epidemiological data were obtained from multiple sources, including Canadian and international sources. The incidence rate, proportion of condition-related events, and mortality rate vary largely across population and ethnic groups. It is uncertain to what extent the results would potentially change had Alberta data been available. Nevertheless, the sensitivity analysis indicates that the results were consistent over a range of assumptions.
3. With the exception of laboratory costs and resources, costs associated with other potential infrastructure requirements, capital equipment, nursing, and other staff including training and education within Alberta Health Services were not included, as an implementation plan has yet to be developed. The budget impact should be considered a conservative estimate.
4. Any potential psychosocial harms associated with a false positive test result were not considered in the analysis.
5. Late hematopoietic stem cell transplantation (HSCT) is associated with higher cost than early HSCT, but the cost between early and late HSCT may vary between healthcare systems.[28] The analysis assumed that the cost of late HSCT is three times higher than early HSCT, based on a United States-based article.[28] Costs of HSCT in Alberta were unavailable at the time of writing this report; the Alberta Children's Hospital is currently extracting cost data regarding HSCT. Note that the one-way sensitivity analysis which was conducted over a

range of cost differences and indicated the cost difference had little impact on the cost-effectiveness results.

6. A cost attribution analysis was conducted to elucidate the resource implications on disparate sectors of the health system. The analysis is conducted from an overall perspective, which is not the same as information generated from a detailed local-level costing exercise.

Conclusion

Given that screening for a single condition or combination of conditions produces additional health benefit but at additional net costs to the system, their adoption is dependent on the availability of funding. In a constrained healthcare system (that is, no new resources), adopting any of these conditions means that either resources must be taken from elsewhere in the system in order to fund their adoption or new resources must be allocated. Adding SCD to the Alberta NMS Program would have an estimated budget impact of $3.61 million over five years. Adding all seven conditions would have an estimated budget impact of $15.82 million over five years. The operational net cost (excluding potential cost savings) in the second through fifth years of adopting all seven conditions is estimated to be $14.06 million, of which $11.86 million (84.3%) is estimated to be for laboratory services.

