MSAC Application 1776

Newborn bloodspot screening for mucopolysaccharidosis Type II (MPS II; Hunter syndrome)

Applicant: Department of Health and Aged Care

PICO Confirmation

Summary of PICO/PPICO criteria to define question(s) to be addressed in an Assessment Report to the Medical Services Advisory Committee (MSAC)

A summary of the PICO criteria to define the questions to be addressed in an Assessment Report to the Medical Services Advisory Committee (MSAC) are shown in Table 1 and Table 2.

Table 1 PICO for newborn bloodspot screening for Mucopolysaccharidosis type II (MPS II): PICO Set 1

Component	Description
Population	All newborn babies in Australia
Prior tests	No prior testing
Intervention	Newborn bloodspot screening (NBS) for detecting Mucopolysaccharidosis type II (MPS II), based on a two-tier screening protocol: 1st tier: quantification of the enzyme iduronate-2-sulfatase (I2S) liquid chromatography (LC) tandem mass spectrometry (MS/MS) or a fluorometric assay 2nd tier: endogenous biomarker method for measuring non-reducing end glycosaminoglycan (GAG) fragment analysis by LC-MS/MS Diagnostic testing of patients with a positive screening result (testing protocol as per the comparator)
Comparator/s	Current practice – Diagnostic testing for MPS II at the point of onset of phenotypic signs and symptoms; no universal newborn screening for MPS II Diagnostic testing: • endogenous biomarker method for measuring non-reducing end GAG fragment analysis by LC-MS/MS in urine • plasma or peripheral blood leukocyte I2S enzyme activity analysis • genetic analysis to identify the causative iduronate-2-sulfatase (*IDS*) gene variant(s)
Reference standard	A confirmed diagnosis using a combination of clinical assessment, diagnostic biochemical testing and genetic testing.
Outcomes	Test performance: • Diagnostic accuracy of the screening test (sensitivity, specificity, positive predictive value, negative predictive value, false positives, false negatives) • Diagnostic accuracy of confirmatory/diagnostic test (sensitivity, specificity, positive predictive value, negative predictive value) • Diagnostic yield of screening and proportion of cases with a genotype predictive of severe phenotype

Component	Description
	Change in management: • Age at diagnosis • Age at treatment initiation (and whether prior to, or after phenotype onset) • Investigations/monitoring/treatments received. • Changes in family counselling Clinical Effectiveness of NBS for MPS II: • Change in morbidity and mortality, quality of life, general functioning and disease manifestations from earlier diagnosis, earlier disease modifying interventions, and avoidance of the diagnostic odyssey (either from studies assessing the impact of comparative change in management, or direct from test to health outcomes evidence) Safety of NBS for MPS II (physical harms to newborn from screening test, diagnostic test or subsequent early vs late treatment): • Impact of false positive screening results (physical harms to the infant or psychological harms to the parents) • Impact of false negative results • Impact of diagnosing late-onset cases at birth, creating "patients in waiting" • Impact of diagnosing mild cases or variants of uncertain significance (VUS) • Safety of (experimental) haematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT) • Any potential risk of harm from ongoing monitoring and surveillance Economic and Financial Implications: • Cost-effectiveness NBS for MPS II (cost per diagnosis; cost per quality adjusted life year (QALY)) • Financial impact of screening, relative to existing practice (including impact of false positives, impact of screening-based treatment compared to treatment following diagnosis at phenotype presentation, impact of ongoing monitoring and surveillance) Other relevant considerations: • Non health outcomes: Value of knowing (emotional benefits and harms to family, social benefits and harms to family) • Ethical considerations (equity of access, considerations regarding consent) • Organisational considerations (incremental impact of NBS on organisations, particularly the impact on services for monitoring late-onset disease, or on the NBS program itself including programmatic implementation considerations)
Assessment questions	What is the comparative safety, effectiveness, cost-effectiveness and financial implications of NBS for MPS II versus current practice (no NBS, diagnosis on presentation of signs and symptoms)?

Table 2 PICO for cascade testing of family members of newborns diagnosed with MPS II via NBS: PICO Set 2

Component	Description
Population	Biological mothers and siblings of an individual (index case) with a pathogenic/likely pathogenic (P/LP) variant(s) in the *IDS* gene Where the mother tests positive, also her parents and siblings
Prior tests	Family history
Intervention	Cascade testing after diagnosis of a newborn due to NBS: • For male family members: Genetic counselling, option of either biochemical testing (urine GAG analysis) or genetic testing for the P/LP variant(s) in the *IDS* gene • For female family members: Genetic counselling and genetic testing for the P/LP variant(s) in the *IDS* gene
Comparator/s	Cascade testing after diagnosis made subsequent to signs/symptoms: • For male family members: Genetic counselling, biochemical testing (urine GAG analysis) or genetic testing for the P/LP variant(s) in the *IDS* gene • For female family members: Genetic counselling and genetic testing for the P/LP variant(s) in the *IDS* gene *(i.e. the same testing strategy as the intervention, differing only in the timing of the testing)*.
Outcomes	Test outcomes: • Number of siblings with early diagnosis of MPS II • Number of family members who uptake cascade testing • Age at diagnosis/treatment of affected siblings Clinical effectiveness: • Effectiveness of early vs late monitoring and treatment for male siblings diagnosed with MPS II following cascade testing • Psychological impact of diagnosis of MPS II (affected sibling or female carrier) Safety: • Physical or psychological harms arising from earlier diagnosis, monitoring and treatment for male siblings diagnosed with MPS II following cascade testing Economic and Financial Implications: • Cost-effectiveness • Financial impact of early vs late cascade testing Other relevant considerations: • Non health outcomes: Value of knowing (family planning, emotional benefits/harms to family, social benefits/harms to family)

Component	Description
	• Ethical considerations (equity of access, considerations regarding consent, considerations regarding cascade testing, especially relating to identification of late-onset MPS II in asymptomatic people) • Organisational considerations
Assessment questions	What is the comparative safety, effectiveness, cost-effectiveness of cascade testing of family members of a male newborn diagnosed with MPS II due to NBS versus cascade testing of the family members following diagnosis of MPS II in the index case after presentation of signs/symptoms?

Purpose of application

An application requesting that mucopolysaccharidosis type II (MPS II; also known as Hunter syndrome) be added to the newborn bloodspot screening (NBS) programs was developed by the Department of Health and Aged Care, following a request from the Hon Mark Butler MP, Minister for Health and Aged Care.

NBS programs are overseen and managed by state and territory governments and operate independently of each other. The Australian Government contributes funding to hospital services, including those for NBS through the National Health Reform Agreement (NHRA). Funding of $39.0 million was announced under the 2022-23 Budget, of which $25.3 million was provided to states and territories to support expansion of NBS programs. This application, as well as applications for NBS for glycogen storage disease type II (GSD II, commonly known as Pompe disease; application number 1774) and for mucopolysaccharidosis type I (MPS I; application number 1775) are proposed to form part of this expansion. All three conditions are lysosomal storage disorders (LSDs) and there is overlap in the tests used to diagnose these three conditions. Therefore, other LSDs may be discussed when considering testing methodologies.

There are five laboratories that conduct tests on bloodspot cards, located in New South Wales, Queensland, South Australia, Victoria and Western Australia. Newborns born in states and territories without NBS testing laboratories have their dried bloodspots sent interstate for testing. All NBS programs are underpinned by the criteria set out in the Newborn Bloodspot Screening National Policy Framework (NBS NPF).

Proposals to add conditions to NBS are considered by MSAC. In providing its advice MSAC considers the safety, effectiveness, cost-effectiveness, and total cost of proposals for public funding, noting that for NBS applications, alignment with the NBS NPF decision-making criteria (see Appendix A) is a key additional policy consideration.

The clinical claim is that NBS for MPS II results in superior health outcomes to the comparator of no newborn screening, with diagnosis of MPS II upon symptomatic presentation. Children presenting with symptoms consistent with MPS II undergo the same diagnostic testing and treatment pathways as children identified via NBS, with the main difference being the time of confirmed diagnosis.

Newborns identified as having MPS II via NBS would be offered further biochemical and genetic testing to confirm their diagnosis. Newborns diagnosed with MPS II would be offered appropriate multidisciplinary clinical management including treatment. Currently, the most common treatment available for MPS II in Australia is enzyme replacement therapy (ERT). There has been very limited use of haematopoietic stem

cell transplantation (HSCT) in Australia as it is currently considered as experimental and routine funding is not available via the Commonwealth. Use has been experimental and restricted to cases with the more severe form of MPS II due to substantial risks associated with HSCT. In the absence of NBS, individuals with MPS II would be diagnosed following symptomatic presentation, clinical suspicion of MPS II, and subsequent diagnostic biochemical and genetic testing to confirm the diagnosis, unless there is a family history of MPS II. Symptoms of MPS II usually become apparent in children with the severe form of MPS II at around 1 to 3 years of age. Individuals with the late onset milder (attenuated) form of MPS II usually start to develop symptoms in their second decade of life. MPS II represents a clinical spectrum and there is an increasing awareness of an attenuated neuropathic form sitting on a spectrum between the severe and milder forms (D'Avanzo et al. 2020).

Many of the early symptoms of MPS II are heterogenous and occur in other childhood disorders. Newborn screening may prevent the diagnostic odyssey and uncertainty associated with investigations of non-specific symptoms. Patients receiving earlier diagnosis and ERT treatment may benefit from a reduction in or stabilisation of their MPS II symptoms. Consequently, they may require fewer multidisciplinary investigations and interventions associated with the systemic manifestations of MPS II over their lifetime.. Conversely, earlier diagnosis of patients with MPS II may mean that the child and their family are exposed to the increased burdens and risks associated with MPS II treatment over a longer period

Newborn screening for the condition may mean that the child and their family can benefit from the value of knowing, whilst acknowledging that earlier knowledge may also result in psychological harm for some patients and families. The child and their family may access support services such as genetic counselling and seek advice from patient support groups before the characteristic symptoms of MPS II develop. Earlier detection of MPS II enables cascade testing of family members (parents and siblings) to confirm either the presence of MPS II in other affected males or the causative familial gene variant(s) in females that are MPS II carriers. Cascade testing will enable parents and other family members to access reproductive technologies for family planning if appropriate.

PICO criteria (PICO Set 1)

Population (PICO Set 1)

The target population for mucopolysaccharidosis type II (MPS II) screening is all newborns in Australia that participate in NBS. Over 99% of all newborn Australian babies participate in NBS screening (Huynh et al. 2022).

PASC noted that MPS II is an ultra-rare X-linked metabolic disorder predominantly affecting males and that females are rarely affected but can be carriers of MPS II.

PASC accepted that the population was all newborn babies who participate in universal NBS programs.

Incidence of MPS II

Incidence in the Australian population

The distribution and incidence of MPS II is variable based on international geographical region and/or ancestry (Çelik et al. 2021), and may potentially differ between relying on diagnosis after symptom-onset compared to detection through a NBS program (although the rate of current under-diagnosis is unknown).

A study of the Western Australian population from 1969-1996 reported an estimated incidence of 0.31 per 100,000 live births and 0.6 per 100,000 male live births. The incidence rate was calculated by dividing the total number of cases diagnosed prenatally and postnatally (due to symptoms or family history) by the total number of live births or total number of male live births during the study period. In all cases, the diagnosis was confirmed by one dimensional electrophoresis of urinary GAGs and/or by enzyme assay on leucocytes or fibroblasts (Nelson et al. 2003).

Another Australian study of the incidence and prevalence of lysosomal storage disorders (LSDs) reviewed data from the national referral laboratory for LSD diagnosis in Australia from 2009 to 2020. The laboratory diagnosis of MPS II was made by a combination of biochemical testing (deficient I2S enzyme and/or elevated GAG biomarkers) and genetic analysis, with patients being investigated due to signs/symptoms or family history. The number of live births was obtained from the Australian Bureau of Statistics. The estimated incidence of MPS II was 0.57 per 100,000 male live births as all cases diagnosed were male (Chin & Fuller 2022).

The Expert Panel for the Life Saving Drugs Program (LSDP) review of ERT idursulfase (ELAPRASE®) reported that the best prevalence estimate for MPS II in Australia is between 0.13 and 0.3 per 50,000 people, which is below the 1:50,000 threshold for an ultra-rare disease under the LSDP (Australian Government Department of Health and Aged Care 2023a).

PASC noted that based on the estimated incidence, approximately 1 - 2 male newborns would be diagnosed with MPS II per year if MPS II was introduced to NBS programs.

Based on data for Australia, the number of babies that would be eligible for NBS for MPS II over the period 2024 to 2028 is shown in Table 3. It is estimated that 312,380 babies would participate in NBS in the 2025-2026 financial year.

Table 3 Estimated number of babies eligible and utilising NBS for MPS II between 2024 and 2028

Financial year	2024-2025	2025-2026	2026-2027	2027-2028
Estimated number of babies born (n) [i]	313,993	314,727	315,462	316,196
Estimated number of babies who uptake NBS (n) [ii]	311,651	312,380	313,109	313,873

[i] ABS registered births (ABS 2022)
[ii] Based on the proportion of babies who took up NBS in 2016−2020, which was 99.3% (Huynh et al. 2022)

Incidence in other countries

In the United States, NBS programs in Illinois and Missouri published preliminary estimates of 0.9 and 1.3 diagnosed MPS II cases per 100,000 newborns screened (Bilyeu et al. 2020; Burton, B. K., Hickey & Hitchins 2020).

A study based on an international assessment of all MPS disorders estimated the birth incidence of MPS II to range from 0.1 (British Columbia) to 2.16 (Estonia) cases per 100,000 births (Çelik et al. 2021). The prevalence in Japan and Taiwan based on clinical identification has been reported to be 0.84 to 1.07 per 100,000 births (Çelik et al. 2021).

The natural history of MPS II

MPS II (Hunter syndrome) is an ultra-rare X-linked recessive LSD. Therefore, the condition occurs predominantly in males; females rarely develop clinical signs of MPS II unless there is abnormal X-

chromosome inactivation (Fang, Deng & Disteche 2021; Guillén-Navarro et al. 2013; Kloska et al. 2011; Lonardo et al. 2014; Tuschl et al. 2005). MPS II is a chronic progressive multisystem condition that affects many tissues and organs. Characteristics and symptoms vary in severity across a continuum ranging from milder late-onset attenuated disease to severe early-onset disease (Ream et al. 2023).

MPS II is caused by pathogenic variants in the *IDS* gene encoding the enzyme iduronate-2-sulfatase (I2S; OMIM 309900). Pathogenic variants in the *IDS* gene lead to a deficiency in the I2S enzyme resulting in the accumulation of two glycosaminoglycans (GAGs), dermatan sulphate (DS) and heparan sulphate (HS), in the lysosomes within cells. Accumulation of GAGs leads to lysosomal hypertrophy and an increase in the number of lysosomes within cells. GAGs are found throughout the body and are a crucial component of the extracellular matrix (ECM). The lack of GAG turnover in the ECM impacts the cellular functions, like cell adhesion, endocytosis, intracellular trafficking of different molecules, intracellular ionic balance, and inflammation (Hampe et al. 2021). This causes progressive, permanent, cellular damage in affected individuals leading to development of somatic manifestations and, in the more severe early-onset cases, neuronopathic manifestations of MPS II. MPS II is a life-limiting disorder with life expectancy determined by symptom severity and neuronopathic involvement. MPS II has historically been broadly divided into two forms, (1) a severe (neuronopathic) form with central nervous system (CNS) involvement and earlier onset and (2) a milder attenuated (non-neuronopathic) form with later onset; however, the characteristics and symptoms of MPS II vary in severity across a continuum (Ream et al. 2023). Two thirds of individuals diagnosed with MPS II have the severe form characterised by earlier onset of symptoms, more rapid disease progression and neuronopathic involvement, leading to symptoms of significant neurological impairment including cognitive disability and behavioural problems (Ayodele et al. 2022).

At birth, newborns with MPS II appear normal. For children affected by the most severe form of MPS II, the first symptoms (e.g., hernia and recurrent otitis media and respiratory tract infections) characteristic of MPS II start to appear as early as 6 to 12 months of age. In the first few years of their life, children with MPS II do not meet normal developmental milestones. As MPS II progresses with age, children regress developmentally losing much of their cognitive function (including speech and language), fine motor skills and develop behavioural issues with hyperactivity. Growth slows at around 5 years old resulting in short stature. Joint contractures develop that significantly affect mobility and multiple skeletal abnormalities called dysostosis multiplex are observed. Motor dysfunction appears between the ages of 6 and 8 years old. Differences in facial features and other somatic symptoms are often the first characteristics prompting parents to seek clinical advice. These include coarseness of facial features (full lips, large cheeks, a broad nose, and a large tongue), delayed development of motor and cognitive milestones, short stature, and abdominal distention. As many organs and tissues are affected in MPS II, there are multiple characteristics and clinical features including a large head, hydrocephalus, short neck, deeper the voice due to enlarged vocal cords, frequent upper respiratory infections, sleep apnoea due to narrowing of the airway, an enlarged liver and spleen, umbilical or inguinal hernia, hearing loss, reduced vision, carpel tunnel syndrome, narrowing of the spinal canal compressing the spinal cord, heart valve abnormalities leading to heart rhythm abnormalities, and skeletal abnormalities (D'Avanzo et al. 2020; Martin et al. 2008; MedlinePlus Genetics 2023). Life expectancy with the severe form of MPS II is between 10 to 20 years of age with death usually caused by obstructive airway disease and/or cardiac failure (Martin et al. 2008).

Compared to the early-onset severe form of MPS II, many of the systemic symptoms of late-onset attenuated MPS II are similar (e.g., characteristic appearance and skeletal abnormalities) but generally milder and neurological involvement characterised by cognitive impairment and severe behavioural problems is limited or absent (D'Avanzo et al. 2020). Life expectancy is significantly greater at around 50 to

70 years. Despite the significant difference between phenotypes of severe and attenuated forms, attenuated patients may ultimately be as greatly affected as severe patients due to somatic manifestations of MPS II over longer life spans. Patients with attenuated disease also face cord compression and some neurocognitive challenges such as, hydrocephalus and deafness, which can affect learning and behaviour. Guffon et al. noted that 25% of attenuated patients also experience mild neurocognitive impairment without regression (Guffon et al. 2015) which can manifest as poor adaptive skills, neurocognitive difficulties, and attention difficulties (Shapiro & Eisengart 2021).

As MPS II is a heterogenous multisystem condition, early characteristics and symptoms can overlap with other childhood syndromes or more common ailments. Consequently, diagnosis of MPS II can be difficult and may be exacerbated by lack of awareness and limited experience of this ultra-rare disorder. This can lead to misdiagnosis and/or delayed diagnosis (Wiśniewska et al. 2022). Patients and their families often endure a long diagnostic odyssey before receiving a definitive diagnosis with a typical diagnostic delay for MPS II of several years. Once a diagnosis of MPS II or similar condition is suspected, the patient is referred to a specialist metabolic disorders clinic for further clinical assessment and diagnostic testing to determine their I2S enzyme levels in blood and GAG levels in urine. These tests may be followed by genetic testing to identify the causative *IDS* gene variant.

Inheritance of MPS II

As an X-linked recessive disorder, MPS II predominantly affects males. Females are usually carriers and rarely develop clinical signs of MPS II unless there is abnormal X-chromosome inactivation (Fang, Deng & Disteche 2021; Guillén-Navarro et al. 2013; Kloska et al. 2011; Lonardo et al. 2014; Tuschl et al. 2005).

It is estimated that between 10 – 33% of *IDS* variants identified in affected individuals are *de novo* rather than familial in origin (Filocamo et al. 2018; NewSTEPs 2022; Pollard, Jones & Wood 2013).

When it is inherited, MPS II is usually inherited from a mother carrying an *IDS* pathogenic variant (carrier). In line with X-linked inheritance patterns, a carrier biological mother and unaffected biological father have a 50% chance that a daughter is a carrier and a 50% chance that a son is affected by MPS II.