Appendix E.A: Markov Process of Screening for Each Condition

Figure E.A.1: Markov process of screening for SCD after true positive results

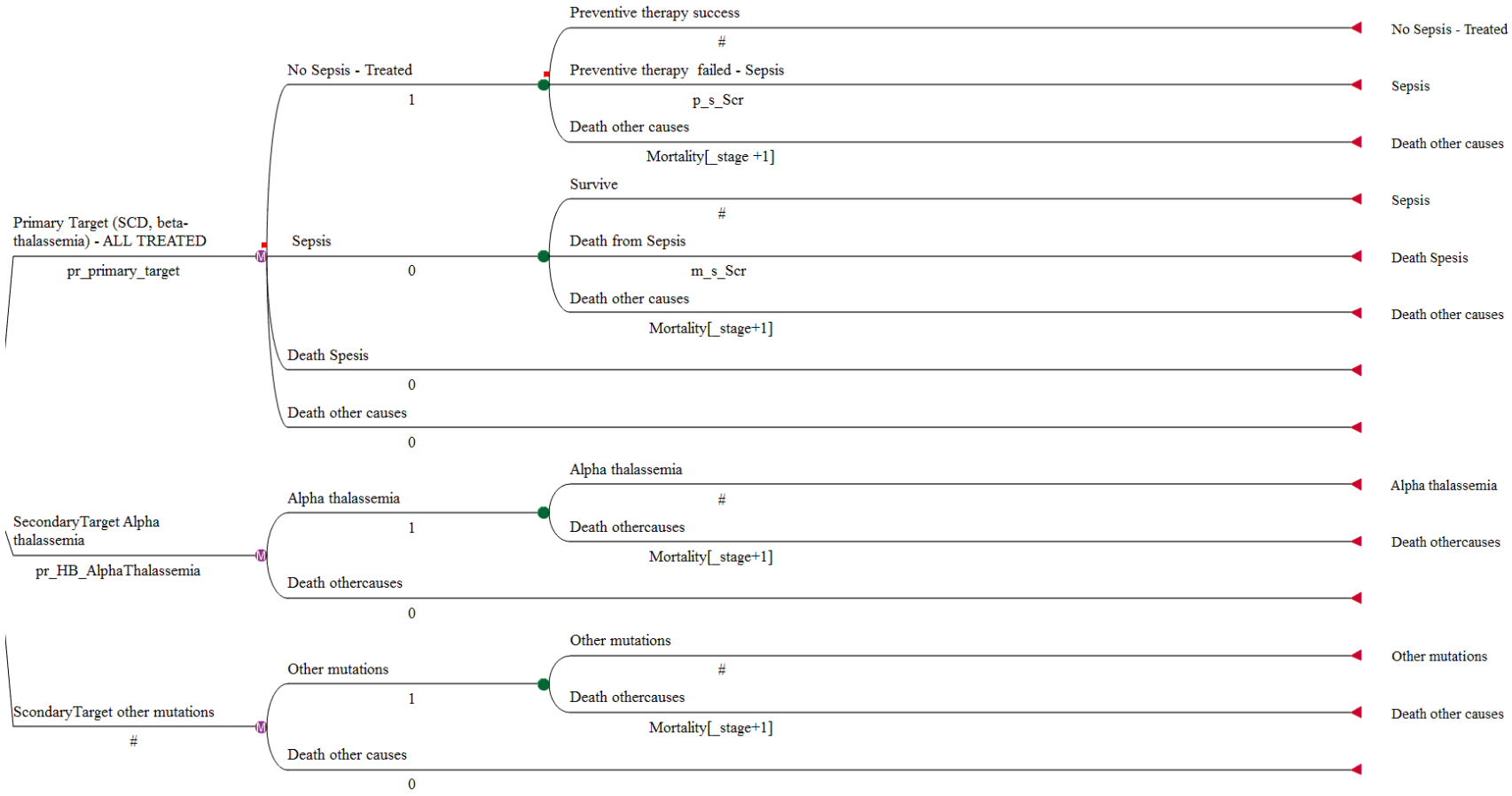


Figure E.A.2: Markov process of screening for SCD after false negative results

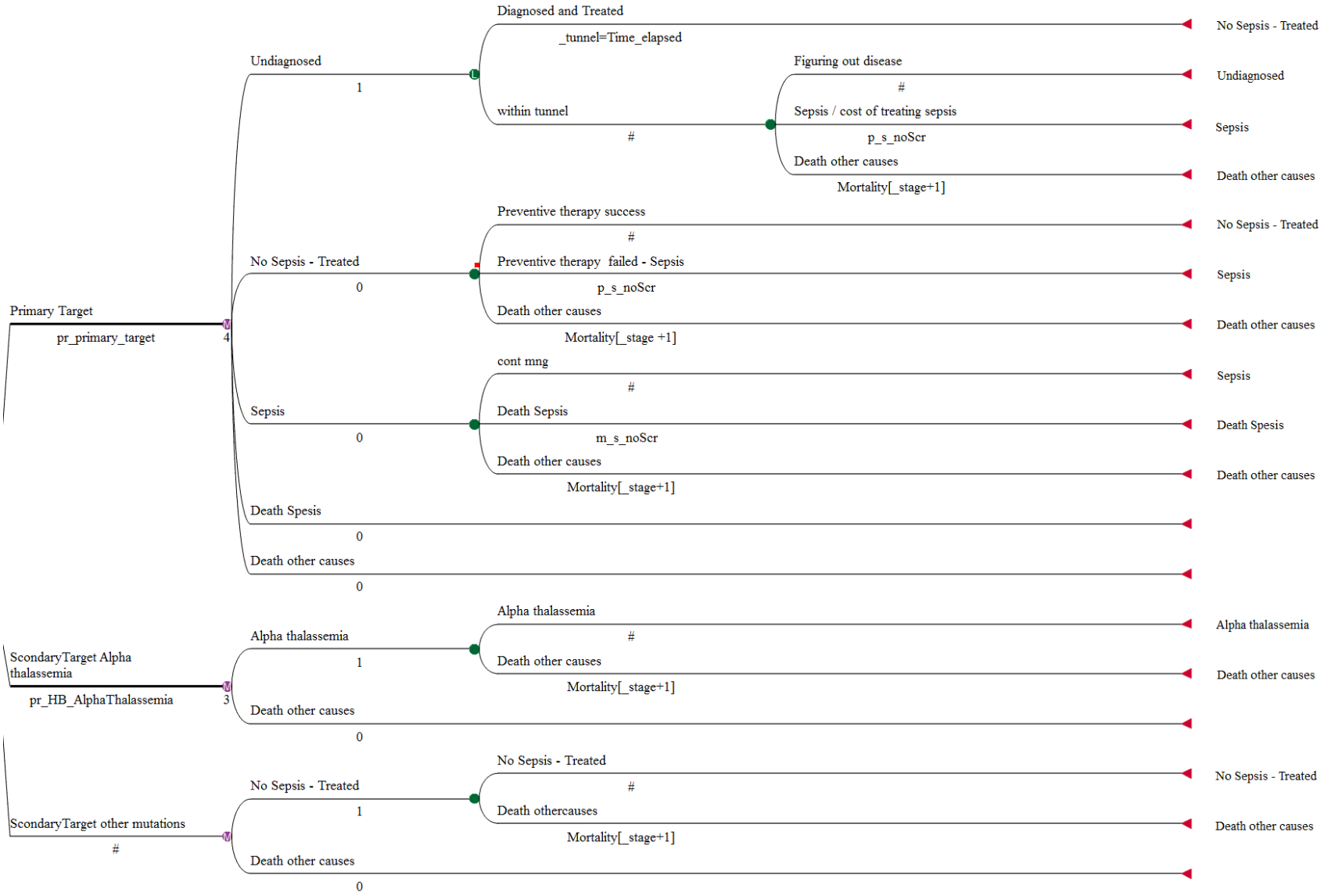


Figure E.A.3: Markov process of screening for HCY after true positive results