Historically, males with the early-onset severe form MPS II have not reproduced due to the severity of their condition and early mortality. Earlier treatment and future improvements in MPS II treatment could increase the possibility of males, particularly those with the late-onset attenuated MPS II, becoming fathers. If an unaffected biological mother and an MPS II-affected biological father have children, all their daughters will be carriers of MPS II and all their sons will be unaffected. If a biological mother who is a carrier, and an MPS II-affected biological father have children, their daughters will have a 50% chance of being a carrier and a 50% chance of being affected by MPS II, while their sons would have a 50% chance of being affected by MPS II.

Genetics of MPS II

The *IDS* gene on the X chromosome spans 44 kb and has nine exons (D'Avanzo et al. 2020). A pseudogene of *IDS* called *IDSP1* is located close to the *IDS* gene. Some regions of the pseudogene are homologous to *IDS*, with exon 3 of the pseudogene being identical to exon 3 of the *IDS* gene (D'Avanzo et al. 2020). This similarity increases the frequency of large gene rearrangements due to homologous recombination and makes genetic analysis of *IDS* variants more complex.

MPS II is characterized by high genetic heterogeneity as most of the *IDS* variants identified are private or novel. More than 700 *IDS* variants have been identified according to the Human Gene Mutation Database. Around 50% of *IDS* variants are missense/nonsense mutations, followed in frequency by small deletions,

splicing variants, gross deletions, complex rearrangements, small indels, and gross insertions (D'Avanzo et al. 2020).

High genetic heterogeneity associated with MPS II means that identifying genotype–phenotype associations has been difficult (Filocamo et al. 2018; Vollebregt et al. 2017). While current understanding of genotype–phenotype associations is limited for MPS II, some variants have been linked to the more severe form of MPS II and may guide treatment decisions. While affected family members with the same variant tend to share a similar phenotype, there are different phenotypes in unrelated MPS II patients carrying the same variant suggesting that genetic modifying processes or environmental factors are also involved (D'Avanzo et al. 2020). However, some variants have been linked to specific phenotypes (Kosuga et al. 2016). For example, complete deletion of the *IDS* gene has been consistently associated with severe MPS II (Kosuga et al. 2016; Seo et al. 2020; Vollebregt et al. 2017).

Individuals with *IDS* gene variants associated with I2S pseudodeficiency exhibit low I2S enzyme levels (~ 5–15% of normal activity) but normal urinary GAG levels and no symptoms of MPS II (NewSTEPs 2022). These patients are asymptomatic because variants in the *IDS* gene reduce the activity of the enzyme as detected *in vitro* but not to the point that it is disease-causing *in vivo (*i.e. they have sufficient I2S activity to prevent accumulation of GAGs). The proposed NBS method with subsequent confirmatory diagnostic testing (and the current diagnostic testing method) for MPS II can differentiate between individuals with MPS II and those that are classed as "pseudodeficient" for the I2S enzyme. This differentiation is important as it increases the positive predictive value (PPV) of NBS by reducing "false positives" from detection of individuals with pseudodeficiency, so preventing misdiagnosis and unnecessary treatment.

PASC considered that phenotype-genotype predictions of disease severity are difficult for MPS II because most families have private variants, and there are no common pathogenic/likely pathogenic IDS variants. Many causative variants are also deletions. PASC noted that MPS II severity can potentially be predicted from genotypes that result in no protein production at all (for example through nonsense-mediated decay), but these may only comprise an estimated one third of patients with a neurological phenotype.

Intervention (PICO Set 1)

The proposed intervention is to add MPS II to universal NBS in Australia. Over 99% of newborns receive NBS, which currently screens for up to 32 rare conditions (Australian Government Department of Health and Aged Care 2023b; Huynh et al. 2022). Universal NBS would be available in addition to targeted preconception and antenatal screening in those at high-risk due to a family history of MPS II. A negative NBS result would not rule out diagnostic testing of clinically symptomatic patients later in life.

The NBS protocol utilises blood spots from a neonate heel prick, collected within 48 to 72 hours of birth, and dried on filter-paper (Guthrie card) (BetterHealth VIC).

Different NBS protocols are possible for screening for MPS II, and state and territory screening laboratories can determine their preferred approach.

PASC supported the use of a two-tier NBS protocol for MPS II, as follows:

- Measurement of I2S enzyme activity using a dried bloodspot sample and LC-MS/MS or fluorometric enzymatic assay (first-tier screening test)

Those patients who are positive on the first-tier screening test, would receive second-tier screening using:

- The endogenous biomarker method for measuring small non-reducing end GAG fragments using LC-MS/MS on a dried bloodspot sample.

Conversely, a single-tier NBS protocol for MPS II is proposed by the National Referral Laboratory, as follows:

- The endogenous biomarker method for measuring small non-reducing end GAG fragments using LC-MS/MS on a dried bloodspot sample.

This method was developed by the National Referral Laboratory. A small case control study has reported that measuring GAG fragments is 100% accurate at distinguishing between different MPS subtypes and healthy individuals when used as a second-tier test (Herbst, Z. M. et al. 2022), but there is currently no published evidence for its use a first-tier or single-tier NBS test. Expert advice has suggested that the reason for its roll-out as a second-tier test rather than single-tier test in the United States, was due to the costs associated with changing methodology and revalidating the approach[1]. Conversely, in Australia, where all the NBS laboratories (except the National Referral Laboratory) do not have a current test for MPS II, there is more flexibility to consider which protocol may be the optimal approach (based on the costs and elimination of false positives).

Expert advice suggested that "all laboratories" in the US are rolling out GAG fragment analysis as their second-tier test for MPS II (following a first-tier enzyme activity testing), as it eliminates the need to do further confirmatory testing[1]. However, genetic testing is still recommended to facilitate cascade testing. If the single-tier screening test (or two-tier screening tests) are positive or borderline positive for I2S deficiency, then the newborn would be referred for confirmatory diagnostic testing using a new blood sample; this would include genetic testing to detect the causative variant in the *IDS* gene.

Expert opinion from the National Referral Laboratory has suggested that the use of GAG fragment analysis would mean confirmatory diagnosis using I2S enzyme activity levels would not be required. However, the current eligibility criteria for idursulfase treatment through the LSDP requires that the diagnosis of MPS II be confirmed through the demonstration of a deficiency of I2S enzyme in white blood cells with the assay performed in a NATA-accredited laboratory; or, for siblings of a known patient, detection of a disease-causing variant.

NBS screening laboratories may determine their own preferred method for screening for MPS II (Table 4). Only the Western Australian and South Australian laboratories had provided feedback at the time of the pre-PASC PICO confirmation.

[1] Expert advice received via email on 23/03/24 from the Head of the National Referral Laboratory

Table 4 Methods of screening for MPS II proposed to be used by different NBS programs in Australia

State	First-tier test	Second-tier test	Third-tier test
Western Australia	Revvity NeoLSD2 using FIA-MS/MS enzymology[1]	Dried bloodspot GAG analysis to be carried out at Adelaide Women's and Children's Hospital National Referral Centre for Lysosomal storage diseases	*IDS* genotyping/sequencing either in WA or AWCH using in-house test (refer to genotyping laboratory)
South Australia	Endogenous non-reducing end GAG method	--	*IDS* genotyping/sequencing

[1]This test is currently for research use only. No information is available on the company website. There was no information provided or available on the company website about this kit including other disorders that are multiplexed with MPS II or the cost of the kit as provided in feedback.
[2]This is currently the method used for diagnosis of MPS II by the National Referral Laboratory.
AWCH=Adelaide Women's and Children's Hospital National Referral Centre for Lysosomal storage diseases; GAG=glycosaminoglycan; I2S=iduronate-2-sulfatase; *IDS*=iduronate-2-sulfatase gene; LC-MS/MS=liquid chromatography tandem mass spectroscopy; NBS=newborn bloodspot screening.

The screening tests will need to be implemented in NBS program laboratories, which are all NATA-accredited to perform human pathology services on patient samples. Each laboratory will need to develop a test protocol adapted to its specific requirements and appropriately validated. Laboratories will require extra staff to accommodate testing and training for laboratory staff on the application of the test protocol will be required. Funding to implement screening will also need to be sought by NBS laboratories from their respective jurisdictional governments, noting that the Commonwealth is providing funding to support expansion (as outlined in the Proposal for public funding section) to purchase any necessary equipment and reagents.

A bloodspot is already collected by health professionals within 48 to 72 hours of birth for babies participating in the universal NBS program. Collection of an additional sample is not required to screen for MPS II as part of universal NBS. However, an additional bloodspot may be used if the results of the initial NBS tests are inconclusive.

The health professionals required may vary from jurisdiction to jurisdiction, however, below is a potential list of key health professionals who may be needed:

- Nurses/midwives who collect blood samples on NBS dried bloodspot cards. This process already occurs routinely to screen for other conditions, therefore, no additional resources are required at this stage.
- Screening laboratory scientist/pathologist – these professionals are needed to undertake the screening and will be required to develop and implement a screening and data analysis protocol for MPS II. This requires a significant amount of work, both in setting up screening and ongoing as it is used.
- Clinical nurse consultants/ screening laboratory support staff will need to assist with recalls, parent notification or early notification of clinicians where there are abnormal NBS results – limited change expected as this process already occurs for other conditions. They will also be required to provide referrals into care.
- If abnormal follow up, confirmatory diagnostic testing may be necessary through the children's hospital, an appropriate physician for diagnosis or through a genetic counsellor. If an external diagnostic lab was to be involved, there would likely not be a change due to the proposed health

technology as diagnostic testing already occurs for MPS II, although the number of individuals requiring diagnostic testing is likely to increase due to screening.

- If MPS II is confirmed, a multi-disciplinary team headed by a metabolic physician will be needed as MPS II affects multiple body systems.

PASC confirmed that the proposed intervention was NBS using a two-tier protocol. The 1st tier would be quantification of the enzyme I2S using LC-MS/MS. The 2nd tier would be a method of GAG fragment analysis by LC-MS/MS such as use of the non-reducing endogenous biomarker method currently used for diagnosis of multiple MPS disorders by the National Referral Laboratory for Lysosomal, Peroxisomal and Other Related Disorders in Adelaide, South Australia.

PASC considered that using the proposed two-tier protocol would enable detection of false positive MPS II screening results caused by I2S pseudodeficiency, and therefore would protect parents of newborns from unnecessary stress and worry associated with diagnostic uncertainty.