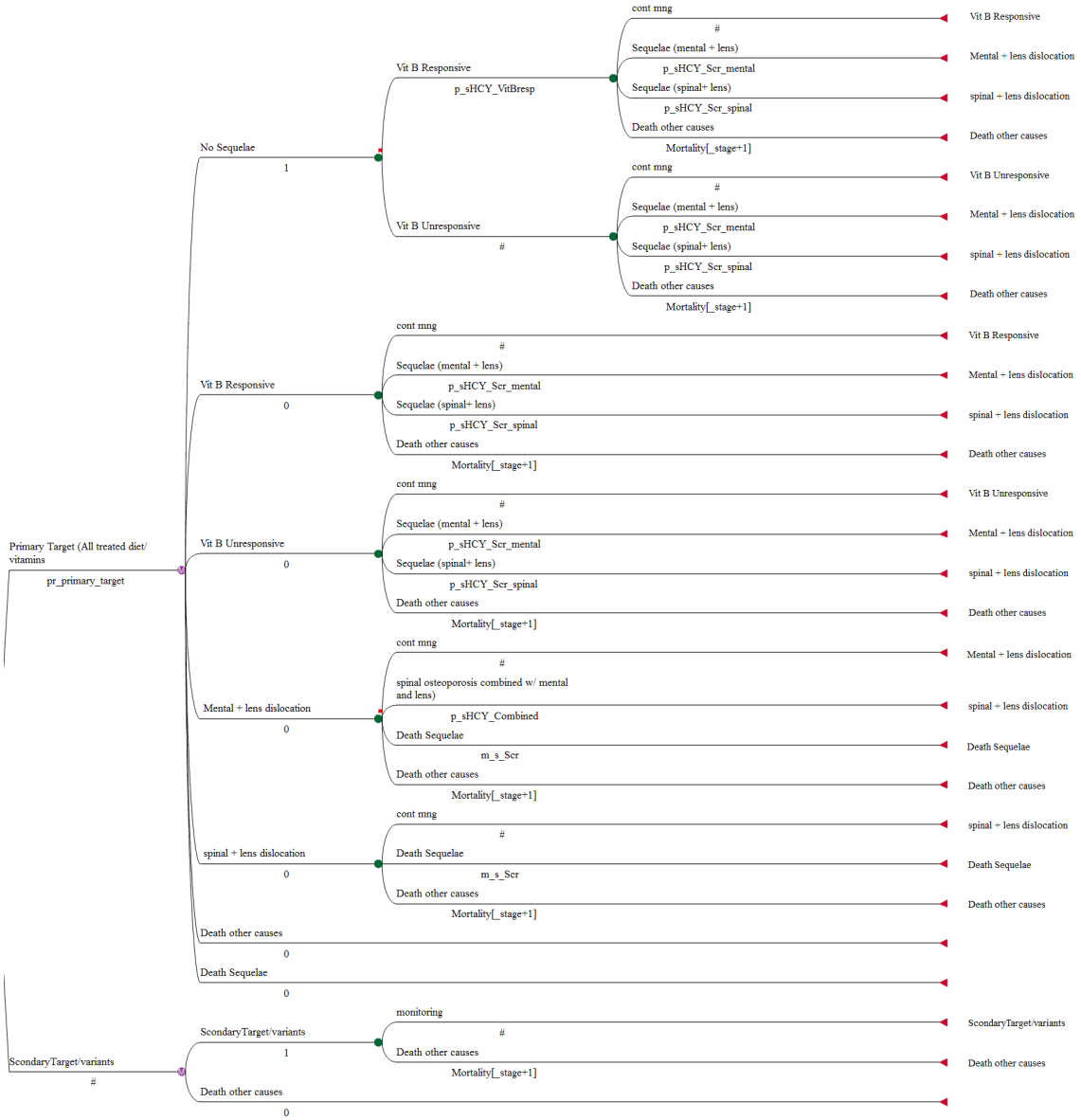


Figure E.A.4: Markov process of screening for HCY after false negative results

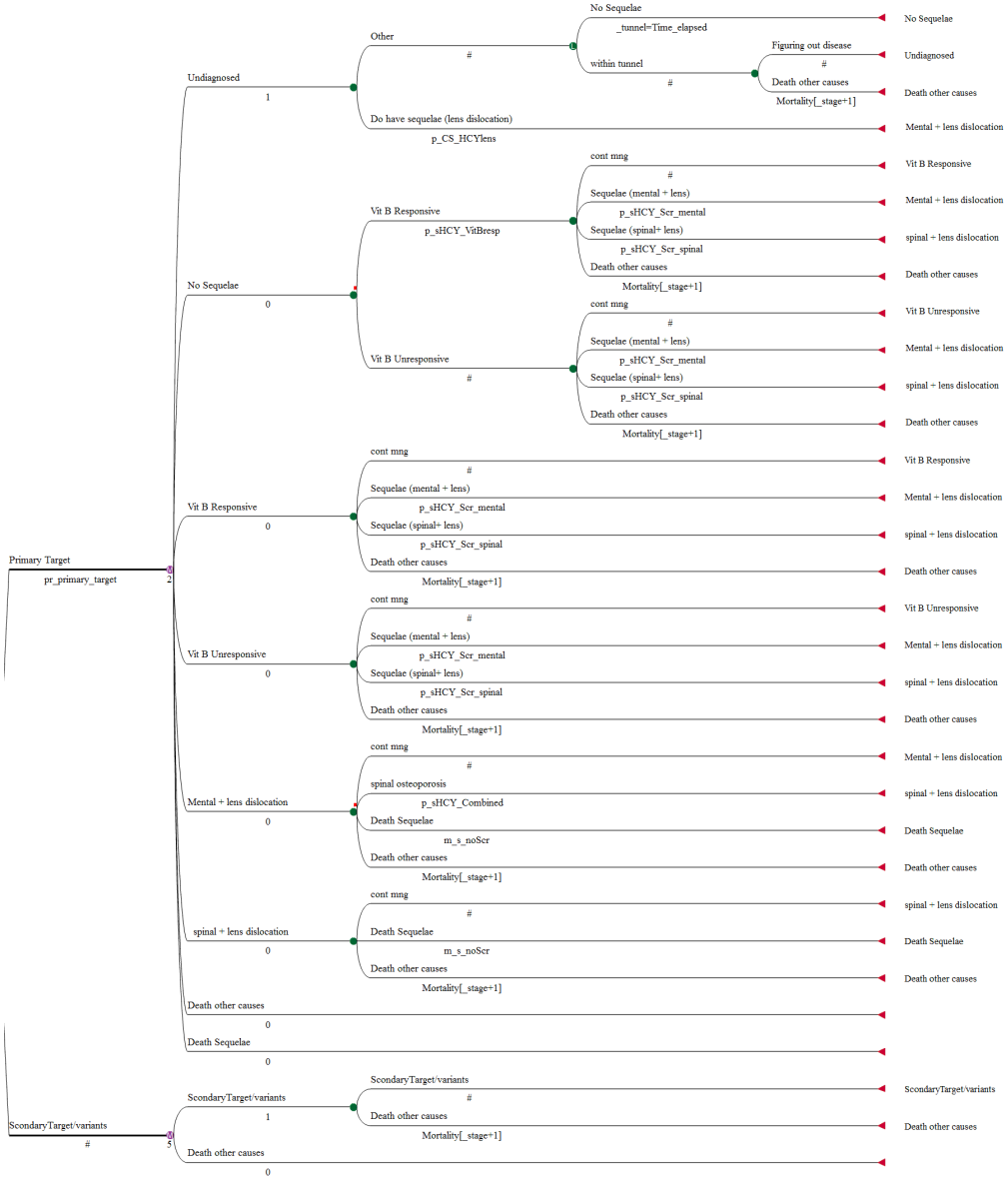


Figure E.A.5: Markov process of screening for TYRI after true positive results

Figure E.A.6: Markov process of screening for TYRI after false negative results

Figure E.A.7: Markov process of screening for GALT after true positive results

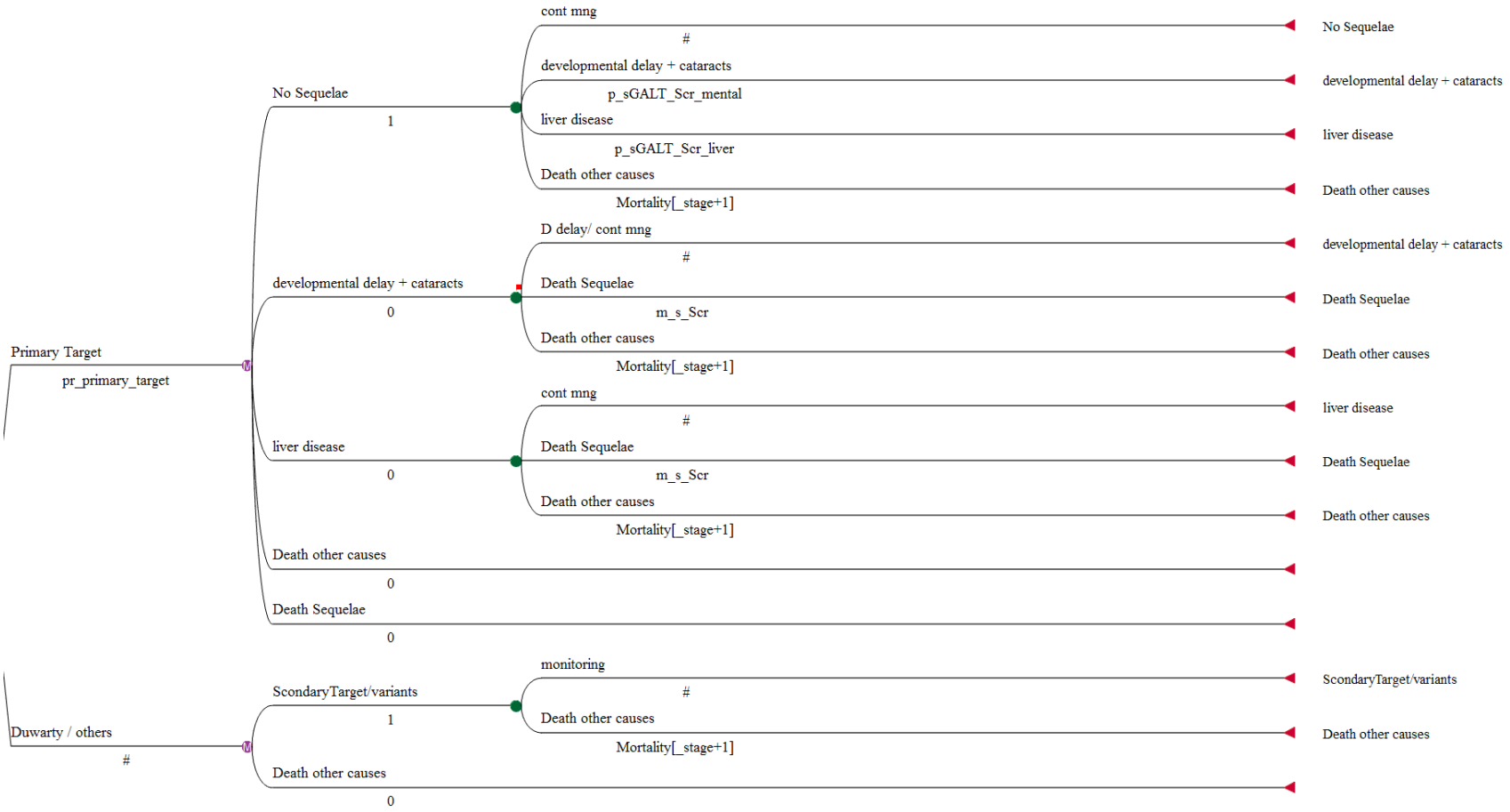


Figure E.A.8: Markov process of screening for GALT after false negative results

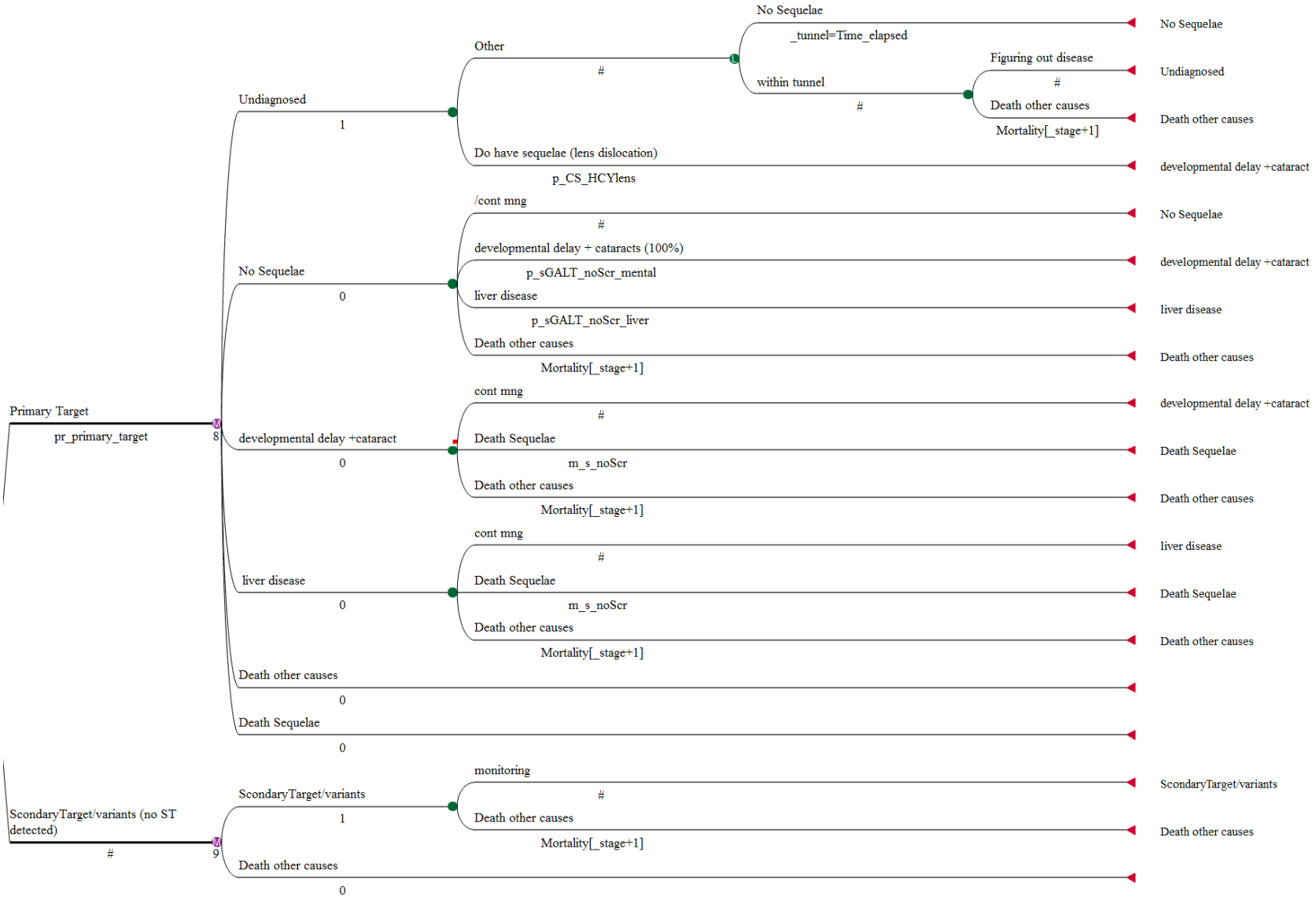


Figure E.A.9: Markov process of screening for SCID after true positive results

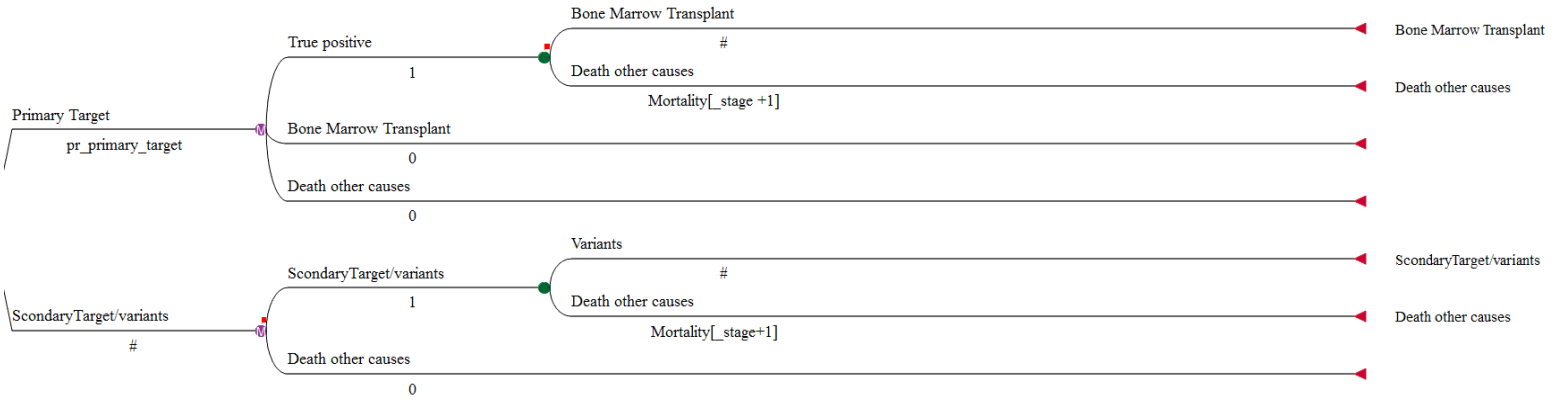
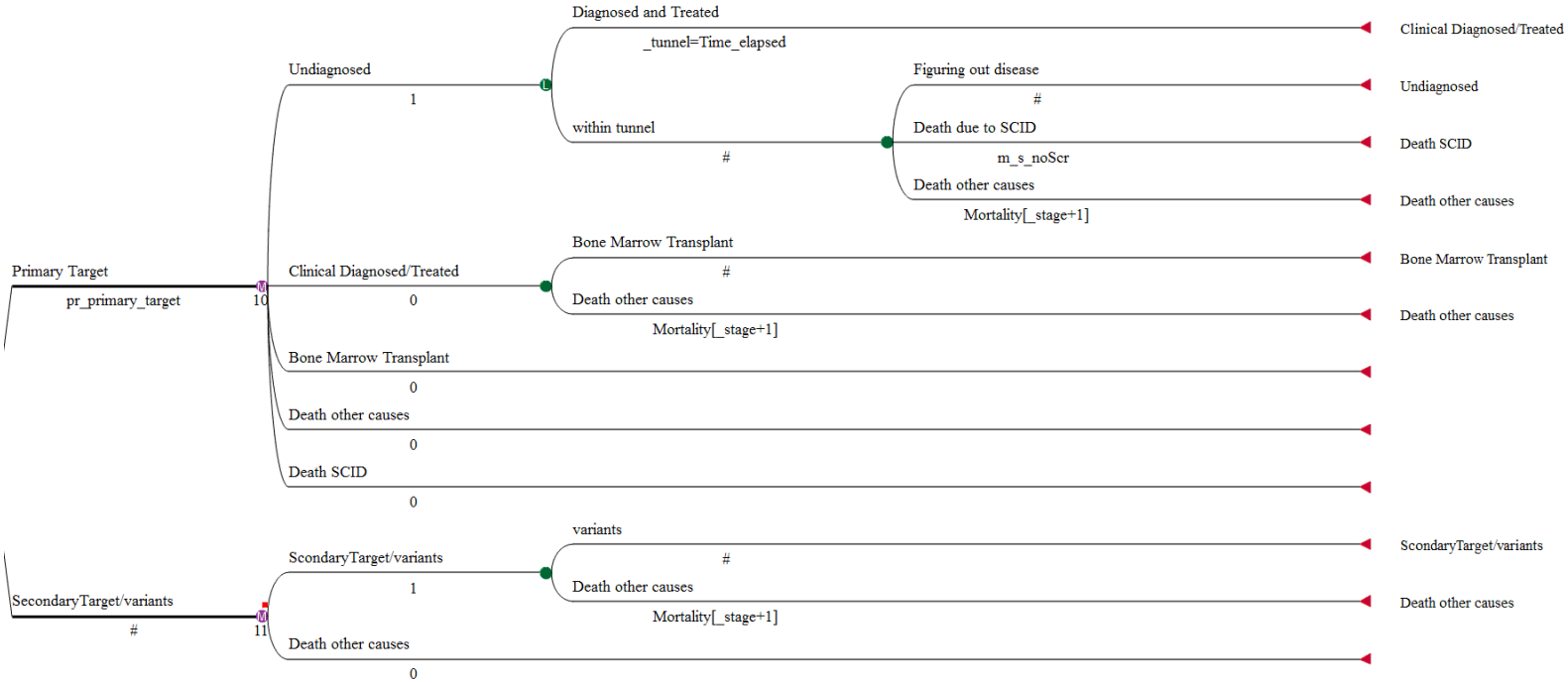