PASC noted that highly specialised quadrupole linear ion trap (Q TRAP) LC-MS/MS equipment was required for the non-reducing endogenous biomarker method of GAG fragment analysis and that this was not currently available in NBS laboratories in Australia. PASC advised additional equipment and resourcing would be required to implement this method in NBS laboratories.

PASC noted that it may be possible to send DBS identified as deficient in I2S enzyme after 1st-tier testing at a NBS laboratory to the National Referral Laboratory for non-reducing endogenous GAG fragment analysis by LC-MS/MS (2nd-tier screening test). However, it is also possible that NBS laboratories could select a different 2nd-tier screening method for testing GAGs in DBS and carry out the test in-house.

PASC advised that the assessment report should also explore the costs of a single tier approach to MPS II NBS using the non-reducing endogenous GAG fragment analysis as carried out by the National Referral Laboratory in Adelaide. This method may have non-inferior sensitivity and specificity to the proposed two-tier screening approach for identifying MPS II and other MPS disorders and cost less than using a two-tier approach where this test is the 2nd-tier screening test. PASC noted that there might be limited or no evidence available for use of this method in NBS as a single-tier approach.

NBS programs for MPS II in other countries

Examples of NBS screening programs available globally for MPS II and their screening approach are summarised in Table 5. NBS for MPS II was added to the United States of America (USA) Recommended Uniform Screening Panel (RUSP) in August 2022; the protocols used for MPS II NBS in the US vary (Burton, Barbara K. et al. 2017; Burton, B. K., Hickey & Hitchins 2020; Burton, Barbara K. et al. 2019). Overseas screening programs use either a one-tier, two-tier or three-tier approach to MPS II screening; all published methods use measurement of the I2S enzyme as their first tier of testing with low I2S activity levels determined by comparison to either mean or median normal activity levels. All tests include internal controls for quantifying enzyme levels and for test validity. The cut-off values below which a newborn is considered to have low I2S activity potentially indicative of MPS II vary between the screening programs. The cut-off value selected is usually conservative to avoid false negative test results that would result in missed detection of MPS II and were established during pilot studies. False positive results, usually attributable to enzyme pseudodeficiency, that occur because of the conservative cut-off for I2S activity are identified either by further tiers of testing during NBS screening or by early referral for confirmatory diagnostic testing using additional test samples (blood, urine) to confirm an MPS II diagnosis.

A single-tier approach to NBS for MPS II using the endogenous biomarker method for measuring GAG fragments is currently not being utilised in any countries globally although this method has been adopted in the US as the second-tier screening test for GAG analysis[2].

Table 5 MPS II newborn screening programs around the world included in the MPS II NBS application

Country	Year of Implementation	Program type	Screening methods
USA (Illinois) (Burton, Barbara K. et al. 2017; Burton, B. K., Hickey & Hitchins 2020; Burton, Barbara K. et al. 2019)	2017	NBS	LC-MS/MS used. 1-tier: I2S activity in DBS (≤10% of median normal activity). If between 10-13% median normal activity, then a second sample was tested. If test is still borderline or positive then referred for diagnostic testing (plasma I2S test, urine GAG analysis, and DNA sequencing).
USA (Missouri) (Bilyeu et al. 2020)	2018	NBS	4-MU test for I2S activity 1st-tier: I2S activity in DBS (25 µmol/L/h (retest cutoff) and 20 µmol/L/h (high risk cutoff). 2nd-tier: GAG analysis in DBS Patients with positive results after 2nd-tier sent for further confirmatory testing (GAG in urine plus other biochemical testing and history)
Taiwan (Lin et al. 2022)	2015	NBS	LC-MS/MS used. 1st-tier: I2S activity in DBS (≤30% of mean normal activity; ≤ 6.5 µmol/L/h) 2nd-tier: I2S activity in DBS (≤10% of mean normal activity; ≤ 2.2 µmol/L/h) 3rd-tier: DNA testing for genotype Positive or potentially positive patients referred for long term follow-up every 6 months.
Japan (Hattori et al. 2023)	2016	NBS; pilots in limited area	1 tier (in duplicate): Fluorescence assay (4-MU) for I2S enzyme activity in DBS. Cut-off level was set as 20% of median normal I2S activity in newborns; subsequently reduced to 10% (5.0 pmol/h/disk) If test positive, referred for further assessment (physical examinations, biochemical tests, imaging studies, DNA sequencing)

DBS=dried bloodspot; GAG=glycosaminoglycan; I2S=iduronate-2-sulfatase; LC-MS/MS=liquid chromatography tandem mass spectroscopy; NBS=newborn bloodspot screening; USA=United States of America; 4-MU=4-methylumbelliferone.

Consideration of reporting for males only versus all newborns

Under *MSAC Application 1710 – Newborn bloodspot screening for X-linked adrenoleukodystrophy*, MSAC considered whether reporting of NBS results for this X-linked condition should be for males only or for all newborns (via the inclusion of an X-counter or equivalent in NBS). For that condition, MSAC "*considered there were arguments each way and, on balance, supported X-ALD NBS results being reported for all newborns, because reporting results for all newborns would equitably allow all families of children identified through screening to make informed reproductive decisions*" (MSAC 1710 PSD, pg 1). However, MSAC also "*considered that its advice to report results for all newborns rather than males only for X-ALD NBS was not necessarily generalisable to other conditions*" (MSAC 1710 PSD, pg 7), so MSAC's previous advice does not necessarily preclude an X-counter being included in NBS for other X-linked conditions and this should be considered on a case-by-case basis.

[2] Expert advice received via email on 23/03/23 from the National Referral Laboratory

However, NBS for MPS II is unlikely to identify female newborns that are carriers of MPS II as their I2S enzyme activity levels should fall within the "normal" range and they will not have GAG fragments indicating MPS II. Therefore an X-counter or equivalent test is not proposed for NBS of MPS II.

PASC noted very rarely females are affected, usually as a result of non-random X-inactivation or Turner syndrome.

Measurement of I2S enzyme activity in DBS samples (potential first-tier screening test)

Most lysosomal enzymes are active in rehydrated dried bloodspot (DBS) samples enabling their activities to be measured. Enzymatic assays generally involve the addition of lysosomal enzyme substrates in buffer to a dried bloodspot punch. The mixture is incubated at 37°C for a prescribed period prior to measuring the enzymatic activity.

Current newborn screening protocols for MPS II include fluorescence-based methods and tandem mass spectroscopy (MS/MS) with sample introduction using either flow injection analysis (FIA) or high-performance liquid chromatography (HPLC) (Gelb, Michael H. et al. 2022; Gelb, M. H. et al. 2019). Gelb et al (2019) considered that while both fluorescence-based methods and MS/MS were not fully automated, they were simple to execute (Gelb, M. H. et al. 2019).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Two tandem mass spectroscopy (MS/MS) methods can be used:

- Flow-injection analysis (FIA) MS/MS, where the sample is introduced as a bolus injection into the mass spectrometer without the prior fractionation of analytes.
- Liquid chromatography (LC) combined with MS/MS (LC-MS/MS) where analytes are fractionated following a liquid–liquid extraction step with ethyl acetate.

MS/MS is the most common biochemical method used for the detection of analytes in DBS and permits multiplexing of tests so that multiple analytes can be extracted from one DBS punch and quantified in a single infusion into the MS. When biochemical assays require separate pre-MS/MS processing of multiple DBS punches, these can be subsequently combined for a single infusion for either LC-MS/MS or FIA-MS/MS analysis. Gelb et al (2022) indicated in their review of LC-MS/MS in NBS laboratories that LC-MS/MS has some advantages over FIA-MS/MS. Importantly for MPS II analysis, the use of LC rather than FIA avoids the problem of I2S-independent breakdown of the I2S substrate to I2S product in the heated electrospray ionization source of FIA-MS/MS which interferes with measurement of I2S activity. Use of LC-MS/MS separates substrates from products via LC prior to MS/MS and therefore in-source cleavage is no longer an issue (Gelb, Michael H. et al. 2022).

Use of LC-MS/MS allows the development of multiplex assays for MPS. Gelb et al (2022) report that LC-MS/MS has the advantage of enabling a larger number of diseases to be cost-effectively screened in a high throughput, multiplex assay with a reasonable turnaround time. They also noted that LC-MS/MS is the 'preferred' method used in commercial production of reagents and kits.

It is necessary to carry out analysis of Mucopolysaccharidosis type I (MPS I) and MPS II in separate enzymatic reactions, which can then be subsequently combined after inactivation for separation and analysis by LC-MS/MS. This is because the enzymatic product of I2S activity is the substrate for the enzyme iduronidase (IDUA) encoded by the MPS I gene. The Illinois NBS laboratory was the first to use LC-MS/MS to quantitate enzyme products as a primary screening test with a 6-plex LSD assay that measured enzymic

activity related to glycogen storage disease type II (GSD II), MPS I, Krabbe, Fabry, Niemann-Pick-A/B, and Gaucher diseases. The test was recently expanded to include I2S for MPS II. A second assay is used for MPS II because, as indicated above, the product of I2S enzymic activity is the substrate for IDUA (MPS I). One 3 mm DBS punch is incubated in an assay cocktail for all enzymes except I2S for MPS II. A second 3 mm DBS punch is used for the MPS II assay. After the enzymatic reactions are stopped, the two assay mixtures are combined and analysed in a single LC-MS/MS run per newborn, with an inject-to-inject time of 2.1 min (Burton, Barbara K. et al. 2017; Burton, B. K., Hickey & Hitchins 2020; Burton, Barbara K. et al. 2019).

Several newborn screening studies have reported multiplexing of MPS II analysis with other disorders using DBS (Table 6).