Figure E.A.10: Markov process of screening for SCID after false negative results



Appendix E.B: Alberta Mortality Rates by All Causes of Death in 2013

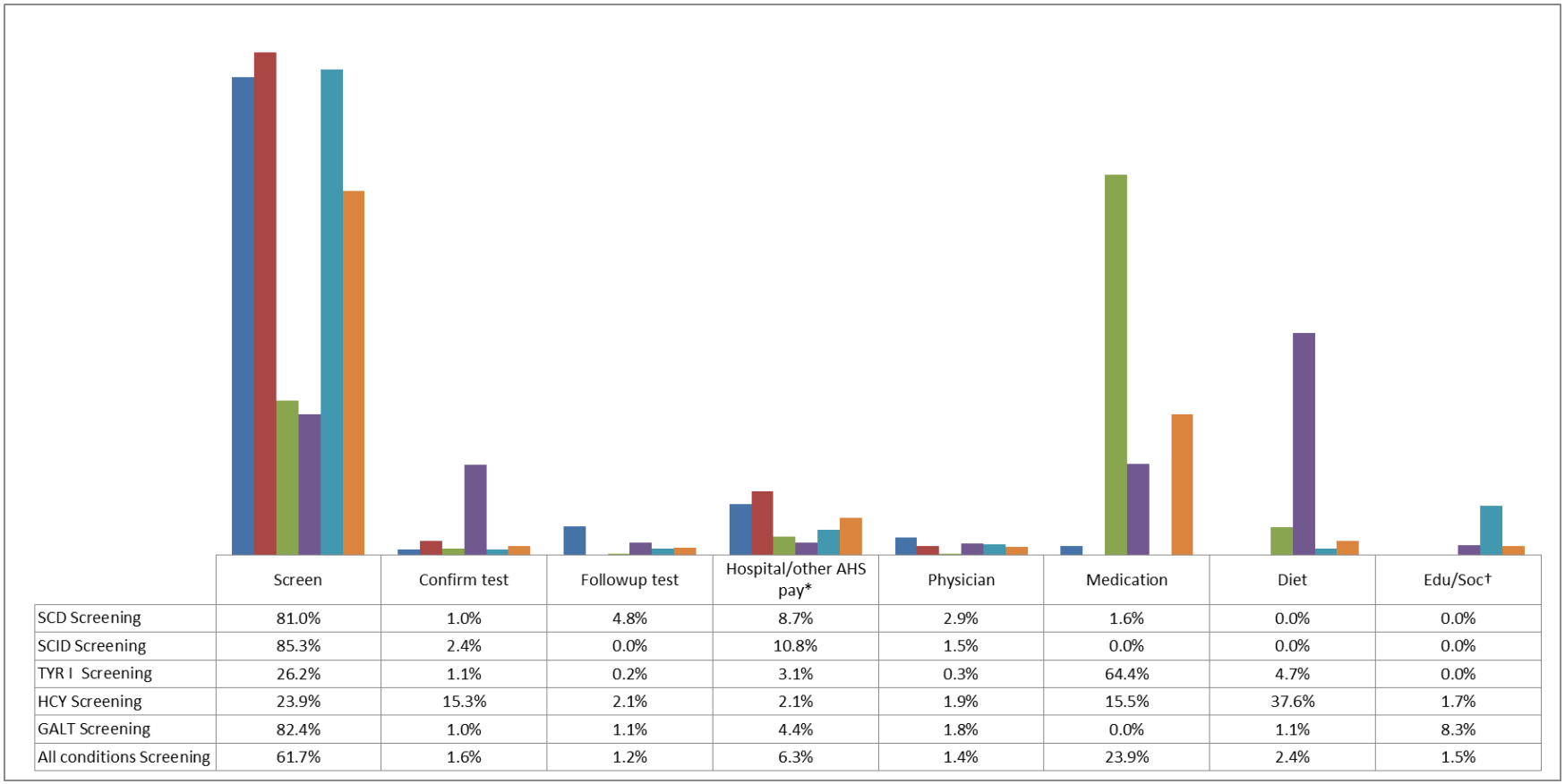
Table E.B.1: Alberta mortality rates by all causes of death in 2013

Age	Mortality rate	Deaths	Population	Standard error
ALL	558.75	22,490	4,025,078	3.73
0	443.46	238	53,669	28.75
1 to 4	28.58	60	209,928	3.69
5 to 9	10.68	26	243,440	2.09
10 to 14	11.49	26	226,342	2.25
15 to 19	39.2	97	247,418	3.98
20 to 24	61.41	180	293,130	4.58
25 to 29	67.41	228	338,212	4.46
30 to 34	66.31	225	339,293	4.42
35 to 39	79.57	238	299,114	5.16
40 to 44	116.7	330	282,771	6.42
45 to 49	210.75	592	280,901	8.66
50 to 54	313.57	934	297,856	10.26
55 to 59	467.21	1,236	264,549	13.29
60 to 64	747.37	1,489	199,233	19.37
65 to 69	1,161.04	1,744	150,211	27.8
70 to 74	1,944.32	2,017	103,738	43.29
75 to 79	3,015.39	2,365	78,431	62.01
80 to 84	5,203.1	3,137	60,291	92.9
85 to 89	9,417.88	3,425	36,367	160.92
90+	19,339.18	3,903	20,182	309.56

Source: Alberta Health - IHDA (*http://www.ahw.gov.ab.ca/IHDA_Retrieval/selectCategory.do*)

Appendix E.C: Results of Cost Attribution Analysis

Figure E.C.1: Proportion of each sector to total cost for adding single conditions



	Screen	Confirm test	Followup test	Hospital/other AHS pay*	Physician	Medication	Diet	Edu/Soc†
SCD Screening	81.0%	1.0%	4.8%	8.7%	2.9%	1.6%	0.0%	0.0%
SCID Screening	85.3%	2.4%	0.0%	10.8%	1.5%	0.0%	0.0%	0.0%
TYR I Screening	26.2%	1.1%	0.2%	3.1%	0.3%	64.4%	4.7%	0.0%
HCY Screening	23.9%	15.3%	2.1%	2.1%	1.9%	15.5%	37.6%	1.7%
GALT Screening	82.4%	1.0%	1.1%	4.4%	1.8%	0.0%	1.1%	8.3%
All conditions Screening	61.7%	1.6%	1.2%	6.3%	1.4%	23.9%	2.4%	1.5%

*These are costs of hospitalization and other services paid by Alberta Health Services including genetic counselling and dieticians.

†Edu/Soc = educational and social services for mental disability

Appendix E.D: Total Costs and Budget Impact by Sectors for Selected Strategies

Table E.D.1: Total costs by sectors ($2015 CAD millions) (excludes potential cost savings)

Strategy	Year	Screening Size[a]	Laboratory Services			Other AHS Services (operational)	Physician Services (operational)	Medication (operational)	Diet (operational)	Education and Social Services (operational)[b]	Software (AH) Implement-ation	Total
			Implement-ation[c]	Screening (operational)[d]	Confirmatory Testing (operational)[d]							
GALT + TYRI + SCD + SCID	2016	63,111	$2.18	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.026	$2.20
	2017 go live	67,435	$0.00	$2.42	$0.073	$0.16	$0.04	$0.13	$0.02	$0.00	$0.00	$2.85
	2018	72,613	$0.00	$2.68	$0.080	$0.19	$0.05	$0.20	$0.02	$0.00	$0.00	$3.22
	2019	78,787	$0.00	$2.99	$0.088	$0.22	$0.05	$0.28	$0.03	$0.01	$0.00	$3.67
	2020	86,138	$0.00	$3.36	$0.097	$0.26	$0.06	$0.38	$0.03	$0.01	$0.00	$4.20
	Total		**$2.18**	**$11.46**	**$0.339**	**$0.83**	**$0.20**	**$0.99**	**$0.09**	**$0.02**	**$0.026**	**$16.14**
All (GALT + TYRI + SCD + SCID + HCY)	2016	63,111	$2.22	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.026	$2.25
	2017 go live	67,435	$0.00	$2.43	$0.084	$0.16	$0.04	$0.13	$0.02	$0.00	$0.00	$2.86
	2018	72,613	$0.00	$2.68	$0.092	$0.19	$0.05	$0.21	$0.02	$0.00	$0.00	$3.25
	2019	78,787	$0.00	$2.99	$0.101	$0.22	$0.05	$0.29	$0.03	$0.01	$0.00	$3.70
	2020	86,138	$0.00	$3.36	$0.111	$0.26	$0.06	$0.40	$0.04	$0.01	$0.00	$4.24
	Total		**$2.22**	**$11.47**	**$0.388**	**$0.83**	**$0.20**	**$1.03**	**$0.11**	**$0.03**	**$0.026**	**$16.31**

Note that costs are a function of the cost inputs (table above), the size of the screening population, the epidemiology associated with each condition (e.g. incidence rates), and the associated health service utilization following the results of screening and confirmatory testing for each condition. A separate economic model was developed for each condition.

[a] Screening size is estimated.

[b] For mental and developmental sequelae.

[c] Includes investment in equipment, laboratory space and labour required for change management.

[d] Includes labour, consumables, laboratory space, and labour for ongoing operations.

Other AHS services refer services provided in an AHS facility including costs of dieticians and genetic counsellors.

Software refers to costs to re-configure the alerting system software.

Table E.D.2: Budget impact by sectors ($2015 CAD millions) (includes potential cost savings)

Strategy	Year	Screening Size[a]	Laboratory Services			Other AHS Services (operational)	Physician Services (operational)	Medication (operational)	Diet (operational)	Education and Social Services (operational)[b]	Software (AH) Implement-ation	Total
			Implement-ation[c]	Screening (operational)[d]	Confirmatory Testing (operational)[d]							
GALT + TYRI + SCD + SCID	2016	63,111	$2.18	$0.00	$0.000	$0.00	$0.00	$0.00	$0.00	$0.00	$0.026	$2.20
	2017 go live	67,435	$0.00	$2.42	$0.073	$0.12	$0.03	$0.10	$0.01	$0.00	$0.00	$2.76
	2018	72,613	$0.00	$2.68	$0.080	$0.14	$0.04	$0.15	$0.02	$0.00	$0.00	$3.10
	2019	78,787	$0.00	$2.99	$0.088	$0.16	$0.04	$0.19	$0.02	$0.01	$0.00	$3.50
	2020	86,138	$0.00	$3.36	$0.097	$0.19	$0.05	$0.23	$0.02	$0.01	$0.00	$3.96
	Total		**$2.18**	**$11.46**	**$0.339**	**$0.60**	**$0.16**	**$0.67**	**$0.07**	**$0.02**	**$0.026**	**$15.52**
All (GALT + TYRI + SCD + SCID + HCY)	2016	63,111	$2.22	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.026	$2.25
	2017 go live	67,435	$0.00	$2.43	$0.084	$0.13	$0.03	$0.10	$0.01	$0.00	$0.00	$2.80
	2018	72,613	$0.00	$2.68	$0.092	$0.15	$0.04	$0.16	$0.02	$0.00	$0.00	$3.15
	2019	78,787	$0.00	$2.99	$0.101	$0.17	$0.04	$0.23	$0.02	$0.01	$0.00	$3.57
	2020	86,138	$0.00	$3.36	$0.111	$0.19	$0.05	$0.30	$0.03	$0.01	$0.00	$4.06
	Total		**$2.22**	**$11.47**	**$0.388**	**$0.65**	**$0.16**	**$0.80**	**$0.09**	**$0.02**	**$0.026**	**$15.82**

Note that costs are a function of the cost inputs (table above), the size of the screening population, the epidemiology associated with each condition (e.g. incidence rates), and the associated health service utilization following the results of screening and confirmatory testing for each condition. A separate economic model was developed for each condition.

[a] Screening size is estimated.

[b] For mental and developmental sequelae.

[c] Includes investment in equipment, laboratory space and labour required for change management.

[d] Includes labour, consumables, laboratory space, and labour for ongoing operations.

Other AHS services refer services provided in an AHS facility including costs of dieticians and genetic counsellors.

Software refers to costs to re-configure the alerting system software.

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SECTION FIVE: CONCLUSION

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Please refer to Table C.1 at the end of this section for a summary of key considerations.

5.1 Research Evidence and the Australian Framework

The following discusses the research evidence from the STE evidence review on screening for the seven conditions within the Alberta context, as it relates to the Australian Framework[1] criteria discussed in section 1.2.

Note that criteria outlined in the Australian Framework that are oriented towards the requisite characteristics of a screening program are not considered in this review, given that we are not evaluating the existing screening program but rather are focusing on the addition of conditions to an already existing program.

The condition must be an important health problem with a recognizable latent or early symptomatic stage

Each of the seven conditions reviewed in the absence of early treatment is associated with increased morbidity and mortality (except HCY, it is not associated with greater mortality). Each of the seven conditions have a well-defined marker for the condition, and it was determined that screening that leads to early diagnosis and treatment had the potential to reduce the burden of disease.

The specific incidence rates are listed in section 2.3 of this report. Note that, according to the EAG clinicians, cases of GALT, TYRI, and HCY in Alberta have remained consistent over time (EAG members, personal communication, July 2015). Of the seven conditions, SCD is the most common in Alberta (followed by SCID), with the program observing an increase in the number of SCD cases each year (EAG members, personal communication, July 2015).

Screening results are reported by the Alberta NMS Program within 10 days, prior to the onset of clinical symptoms. For example, of the seven conditions, GALT is associated with the earliest manifestation of clinical symptoms, with infants may present within the first week of life; approximately 93% of samples are reported within the first week.

The test for each condition must be highly sensitive and specific, be validated and safe, have a relatively high positive and negative predictive value, and be acceptable to the target population, including important subgroups

The screening test for all conditions, with the exception of HCY, had a sensitivity and specificity of 99%. Negative predictive values for all conditions were over 99%. The PPV for all conditions except for SCD were generally low (most studies reported a PPV less than 5%) and varied in the included studies, reflecting the differences in the cut-offs for the screening test, selection of confirmatory test, and incidence of the disease. The high sensitivity and specificity suggest that the screening test is highly accurate, and the low PPVs are primarily due to the low incidence of disease, which is not surprising in the context of rare diseases.

There were no significant adverse events associated with screening reported in the literature. The most notable harm from screening is the potential anxiety for families caused by false positive results. While false positives cannot be completely eliminated, a specificity of 99% greatly minimizes

the harm. It is also important to note that a sensitivity of 99% will also minimize the harms associated with false negative results.

For HCY, both sensitivity and specificity could not simultaneously approach 99%. Sensitivity can be increased at the expense of reduced specificity, and vice versa, using different cut-off thresholds. Having a low sensitivity but high specificity will reduce the number of false positives but increase false negatives; infants with false negative results may be worse off than if they had not been screened, because the condition may be mistakenly ruled out as a cause of their symptoms. On the other hand, having a high sensitivity but low specificity in the context of a rare condition will increase the number of false positives, and yet potentially not identify any cases despite the high sensitivity.

Screening for all of the conditions reviewed was deemed acceptable to providers according to the EAG clinicians. The literature suggests that newborn screening in general is acceptable to families; the program currently has a coverage rate of 99%.[2]

The treatment for each condition must be effective, available, easily accessible, and acceptable to all patients with the recognized disease or condition; there should be clear evidence that screening and treatment leads to better outcomes than finding and treating the disease at a later stage.

For all of the conditions reviewed, there is evidence to suggest that early treatment is effective in leading to better clinical outcomes, compared to treatment after onset of symptoms. Treatments were also deemed to be available within Alberta and acceptable to patients. Acceptability of HSCT for SCD was not identified to be a significant challenge in Alberta.