Table 6 Studies reporting multiplexing of MPS II with other conditions for NBS

Study author	Conditions
(Scott et al. 2020)	MPS II, MPS IIIB, MPS IVA, MPS VI, and MPS VII
(Kelly et al. 2024)	ASMD, CLN2, CTX, Fabry, GM1 gangliosidosis, Gaucher, LAL–D, MLD, MPS II, MPS IIIB, MPS IVA, MPS VI, MPS VII, NPC
(Arunkumar et al. 2021)	MPS I, MPS II, MPS IIIB, MPS IVA, and MPS VI
(Liu et al. 2017)	MPS I, MPS II, MPS IIIB, MPS IVA, MPS VI, and MPS VII, LINCL
(Mashima et al. 2018)	MPS II, MPS IVA, and MPS VI
(Oguni et al. 2020)	MPS I, MPS II, MPS IIIB, MPS IVA, and MPS VI
(Chien et al. 2020)	GSD II, Fabry, Gaucher, MPS I, MPS II, MPS IIIB, MPS IVA, and MPS VI

ASMD=Acid Sphingomyelinase Deficiency (Niemann-Pick type A and B), CLN2=Ceroid Lipofuscinosis type 2, CTX=Cerebrotendinous Xanthomatosis, GSD II=glycogen storage disease type II (Pompe); LAL–D=Lysosomal Acid Lipase deficiency, MLD=Metachromatic Leukodystrophy, MPS II=Mucopolysaccharidosis II (Hunter Syndrome), MPS IIIB=Sanfilippo type 3b, MPS IVA=Morquio syndrome, MPS VI=Maroteaux-Lamy syndrome, MPS VII=Sly Syndrome, NPC=Niemann-Pick Disease Type C, LINCL=type 2 neuronal ceroid lipofuscinosis.

Fluorometric assays

Fluorometric enzyme assays identify samples with reduced enzyme activity by using an artificial substrate with a fluorescent tag, 4-methylumbelliferyl (4-MU)-glycoside. 4-MU-glycosides are acted on by lysosomal enzymes enabling quantification of the enzyme products by fluorescence.

Pilot results from Taiwan, Illinois, and Missouri for MPS II NBS indicate that both tandem MS/MS and fluorometry can be effective (Arunkumar et al. 2020). The Missouri USA newborn screening lab uses fluorometric assays with a digital microfluidics platform (Bilyeu et al. 2020). Bilyeu et al (2020) reported the validation and pilot testing of an MPS II assay (Bilyeu et al. 2020). Enzyme activity was measured using 4-methylumbelliferone (4MU) with fluorescence of the plate read in a BioTek Synergy HTX microtiter plate reader (Agilent; Santa Clara, CA) with 400 (±15) nm excitation and 485 (±20) nm emission filters; the fluorescence was measured as relative fluorescence units (RFUs) and subsequently converted to activity units (µmol/L/h). Hattori et al (2023) reported that since 2019 screening in Japan was carried out using a fluorometric enzyme assays kit, designed for multiplex screening for LSDs and hypophosphatasia (Enzaplate LSD; distributed by Siemens Healthcare Diagnostics K.K., Tokyo, Japan). A single 3.2-mm diameter disk punched from a DBS card is incubated in a 96-well plate with 200 µL of extraction solution. A 20 µL aliquot of the extract is then added to 40 µL of substrate solution and incubated at 38 ◦C for 3 hours, after which 200 µL of reaction stop solution is added. Fluorescence intensity is analyzed at excitation and emission wavelengths of 370 and 465 nm, respectively. Enzyme activity was calculated as pmol 4-MU/h/disk. Enzyme activity is expressed in different units by the Taiwan and Missouri screening laboratories.

Kumar et al (2015) concluded from their study that MS/MS assays of lysosomal enzymes outperform 4MU fluorometric assays in terms of analytical range. In their study, they designed new MS/MS assays with a larger analytical range for use with dried blood spots (DBS) for detection of MPS II, MPS IVA, and MPS VI. Following a comparison with fluorometric assays using 4MU-substrate conjugates, they reported that the MS/MS assay for MPS II, MPS-IVA, and MPS-VI had an analytical range that was 1–2 orders of magnitude higher than for the corresponding fluorometric assay. The intrinsic fluorescence of the 4MU-glycoside substrates resulted in higher background noise, reducing the analytical range of the fluorometric assays (Kumar et al. 2015). Liao et al (2014) also concluded that compared with the 4-MU method, the MS/MS method provided more specific and less laborious multiplex high-throughput screening for lysosomal storage diseases (Liao et al. 2014). The greater analytic range of MS/MS over fluorometric assays improves the separation of enzymatic products and therefore the ability to distinguish between "normal" levels of I2S and low levels of I2S associated with MPS II.

Further testing for MPS II biomarkers (usually GAG testing of urine or a DBS) is required to distinguish between patients that are positive for MPS II and those with low enzyme levels due to pseudodeficiency.

Analysis of GAGs (dermatan sulfate and heparan sulfate) levels and/or subspecies in DBS (Second-tier screening test or potential single-tier test)

As the assay to detect I2S enzyme activity has a low PPV for MPS II due to the detection of individuals with pseudodeficiency, samples with a positive first-tier test result (i.e., samples with low I2S enzyme activity) would undergo a second NBS test (second-tier test) to determine the either the level of GAGs or the GAG subspecies in the dried bloodspot samples. LC-MS/MS assays are used by most NBS programs as a second-tier test to improve the specificity of I2S screening tests with a low PPV. This approach reduces the number of false-positives as a second-tier test result in the normal range indicates the first-tier test result was a false positive result and usually a consequence of I2S pseudodeficiency. For MPS II, a high proportion of below-cutoff enzyme activity levels detected by the first-tier I2S activity assay are due to I2S pseudodeficiency. The analysis of GAGs in a separate punch from the same dried bloodspot differentiates between "true" I2S deficiency due to MPS II and pseudodeficiency (Herbst, Z. M. et al. 2022). Individuals with pseudodeficiency have normal levels of GAGs in the DBS sample whereas individuals with I2S deficiency due to MPS II have elevated GAG levels.

While current diagnostic pathways for MPS II and confirmatory diagnostic testing following NBS would utilise a urine sample for measurement of GAGs, it is proposed that NBS for MPS II would assess GAGs in the DBS obtained for NBS. This removes the need to recall the patient to obtain a urine sample and protects parents from unnecessary stress and worry associated with uncertainty.

In their recent review, Saville et al (2023) reported that studies had confirmed that the GAG analysis method using native GAG, non-reducing end fragments (endogenous biomarker method) is superior as a second-tier NBS test for MPS in comparison to the method where internal disaccharides are quantified after enzymatic digestion of GAG polymers (Herbst, Z. M. et al. 2023; Herbst, Z. M. et al. 2022; Herbst, Zackary M. et al. 2020; Saville, J. T. et al. 2023). The key difference is that the native GAG, non-reducing end fragments are essentially undetectable in DBS from newborns that are not at risk for MPS disorders (carriers, pseudodeficiencies, and those containing no variants). In contrast, dried blood spots from non-MPS newborns contain elevated internal disaccharide markers with a false positive rate of ~5–10% across the different MPS types (Herbst, Z. M. et al. 2023).

Herbst et al (2022) assessed whether GAG-derived biomarkers in newborn DBS could be used for second-tier testing in NBS to follow the measurement of I2S activity in DBS. They considered two methods: the

internal disaccharide and endogenous biomarker methods. The internal disaccharide method is the most commonly used GAG assay where GAG polymers are cleaved by bacterial lyases and hydrolases to yield a set of disaccharides from the GAG polymer (Tomatsu, Shunji et al. 2013; Tomatsu, S. et al. 2014). The endogenous biomarker method does not involve *in vitro* enzymatic digestion of the GAG polymers but instead measures endogenous fragments generated *in vivo* by endohydrolases and exohydrolases (Fuller et al. 2004; Saville, Jennifer T. et al. 2019). Herbst et al (2022) assessed how selective GAG-derived biomarker measurement was in differentiating between DBS from healthy newborns, newborns with I2S pseudodeficiency, and newborns diagnosed with MPS II. LC-MS/MS was used to detect the GAG fragments because it is considered more sensitive and specific than methods that detect intact GAG polymers (e.g., fluorometry) (Herbst, Z. M. et al. 2022). The authors reported that the endogenous disaccharide method exhibited greater differentiation between control newborns DBS and newborns with pseudodeficiency compared with MPS II newborns DBS than when using the internal disaccharide method (Herbst, Z. M. et al. 2022).

There are no data concerning levels of internal disaccharide and endogenous GAG biomarkers for MPS II-diagnosed female newborn DBS (Herbst, Z. M. et al. 2022).

Genetic testing for causative IDS gene variants

Analysis of GAGs in DBS is considered to be more powerful than deoxyribonucleic acid (DNA) sequencing as a second-tier NBS test to reduce false positive results after first-tier testing. Although the same newborn DBS sample can be submitted to DNA sequencing analysis, genotypes are often inconclusive due to the large number of private variants meaning many variants of uncertain significance (VUS) are found, and also because of partially penetrant pathogenic variants. However, MSAC did advise in relation to X-linked adrenoleukodystrophy that despite most variants in the relevant gene being private, "*genetic testing was unlikely to result in a variant of uncertain significance (VUS) because the abnormal VLCFA [very long chain fatty acid] levels from the prior tier of screening can be taken into account when classifying the pathogenicity of the detected genetic variant*" (MSAC 1710 PSD, pg 5). Therefore, it is suggested that any novel variants or variants previously classified as VUS could be reclassified as pathogenic/likely pathogenic if NBS has identified either reduced enzyme activity or GAG fragments able to identify MPS II.

Currently, genetic testing for the causative MPS II variant in an individual with symptoms of MPS II is carried out during confirmatory diagnostic testing. Following the introduction of NBS for MPS II, it is similarly proposed that genetic testing would be carried out during confirmatory diagnostic testing for newborns with a positive NBS result for MPS II. Clinical expert advice is that genetic testing could be recommended as a third tier NBS test, and that consideration should be given to genetic testing being completed prior to a newborn's family being recalled for a clinical consultation. This approach would provide the recalling physician with as much information as possible when counselling the family. However, it is acknowledged that this approach may extend the time from screening to recall depending on the speed with which the genetic testing can be performed.

Clinical assessment and confirmatory diagnostic testing

Newborns receiving a positive or indeterminate screening result for MPS II will be referred for clinical assessment and confirmatory diagnostic testing, including analysis of GAGs in urine, plasma or peripheral blood leukocyte I2S enzyme activity analysis, testing for another sulfatase to exclude multiple sulfatase deficiency and genetic analysis to identify the causative *IDS* gene variant.

The outcomes of MPS II screening and the number of babies requiring referral for further diagnostic testing were estimated for the 2025-2026 financial year based on the estimated number of babies who

uptake NBS in that period (312,380 babies; Table 3) and published USA data on the outcomes of NBS for MPS II (Burton, B. K., Hickey & Hitchins 2020). The estimates for the two-tier screening test proposed are as follows:

- Burton et al (2020) screened 339,269 infants and identified 28 male infants with low I2S activity.
- When Burton et al (2020) further tested the 28 babies, 3 babies were confirmed with MPS II and the remaining 25 babies were pseudodeficient for I2S. These would have been identified as pseudodeficient by a second tier NBS test (GAG analysis) as proposed in this application.

If the data from Burton et al (2020) are extrapolated to the 312,380 babies that would be tested in the 2025-26 financial year in Australia, then 2 babies would be confirmed with MPS II and 23 would be identified as pseudodeficient for I2S (these pseudodeficient babies would not require subsequent referral for further diagnostic testing). Please note, the above data did not include any data for false positive/borderline results for NBS tests as the accuracy of screening tests used for NBS may vary.

The incidence of MPS II based on symptomatic diagnosis (not NBS) is 1/320,000 for Western Australia for the period 1969–1996 (Nelson et al, 2003) and 1/176,000 live births for Australia based on data from patient referrals and prenatal testing for period 2009 to 2020 (Chin & Fuller, 2022).

Chin & Fuller (2022) acknowledged that the reported incidence was higher than previously reported. Of 3,693,759 live births from 2009-2020 in their Australian study, 23 males were identified with MPS II. Based on the proposed figures for births for the 2025-2026 financial year (312,380 babies), two babies would be diagnosed with MPS II, which aligns with the calculations above based on data from Burton et al (2020) for NBS in the US.

Genetic testing is not essential for a confirmed diagnosis of MPS II but facilitates cascade testing. Identification of the causative variant allows genetic cascade testing to aid identification of other affected male family members or female genetic carriers of MPS II. This may inform future treatment and reproductive options.

PASC noted that genetic testing has limited utility for NBS outside cascade testing, given the high proportion of private variants in the IDS gene.

Where a MPS II diagnosis is confirmed following NBS but treatment is declined or uncertainty remains regarding the diagnosis following confirmatory testing, expert advice has indicated that regular clinical surveillance of the baby would be carried out during childhood (3 monthly for the first 1-2 years and then maybe 6 monthly until 6 and then 1-2 yearly if deemed to have an attenuated form) to monitor for symptoms consistent with development of the severe form of MPS II. Many patients with the severe form of MPS II will develop potentially life-threatening manifestations by the second decade of life. The clinical expert suggested that beyond childhood, at least 1 -2 yearly reviews are recommended to keep patients in touch with clinical services, as patients would be more likely to have the later onset slowly progressing attenuated form of MPS II. Regular monitoring during childhood and uncertainty around the implications of an MPS II diagnosis (e.g. severity at presentation) places an additional burden on both the patient and family particularly as a newborn with MPS II appears normal at birth when NBS is carried out. The monitoring protocol will need to be closely developed with the current clinical experts.

Treatment and ongoing management

Once a diagnosis of MPS II is confirmed, the patient and their family would be referred to a metabolic disorders clinic to discuss the MPS II diagnosis, treatment options and cascade genetic testing for family members (for parents and siblings initially) with a metabolic disease specialist and clinical geneticist.

Current treatments for MPS II are not curative but aim to manage the symptoms of MPS II, improve quality of life, slow down progression, and prevent/reduce permanent tissue and organ damage (Scarpa et al. 2011). Early intervention may help prevent or reduce irreversible damage. While patients may benefit from earlier diagnosis and treatment, they are also exposed to the burdens and risks of treatment prior to the appearance of symptoms associated with MPS II and this also places an additional burden on the family.

Treatments currently available in Australia for patients with MPS II are enzyme replacement therapy (ERT) and in some cases hematopoietic stem cell transplantation (HSCT).

Enzyme replacement therapy (ERT)

Intravenous ERT with human recombinant idursulfase (I2S; ELAPRASE®; ARTG 129481) is currently the main treatment for MPS II in Australia. The recommended dosage regimen is 0.5 mg/kg of body weight administered every week as an intravenous infusion via a central venous catheter (CVC), with an infusion time of 3 to 8 hours. A CVC is required life-long for idursulfase administration. Treatment should be supervised by a physician or healthcare professional experienced in the management of patients with MPS II or other inherited metabolic disorders (Therapeutic Goods Administration 2008). ERT is generally well tolerated. The most common treatment-related adverse events associated with use of ERT are infusion-related reactions and hypersensitivity reactions that can in some cases be life threatening (Therapeutic Goods Administration 2008). Patients may also develop antibodies, including neutralising antibodies, to idursulfase. Whether these antibodies affect treatment efficacy is uncertain.

The clinical studies on idursulfase included patients with attenuated MPS II and excluded patients with neuronopathic involvement (Muenzer et al. 2011; Muenzer et al. 2007; Muenzer et al. 2006). Idursulfase does not cross the blood-brain barrier and therefore does not prevent or treat the cognitive and behavioural manifestations associated with the severe form of MPS II (Scarpa et al. 2011). ERT has been associated with somatic improvements in the most severe patients but has not resulted in cognitive benefits (Muenzer et al. 2012). When the PBAC considered idursulfase in 2007, "it concluded that it would advise the Government to consider limiting treatment under the LSDP to patients without severe CNS involvement"[3], although the eligibility requirements for idursulfase through the LSDP do not explicitly prohibit its use in this population (Australian Government Department of Health and Aged Care 2022). Novel approaches (e.g., intraventricular or intrathecal, CNS penetrating ERT) of ERT delivery and newer therapies currently under development may improve treatment outcomes for the neuronopathic aspects of severe MPS II (Kemper 2022).

In its review of idursulfase treatment funding through the LSDP, the Expert Panel noted there were positive aspects to idursulfase treatment as experienced by patients, their families and treating physicians. Important outcomes for adult and paediatric patients with MPS II were stabilisation and reduction of respiratory symptoms, improved mobility and range of motion, improved tolerance for and reduced need for surgery, reduced liver size, improved quality of life (QoL), improved sleep, and increased life

[3] https://www.pbs.gov.au/pbs/industry/listing/elements/pbac-meetings/psd/2007-11/pbac-psd-idursulfase-rhu-nov07

expectancy (Australian Government Department of Health and Aged Care 2023a). These observations are in line with other evidence reviews of ERT treatment (da Silva et al. 2016; Kemper 2022; McBride, Berry & Braverman 2020; Ream et al. 2023; Scarpa et al. 2011; Żuber et al. 2023).

NBS for MPS II would allow treatment of newborns diagnosed with MPS II to commence prior to displaying clinical characteristics and manifestations of I2S deficiency. However, it is not possible to reliably distinguish between the severe and attenuated forms of MPS II based on biochemical and genetic testing. Therefore, it is not possible to predict whether the condition will progress to the severe form prior to a child becoming symptomatic given that newborns identified by NBS appear phenotypically unaffected at birth. At present, two thirds of individuals diagnosed with MPS II have the severe form (Ayodele et al. 2022). There are limited data on the benefits and risks of ERT treatment in babies aged <1 year as most data published for young children are derived from retrospective studies, mainly family and matched sibling case reports and case series (Kemper 2022). However, as MPS II is an ultra-rare disorder these studies provide some positive insight into the benefits of treatment for individual patients.

There are no Australian treatment guidelines for MPS II, therefore, it would be important to develop Australian guidelines for the monitoring and treatment of patients identified through NBS. Guidance has been developed for Europe and the USA (McBride, Berry & Braverman 2020; Scarpa et al. 2011). The current European guidelines were developed in 2011 by the Hunter Syndrome European Expert Council (HSEEC). There is currently no standardised severity scoring system for MPS II. The guidance recommends that patients are closely monitored and undergo a comprehensive physical, biochemical, and behavioural evaluation, ideally at a specialist LSD clinic, 3 monthly for the first 1-2 years and then maybe 6 monthly until 6 and then 1-2 yearly if deemed to have an attenuated form. Assessments should include evaluation of the musculoskeletal and cardiovascular systems, ears, airways, eyes, skin, nervous system, abdominal and gastrointestinal system (Scarpa et al. 2011). These may be difficult in very young children or those with cognitive dysfunction.

The European guideline considers that because there is a clear relationship between progressive GAG storage and clinical manifestations in MPS II, ERT should be initiated as early as possible after diagnosis. The consensus opinion of the authors was that due to the heterogeneous nature of MPS II and the variable rate of progression, it would be reasonable to offer ERT to all patients for at least 12-18 months, regardless of MPS II phenotype, after which a decision would be made as to whether to continue in consultation with the parents. The primary concern would be impact on the patient's quality of life. Evidence of central nervous system (CNS) disease progression would be taken into consideration when deciding to continue treatment (Scarpa et al. 2011).

There are no predefined discontinuation rules for ERT. The decision to stop treatment is based on a patient's individual circumstances and clinical judgement. A decision in the best interests of the patient is made following discussions with the patient and/or their family (Scarpa et al. 2011).

PASC noted that in practice, due to parental preference treatment is rarely discontinued.

Recent US guidance on treatment of MPS II from a Delphi derived practice resource from the American College of Medical Genetics and Genomics (ACMG) made consensus-based treatment recommendations as a previous systematic evidence-based review of treatment for MPS II was unable to create a definitive practice guideline based solely on published evidence (McBride, Berry & Braverman 2020). Regarding ERT treatment, the Delphi study recommended:

- All individuals with severe MPS II or predicted to have severe MPS II based on genotype warrant starting ERT, prior to showing signs or symptoms.
- Individuals with signs or symptoms with either attenuated or severe MPS II warrant ERT.
- Individuals with attenuated MPS II who are not showing signs or symptoms of disease do not warrant ERT. However, the clinical expert suspected that this may not reflect clinicians' views in Australia.