Systems should be in place for evidence-based follow-up assessment of all people with a positive screen, regardless of rurality, ethnicity, socioeconomic status, or disadvantage status

The current Alberta NMS Program has established referral and assessment protocols and procedures for positive screens for metabolic disorders. Referral and assessment protocols for SCD and SCID would need to be developed and coordinated with existing pediatric speciality clinics.

Ongoing management referral protocols must be established for individuals who have the disease or condition detected through the screening program

The adoption of any of the seven conditions would be leveraging an existing population-based screening program with established protocols and procedures. There are existing pediatric speciality clinics that already manage the short- and long-term clinical management for patients who present clinically. As previously mentioned, referral and assessment protocols for SCD and SCID would need to be developed and coordinated with these pediatric and adult speciality clinics. However, there are important system readiness considerations (refer to section 5.2 below).

Overall benefits of screening outweigh the harm

GALT, TYRI, SCD, and SCID are associated with significant morbidity and risk for mortality, have screening tests with high sensitivity and specificity, and have evidence supporting the clinical benefit of early intervention. The most notable harm from screening is the potential anxiety for families caused by false positive results, which are minimized given that specificity is 99%.

Another consideration are secondary conditions/incidental findings[xxiv] which are conditions that may not meet the specified criteria for screening, as reviewed in this report, but are identified as part of the differential diagnosis. Secondary conditions/variants are a concern (with the exception of TYRI) where scarce health resources are invested in screening and a greater number of secondary conditions/variants are identified than target conditions, given the rarity of the condition:

- TYRI: Screening with succinylacetone is highly specific for TYRI only.
- GALT: Duarte variant may be ten times more frequent (2.48 per 10,000 infants screened; see Table E.3).[3]
- HCY: MAT I/III may be nine times more frequent (0.14 per 10,000 infants screened; see Table E.3).[4]
    - In five years of testing, British Columbia has found five secondary conditions and no cases of HCY (BC Children's Hospital, personal communication, June 2015).
- SCD: α-thalassemia/other mutations may be 1.7 times more frequent (2.99 per 10,000 infants screened; see Table E.3).[5]
    - There are over 700 Hb variants that can be identified via screening, with 25 to 30 variants considered clinically significant.[6]
- SCID: Syndromes with T-cell impairment/secondary T-cell impairment/variant SCID may be eight times more frequent (0.87 per 10,000 infants screened; see Table E.3).[7]

Nonetheless, given the burden of disease as well as the availability of accurate screening and effective treatment, it is deemed that the overall benefits of screening for GALT, TYRI, SCD, and SCID outweigh the harm. HCY is also associated with significant morbidity, and has evidence showing the clinical benefit of early intervention. Despite the high sensitivity (greater than 96%) and specificity (99%) reported in two of the three primary studies on HCY, their reported sensitivity and specificity are unreliable, given the significant limitations in study design. The experience of HCY screening in other Canadian jurisdictions has shown that HCY cannot be screened with both high sensitivity and specificity. Consequently, there can be an increase in unintended health system utilization resulting from the identification of secondary conditions; such will likely not be outweighed by the number of patients who could benefit, given the rarity of the condition. The overall benefit of screening for HCY is therefore deemed to not outweigh the harm.

5.2 Implementation and System Readiness

Laboratory Impact

If all seven conditions were added to the Alberta NMS Program, it would have a significant impact on laboratory resources. The net cost (excluding potential cost savings) over five years was estimated to be $16.31 million, with an estimated implementation net cost of $2.25 million in the first year and an estimated operational net cost of $14.06 million in the second through fifth years. Of the $14.06 million operational net cost, the proportion required for screening and confirmatory testing is

[xxiv] Variants of the primary target that may not meet the same criteria, or different conditions that are discovered as part of the differential diagnosis (that is, secondary targets). It may be a consequence of multiplex testing (that is, the technology used generates information beyond the specific markers for the targeted disease) or because the additional diseases share the same biomarkers as the targeted disease.

estimated to be approximately 84.3% (refer to Appendix E.D for specific details of the estimated budget impact). The condition-specific and overall needs are listed below:

- GALT: Purchase GSP® or add functionality to existing VICTOR^{2}D™.
- TYRI: Add succinylacetone to existing MS/MS.
- HCY: Add methionine to existing MS/MS.
- SCD: Purchase two HPLC machines (one for redundancy) capable of blood spot analysis.
- SCID: Assume lab development test using a single-wash, in-situ real-time PCR (qPCR), which would also require purchasing a second QuantStudio™ for redundancy purposes.
    - Laboratory services have suggested that it would employ the single-wash, in-situ qPCR method due to quality assurance issues that have been identified with the commercial method (VICTOR™ EnLite™).
    - Ontario is currently employing single-wash, in-situ qPCR,[8] based on the method developed by the Centers for Disease Control in the United States (Genetic Laboratory Services, Alberta Health Services, personal communication, July 2015).
        - Whatever method is adopted, the specifics around what would be required and other impacts to the lab remain to be addressed.
- Configuration of lab software (Specimen Gate Office), and integration with Netcare and the NMS Application[xxv] for the expanded panel.
- 1.0 FTE Information Technology Analyst I (implementation phase and ongoing).
- 1.0 FTE Laboratory Technologist I, 3.0 FTE Medical Laboratory Scientists I, and training of existing staff (implementation phase and ongoing).
- 2.0 FTE Geneticists (implementation phase and ongoing), as well as a 1.0 FTE Geneticist Trainee (CCMG) (implementation phase and ongoing).
- 0.5 FTE Lab Genetic Counsellors I (implementation phase and ongoing).
- Need to also consider resources needed for the maintenance, updating, and/or replacement of equipment.
- May need to add an additional blood spot during sample collection.
- May require additional laboratory space to accommodate additional equipment.

Treatment and Management

If all seven conditions were added to the Alberta NMS Program, this would leverage an existing program with established protocols and procedures. Care pathways are available for the treatment and management of the conditions. However, there would be impacts to providers and other resources within Alberta Health Services.

- It is anticipated that screening for GALT, TYRI, or HCY would not add significant strain to clinics, due to the rarity of the conditions. However, additional resource utilization would

[xxv] The NMS Application is a secure, web-based application that reconciles registered births and newborn screen results and generates alerts that assist in identifying and tracking infants who require follow-up.

occur, resulting from the identification of secondary conditions/variants. The cost of dietary supplements for TYRI and HCY, paid for by operational budgets at the metabolic clinics, would also be impacted.

- Require adding 0.2 FTE Genetic Counsellors for clinical work. May require 2.5 FTE to account for SCD variants and carriers (if reported).
- Careful consideration regarding medical manpower within Alberta Health Services would be important to maximize the existing physician capacity to manage SCD and their secondary conditions/variants.
    - Pediatric hematologists are at capacity (EAG members, personal communication, August 2015). Edmonton is in the process of recruiting one pediatric hematologist.
- Current capacity in pediatric immunology can manage the expected number of SCID patients and their secondary conditions/variants identified through screening.
    - Edmonton is in the process of recruiting an additional pediatric immunologist. Calgary has two pediatric immunologists.
- For SCD, additional resources would be needed for blood transfusion (including outpatient space) as well as HSCT.
- For SCID, additional resources would be needed for inpatient and outpatient isolation space, enzyme replacement therapy, and HSCT. Temporary housing (for example, Ronald McDonald House), which may not be funded by government, is heavily relied upon by families traveling to receive treatment.

5.3 Conclusion

Given the evidence and considerations of the Australian Framework and system readiness in the Alberta context, in principle we conclude that the clinical benefits associated with screening for many of these seven conditions outweigh the potential harms (which can be managed). Expanding a newborn blood spot screening program such as the Alberta NMS Program, however, is ultimately dependent on both the availability of funding (given that the economic evaluation has established that screening produces additional health benefits, but at additional cost to the system) and on the ability to increase service capacity and provision.

Not adding any of the seven conditions would have, of course, the most minimal impact to the existing health system, but would forgo adding conditions for which screening is highly accurate and early intervention is associated with improved clinical outcomes. Adding all seven conditions would be associated with the greatest health system impact and not necessarily the greatest value. This is due to the fact that, with current methods, HCY cannot be screened with high sensitivity and specificity, resulting in an increase in unintended health system utilization from the identification of secondary conditions and variants that would likely not be outweighed by the number of patients who could benefit. Screening for HCY was also associated with the lowest cost-effectiveness among the seven conditions reviewed.

Adding a subset of conditions, on the other hand, could balance considerations of clinical benefit and value for the health system. This subset of conditions could include GALT, TYRI, SCD (Hb SS, Hb S/β-thal, and Hb SC), and SCID. Note that the value for money associated with adopting these conditions as a group appears to be acceptable but is completely driven by the high technical

efficiency of SCD, which compensated for the low technical efficiency of the other conditions. GALT, TYRI, SCD, and SCID were deemed to: be important health burdens; have screening tests with high sensitivity and specificity; have evidence supporting the clinical benefit of early intervention; have established care pathways (from screening to long-term management); and, if added to the current panel as a group, not be associated with unreasonable value for money. This subset would also make Alberta more similar to other Canadian jurisdictions in terms of newborn blood spot screening (refer to Table S.1 for a jurisdictional scan).

Nevertheless, in the Alberta context, in screening for these conditions, system readiness and capacity would be a challenge, particularly for SCD. If the Alberta NMS Program were to expand to include all or a subset of the seven conditions, it is imperative that an implementation plan be developed to ensure that the necessary funding, resources, providers, and training be established either prior to their adoption or through a staged implementation strategy. It is also recommended that such an implementation plan include a strategy for monitoring the impact on these conditions in terms of risks, benefits, and value for money. The incidence rates of these conditions in Alberta remain unknown, and yet they are a strong driver of the value proposition. Moreover, the methodological quality of the evidence examining effectiveness was rated to be poor in general, but is not expected to improve due to the rarity of these conditions. Therefore, the only mechanism of addressing these uncertainties in the Alberta context and determining the added value of expanding the program, as well as identifying both intended and unintended consequences (for example, whether screening coverage will be impacted from the adoption of SCD), is to prospectively monitor and evaluate the impact of screening for these conditions.

Table C.1: Summary of key considerations

Condition	Australian Framework (informed by STE evidence review)				System Readiness[b]			
	Important health problem/ Rationale	Test Sn and Sp/ Benefit of early treatment	Follow-up of positive screens/ Management protocols	Overall benefits outweigh the harm	Value for money	Budget impact (5 year cumulative)	Laboratory[c]	Treatment/ Management/ Acceptability
GALT	May prevent life-threatening symptoms. Risk of cataracts may also be reduced.	Sn ~99% Sp ~99% Early treatment improves clinical outcomes[a]	The current Alberta NMS Program has established referral and assessment protocols and procedures for positive screens for metabolic disorders.	Yes	Not cost-effective ICER=$122,749	~$3.46 million	Add GSP®; or add functionality to existing VICTOR^{2}D™	Minimal impact to clinics due to rarity Screening/ treatment is acceptable to providers and patients[e]
TYRI	May reduce risk of liver damage/need for liver transplant, kidney disease, and carcinoma.	Sn ~99% Sp ~99% Early treatment improves clinical outcomes[a]		Yes	Not cost-effective ICER=$31,724	~$2.93 million	Add succinylacetone to existing MS/MS	Minimal impact to clinics due to rarity Screening/ treatment is acceptable to providers and patients[e]
HCY	May reduce risk of thrombosis, visual problems, and cognitive deficiency. No reduction in the risk of death.	Trade-off between Sn and Sp Early treatment improves clinical outcomes[a]		No	Not cost-effective ICER=$25,453	~$0.17 million	Add methionine to existing MS/MS	Minimal impact to clinics due to rarity Screening/ treatment is acceptable to providers and patients[e]

Condition	Australian Framework (informed by STE evidence review)				System Readiness[b]			
	Important health problem/ Rationale	Test Sn and Sp/ Benefit of early treatment	Follow-up of positive screens/ Management protocols	Overall benefits outweigh the harm	Value for money	Budget impact (5 year cumulative)	Laboratory[c]	Treatment/ Management/ Acceptability
SCD	May prevent infection from sepsis. Parental education may reduce risk of infection and splenic sequestration.	Sn ~99% Sp ~99% Early treatment improves clinical outcomes	Referral/assessment protocols for SCD and SCID will need to be developed and coordinated with existing specialty clinics.	Yes	Potentially acceptable ICER=$2,621	~$3.61 million	Add 2 HPLC machines (one for redundancy) capable of blood spot analysis	Already at capacity[d] Blood transfusion/HSCT resources & outpatient space Screening/ treatment is acceptable to providers and patients[e]
SCID	Reduce the risk of severe infections that can cause death. Treatment may be curative.	Sn ~99% Sp ~99% Early treatment improves clinical outcomes – treatment can be curative		Yes	Not cost-effective ICER=$332,360	~$5.65 million	Assume lab development test using qPCR and add a second QuantStudio™	Blood transfusion/HSCT resources & outpatient space Screening/ treatment is acceptable to providers and patients[e]

a. Quality of the studies was poor, primarily due to rarity of the conditions and study design. EAG clinicians advocate that the available treatments are effective.

b. Add up to 2.5 FTE Genetic Counsellors if carrier status for SCD is reported.

c. Lab software (Specimen Gate Office) requires configuration and integration with Netcare and the NMS Application for the expanded panel. Add information technology specialist and consider resources for maintenance, updating, and/or replacing equipment.

d. No capacity in pediatric hematology. Careful planning by medical manpower within AHS will be needed.

e. Based on the literature.

ICER: incremental cost-effectiveness ratio; Sn: sensitivity; Sp: specificity

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Appendix A: Expert Advisory Group and Project Team

Expert Advisory Group, Members' Discipline/Affiliation

- Ethics, Alberta Health Services
- General Practice – Primary Care, Alberta Health Services
- Health Services Research, University of Calgary
- Laboratory Medicine – Clinical, Alberta Health Services
- Laboratory Medicine – Administration, Alberta Health Services
- Medical Biochemistry, Alberta Health Services
- Medical Genetics, Alberta Health Services
- Medical Genetics – Inherited Metabolic Disorders, Alberta Health Services
- Neonatology, University of Calgary and Alberta Health Services
- Pediatric Genetics, Alberta Health Services
- Pediatric Hematology/Immunology, Alberta Health Services
- Pediatric Respirology, Alberta Health Services
- Population and Public Health, Alberta Health Services
- Screening Programs – Healthy Living, Population, Public & Aboriginal Health, Alberta Health Services
- Senior Administration, Alberta Health Services

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- Ms. Barbara Smith
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Author Contribution Statements

Arianna Waye was the principal lead for conducting the social system and demographics assessment (S section).

Bing Guo and *Mohamed El Shayeb* were the principal leads for the design and conduct of the safety and technological efficacy/effectiveness assessment (T section).

Paula Corabian assisted in conducting the quality assessment of evidence reviewed in the T section.

Charles Yan was the principal lead for the economic analysis (E section).

Ilke Akpinar assisted in conducting the economic analysis.

Anderson Chuck was the project lead for the review.

Dagmara Chojecki conducted the literature searches.

This STE report examines the safety, screening accuracy, therapeutic efficacy/effectiveness, cost-effectiveness, budget impact, and health system readiness of newborn screening for seven conditions (galactosemia, tyrosinemia type I, homocystinuria, sickle cell anemia, sickle cell/beta-thalassemia, sickle cell/hemoglobin C disease, and severe combined immunodeficiency), contextualized to the Alberta setting.

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