Eligibility for subsidised ERT (idursulfase; ELAPRASE®) treatment in Australia

ERT (idursulfase; ELAPRASE®) for MPS II has been funded in Australia since 2008. Funding is provided for eligible individuals diagnosed with MPS II through the Commonwealth LSDP. A patient must continually meet the LSDP funding conditions in order to remain eligible for ERT (Australian Government Department of Health and Aged Care 2022). The LSDP for idursulfase treatment was reviewed by the Expert Panel in 2020 (Australian Government Department of Health and Aged Care 2023a). The patient must present with at least one of the following complications of MPS II to be eligible for treatment with idursulfase:

- Sleep disordered breathing: Patients with an Apnoea/Hypopnoea Incidence of >5 events/hour of total sleep time or more than 2 severe episodes of desaturation (oxygen saturation <80%) in an overnight sleep study.
- Respiratory function tests: Patients with FVC less than 80% of predicted value for height.
- Cardiac: Myocardial dysfunction as indicated by a reduction in ejection fraction to less than 56% (normal range 56-78%) or a reduction in fraction shortening to <25% (normal range 25-46%).
- Joint contractures: Patients developing restricted range of movement of joints of greater than 10 degrees from normal in shoulders, neck, hips, knees, elbows or hands.

Applications may be submitted for ERT under the LSDP for infants and children aged less than 5 years not yet demonstrating symptoms consistent with the other LSDP eligibility criteria above, where there has been a diagnosis of MPS II (e.g., by genotyping) with clear prediction of progress of the disease, or if severe disease can be predicted based on a sibling's disease progression.

Hematopoietic Stem Cell Transplantation (HSCT)

ERT cannot cross the blood-brain barrier and therefore has a limited impact on CNS symptoms. HSCT has been used to treat patients with the severe form of MPS II characterised by CNS involvement because peripheral blood monocytes can cross the blood–brain barrier and may establish in the CNS as microglial cells (Taylor et al. 2019). Recipients of HSCT can use donor cells from bone marrow, peripheral blood, or umbilical cord blood. An allogeneic HSCT (i.e., using bone marrow from a donor who does not have MPS II) can increase I2S activity. Patients with MPS II who receive HSCT may need additional treatment including ERT.

The number of patients that have received HSCT for MPS II in Australia is very small based on expert advice. It is currently considered experimental and funding is not available via the Commonwealth. Use has been restricted to cases with the more severe form of MPS II due to increased risks associated with HSCT which includes death due to serious infections, graft versus host disease and other early and late effects.

Authors of a recent review of early versus late treatment with HSCT therapy concluded that HSCT has a disease modifying effect and that early HSCT can positively impact on neurological disease progression in

patients with severe MPS II. It may offer an effective treatment strategy for children diagnosed with the severe form of MPS II through NBS if clinical assessment, biochemical testing, and genetic testing have utility for predicting severe MPS II. However, further research is required to establish how long HSCT remains effective in children with MPS II (Sreekantam et al. 2022).

A retrospective study in Japan to evaluate the efficacy and benefit of HSCT in MPS II patients assessed activities of daily living (ADL), intelligence quotient (IQ), brain magnetic resonance image (MRI) lesions, cardiac valvular regurgitation, and urinary GAG over a follow-up period of 9.6±3.5 years. They concluded that the utility of HSCT should be re-evaluated for the treatment for MPS II and that HSCT may be beneficial when it is performed before signs of brain atrophy appear on MRI and before heart valvular regurgitation occurs (Tanaka et al. 2012). It is difficult to estimate the cost of HSCT because of many factors impacting on the cost (e.g., age at transplantation, the type of donor, preconditioning regimen used, potential complications, and other out-of-pocket expenses).

Ongoing clinical management

Individuals with a confirmed diagnosis of MPS II require ongoing clinical management and monitoring of symptoms by a multidisciplinary team (Muenzer et al. 2009). Common interventions required as the symptoms of MPS II become apparent during disease progression include developmental, occupational, and physical therapy; shunting for hydrocephalus; tonsillectomy and adenoidectomy; continuous positive pressure ventilation; carpal tunnel release; cardiac valve replacement; inguinal hernia repair; and hip replacement (Joseph, DiCesare & Miller 2018).

Comparator(s) (PICO Set 1)

The comparator for the proposed health technology is no screening for MPS II through the universal NBS program. Under the comparator, diagnosis of MPS II is as per current clinical practice: following clinical presentation with signs and symptoms consistent with MPS II, or a family history of MPS II. Those investigated due to symptoms are considered under PICO Set 1, and those investigated due to having a family history of MPS II are considered under PICO Set 2.

PASC confirmed that the comparator was no screening for MPS II through the universal NBS program.

Current diagnostic tests carried out on children or adults presenting with symptoms of MPS II in the absence of NBS are:

- The endogenous biomarker method for measuring small non-reducing end GAG fragments on LC-MS/MS in urine
- plasma or peripheral blood leukocyte I2S enzyme activity analysis and
- genetic analysis to identify the causative *IDS* gene variant(s)

The same tests are proposed for confirmatory diagnostic testing of babies found to be positive for MPS II through NBS.

Expert opinion from the Head of the National Referral Laboratory has suggested that the use of GAG fragment analysis would mean confirmatory diagnosis using leukocyte I2S enzyme activity level would not be required. However, the current eligibility criteria for idursulfase through the LSDP requires that the diagnosis of MPS II be confirmed through by the demonstration of a deficiency of I2S enzyme activity in white blood cells with the assay performed in a NATA-accredited laboratory; or, for siblings of a known

patient, detection of a disease-causing variants. Genetic testing for IDS variants is not required for diagnosis but is performed once diagnosis is confirmed to facilitate cascade testing of family members.

Without NBS, patients may experience a long "diagnostic odyssey" with an extended period between symptom onset and diagnosis because the early manifestations of MPS II at clinical presentation are common to other conditions. A range of other tests may therefore be used to rule out other conditions/disorders that have similar symptoms (e.g., childhood syndromes, other LSDs or metabolic disorders).

PASC noted that currently, diagnosis of MPS II is established following clinical presentation with signs and symptoms consistent with MPS II and subsequent biochemical testing using the non-reducing endogenous biomarker method of GAG fragment analysis on urine, or through cascade testing due to family history. A leukocyte I2S deficiency must also be confirmed via enzyme assay to meet ERT eligibility on the LSDP. Genetic testing for IDS variants is not required for diagnosis but is performed once diagnosis is confirmed to facilitate cascade testing of family members.

PASC noted that the diagnostic tests for MPS II (leukocyte I2S enzyme assay and non-reducing endogenous biomarker method of GAG fragment analysis on urine) are not funded by the MBS and are currently performed at the National Referral Laboratory in Adelaide.

Reference standard (PICO Set 1)

Diagnosis of MPS II in an individual based on clinical assessment of manifestations of MPS II, biochemical testing (GAG analysis, I2S deficiency) and/or genetic testing by DNA sequence analysis (i.e. identification of a pathogenic variant in the *IDS* gene).

PASC noted that the reference standard is clinical diagnosis.

Outcomes (PICO Set 1)

Outcomes for evaluation include:
Test performance:

- Accuracy of the screening test (sensitivity, specificity, positive predictive value, negative predictive value, false positives, false negatives)
- Diagnostic accuracy of confirmatory/diagnostic test (sensitivity, specificity, positive predictive value, negative predictive value)
- Diagnostic yield of screening and proportion of cases with a genotype predictive of severe phenotype

Change in management:

- Age at diagnosis
- Age at treatment initiation (and whether prior to, or after phenotype onset)
- Investigations/monitoring/treatments received.

Clinical Effectiveness of NBS for MPS II:

- Change in morbidity and mortality, quality of life, general functioning and disease manifestations from earlier diagnosis, intervention, and/or avoidance of the diagnostic odyssey (either from studies assessing the impact of comparative change in management, or direct from test to health outcomes evidence)

Safety of NBS for MPS II (physical harms to newborn from screening test, diagnostic test or subsequent early vs late treatment):

- Impact of false positive screening results (physical harms to the infant or psychological harms to the parents)
- Impact of false negative results
- Impact of diagnosing mild cases or private variants not previously associated with MPS II
- Safety of (experimental) haematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT)
- Any potential risk of harm from ongoing monitoring and surveillance

Economic and Financial Implications:

- Cost-effectiveness NBS for MPS II (cost per diagnosis; cost per quality adjusted life year (QALY))
- Financial impact of screening, relative to existing practice (including impact of false positives, impact of screening-based treatment compared to treatment following diagnosis at phenotype presentation, impact of ongoing monitoring and surveillance)

Other relevant considerations:

- Value of knowing (emotional benefits vs harms to family, social benefits vs harms to family)
- Ethical considerations (equity of access, considerations regarding consent)
- Organisational considerations (incremental impact of NBS on organisations, particularly the impact on services for monitoring late-onset disease, or on the NBS program itself including programmatic implementation considerations)

PASC noted that the one third of patients with non-neurological MPS II are most likely to derive benefit from NBS. For these patients, management decisions such as earlier initiation of ERT may result in superior treatment outcomes because ERT has an impact on some of the somatic manifestations of MPS II even though it has no impact on neurological symptoms of MPS II.

PASC noted comments from the clinical expert co-applicants and consultation feedback that the LSDP may need to be amended to allow treatment of pre-symptomatic patients, if this condition is added to NBS. PASC accepted that changes to eligibility criteria for ERT funding via the LSDP were out of scope for the assessment of this application, so the DCAR should assume current LSDP eligibility criteria remain in place. However, PASC also advised that in future, where NBS applications involve a corresponding change to a therapy, a codependent application is required.

PASC acknowledged clinical advice that treatment with ERT may improve cognition, prolong life and reduce disease burden. However, the clinical advice also included that all families want to treat their child despite the significant burden ERT places on families, and it can be difficult for a clinician to withdraw access to LSDP-funded ERT even when a patient with MPS II starts to show evidence of neurological decline for which ERT has no clinical benefit.

Patients with MPS II may benefit from early HSCT to reduce life-long dependency on ERT and it may positively impact on neurological disease progression in patients with severe MPS II. Evidence for use of HSCT in MPS II is currently limited.

PASC noted that the clinical co-applicants had commented that the lifetime morbidity of attenuated forms may be more common than currently accepted.

PASC also noted that family planning options should be considered as a secondary outcome for impacted family members.

PASC discussed that screening for MPS II and detection of attenuated cases has the disadvantage of, creating "patients in waiting", and there are extensive ethical issues related to screening for a condition that does not require early intervention.

PICO criteria (PICO Set 2)

Population (PICO Set 2)

Biological Parents

Based on the X-linked inheritance pattern of MPS II, it is proposed that cascade genetic testing of the biological parents will be offered only to the mother of male newborns diagnosed with pathogenic or likely pathogenic variants of MPS II. The clinical expert co-applicants' recommendation was that cascade testing of females for MPS II carrier status should be extended beyond immediate female family members to include maternal aunts and grandmother.

PASC considered that if the mother tests positive, then cascade testing of her parents and siblings is indicated (i.e. the newborn's maternal grandparents, aunts and uncles). PASC further considered testing of maternal relatives including the maternal grandfather and potentially her brothers may be indicated to evaluate an I2S *variant identified in the proband and mother, and this may already take place for this purpose to some extent. PASC therefore advised the population to be assessed for cascade testing should include maternal grandparents, aunts and uncles, and noted the MSAC Guidelines describe the presentation of economic scenario analyses to explore expanding the cascade testing population to also include second-degree relatives. It is expected that in 70 to 90% of cases, expanded cascade testing would be indicated because of identification of a maternally inherited* I2S *variant.*

As described in the "Population" section of PICO set 1, female carriers of MPS II usually have normal levels of I2S activity and rarely display symptoms of MPS II. Where they do have disease manifestations, these are usually due to abnormalities in the structure of the X-linked chromosome or the inactivation process of the X-chromosome (Fang, Deng & Disteche 2021; Guillén-Navarro et al. 2013; Kloska et al. 2011; Lonardo et al. 2014; Tuschl et al. 2005). It is therefore proposed that cascade genetic testing would be offered to the biological mother; however, the appropriateness of offering cascade testing (biochemical or genetic testing) to the father, who could have a late onset attenuated form of MPS II, should also be explored during the assessment.

Siblings

MPS II is an X-linked recessive disorder, primarily affecting males. It is familial (inherited) in most cases, although *de novo* mutations in the *IDS* gene have been identified. When MPS II is familial, the biological mother of affected male offspring with a pathogenic variant is a carrier, with a 50% chance that other male offspring would be affected.

Older male siblings, with the same mother as the index case, born prior to the implementation of NBS for MPS II may not have presented with symptoms and remain undetected as the median age at symptom onset of severe disease ranges from 1 to 3 years of age, or later in life for the attenuated form. It is proposed that these individuals receive biochemical testing (urine GAG analysis). If the test is positive for MPS II, the individual would be offered further diagnostic testing including cascade testing. As per the expert clinical advice, clinicians can choose to conduct urine GAG analysis or genetic cascade testing. It is anticipated that in most cases, urine GAG analysis will be preferred due to quick turnaround time and cost. In males, only a single variant is required in order to be affected, so genetic testing would not identify any carriers.

It is proposed that cascade genetic testing is not offered to female siblings. The Human Genetics Society of Australasia (HGSA) advised in its 2022 position statement that "unless there is a direct medical benefit in the immediate future, the default position should be to postpone carrier testing until the child or young person can be supported to make an informed decision". Whilst this is the HGSA position, the clinical expert suggested that with appropriate genetic counselling, cascade testing could be performed at any age. However, there is a need of guidance with appropriate ethical considerations on who will let the female sibling know the results of their carrier testing. In current clinical practice, clinicians make it clear to the parents that if carrier testing in a child is undertaken, it is their responsibility to inform the child when they are of an appropriate age. Other options include recalling the child for a follow-up appointment when they are 18, however this would likely be very difficult administratively and many would be lost of follow-up.

Follow-up would be required to ensure that female siblings are offered a consultation, genetic counseling and testing prior to reaching reproductive age as the primary purpose of cascade testing of females would be to inform future reproductive planning.

PASC acknowledged the potential benefits of cascade testing for biological mothers and siblings of male newborns diagnosed with pathogenic or likely pathogenic variants of MPS II.

PASC considered that in the rare situation where the mother had gonadal mosaicism she could pass on the variant without it being detectable in her genotyped sample, and so advised all male siblings should be offered cascade testing, even when maternal cascade testing appears to indicate a de novo variant.

Intervention (PICO Set 2)

The proposed intervention is cascade testing for the biological mother of newborns diagnosed with MPS II following NBS, older male sibling(s) with the same biological mother, and female siblings with the same biological mother (once old enough to provide informed consent and before reaching reproductive age). Cascade testing would require referral from a clinician following diagnosis of the index case[4].

The intervention for the biological mother of a newborn diagnosed with MPS II through NBS is genetic testing for the specific pathogenic/likely pathogenic *IDS* variant(s) identified in the newborn and genetic counselling for family planning. Cascade testing to determine the presence of specific pathogenic variants is usually conducted using targeted sequencing methods. Where required, the method utilised should allow for detection of large gene rearrangements or deletions due to the presence of the *IDS* pseudogene.

Male siblings would be offered biochemical testing (initially urine GAG analysis) if considered at risk of having MPS II. Genetic testing would only occur if the biochemical tests were positive for MPS II. Affected older male siblings would subsequently require referral for appropriate management and treatment (see PICO set 1). The clinical expert advised that clinicians should be given the option of urine GAG analysis or genetic testing and anticipated that in most cases the clinicians would choose to conduct urine GAG analysis due to its quicker turnaround time.

The overall number of individuals cascade tested may increase slightly if MPS II is currently being underdiagnosed. Additionally, cascade testing would potentially be carried out at an earlier stage if screening for MPS II is added to NBS programs. Health professionals that would provide cascade testing

[4] Note: "index case" is used in this document to mean the first person in a family detected as having the condition (through diagnostic testing after NBS, but not following symptoms or cascade testing)

are the same as *per* current practice, including genetic counsellors, clinical geneticists, and laboratory scientists/geneticists. Training and qualifications required to deliver cascade testing would also be the same as per current practice.

Cascade testing of mothers and female siblings provides the value of knowing and helps to inform future reproductive decision-making. It may also support earlier diagnosis and management of disease in MPS II affected older male siblings, who have not presented clinically or been screened via NBS for MPS II.

Where the index case has a *de novo* P/LP *IDS* variant (~10–30%), cascade testing would exclude family members as being affected by or carriers of MPS II.

PASC noted that cascade test methods for the intervention and the comparator are the same.

Comparator (PICO Set 2)

The comparator is cascade testing (mother, female siblings, and older male siblings) with genetic counselling after a symptomatic (phenotypic presentation) male child or adult is diagnosed with MPS II. The comparator is the same as the proposed intervention except the time of cascade testing would be later than if NBS for MPS II was available.

If a male with symptoms of MPS II (phenotypic presentation) is diagnosed with MPS II, then the biological mother and male sibling(s) of the proband[5] are offered cascade testing. Male siblings are offered biochemical testing (initially urine GAG analysis). Genetic testing of male siblings would only occur if the biochemical tests were positive for MPS II.

For the biological mother, cascade testing would be genetic analysis for the P/LP *IDS* variant identified in the index case. Female siblings are potentially carriers of MPS II and would be offered cascade testing when they reach an age considered able to provide informed consent, and before reaching reproductive age. Females have very rarely been diagnosed with MPS II.

Where the proband has a *de novo* P/LP *IDS* variant (~10–30%), cascade testing of the family members would exclude them as being affected by or carriers of MPS II.

PASC noted that the comparator was the same as the intervention, except the time of cascade testing would be later than if NBS for MPS II was available.

Reference standard (PICO Set 2)

The reference standard is clinical diagnosis based on clinical assessment of manifestations of MPS II, biochemical testing (GAG analysis, I2S deficiency) and/or genetic testing by DNA sequence analysis (i.e. identification of a pathogenic variant in the *IDS* gene).

Outcomes (PICO Set 2)

For biological mothers tested, the expected test results will be that they either are or are not a carrier of MPS II. The major benefit of cascade testing for carriers is to inform reproductive decision-making.

[5] Note: "proband" is used in this document to mean an affected individual (i.e. a person who has signs and/or symptoms consistent with the disease phenotype) who has received a confirmatory (genetic, and/or other accepted diagnostic test) diagnosis.

Male siblings who were not tested for MPS II through newborn screening (due to being born outside of Australia or born prior to the introduction of MPS II to the NBS program) may be identified as being clinically affected with MPS II due to cascade testing. Affected male siblings may benefit from and/or be exposed to the harms of monitoring and treatment at an earlier time than if they had been diagnosed after presenting with signs and symptoms of MPS II.

Cascade testing will not be informative regarding whether the cascade tested male sibling has the severe or attenuated form of MPS II unless clinical assessment indicates that symptoms consistent with MPS II are present in a male in the same maternal line, or if a genotype/phenotype association has been determined previously for the pathogenic variant in the *IDS* gene identified in the index case (e.g., a large deletion or rearrangement).

The following outcomes are relevant to cascade testing:

Test outcomes:

- Number of family members who uptake cascade testing
- Age at diagnosis/treatment of affected siblings

Clinical effectiveness:

- Effectiveness of early vs late monitoring and treatment for male siblings diagnosed with MPS II following cascade testing
- Psychological impact of diagnosis of MPS II (affected sibling or female carrier)

Safety:

- Physical or psychological harms arising from earlier diagnosis, monitoring and treatment for male siblings diagnosed with MPS II following cascade testing

Economic and Financial Implications:

- Cost-effectiveness
- Financial impact of early vs late cascade testing

Other relevant considerations:

- Value of knowing (family planning, emotional benefits/harms to family, social benefits/harms to family)
- Ethical considerations (equity of access, considerations regarding consent, considerations regarding cascade testing, especially relating to identification of late-onset MPS II in asymptomatic people)
- Organisational considerations

PASC noted the outcomes for PICO Set 2.

Assessment framework

The NBS programs are a form of universal (or population) screening. As universal screening programs are considered to be associated with a high financial risk, MSAC has a clear preference for 'direct from test to health outcomes' evidence (MSAC 2021). However, the NBS NPF also provides a set of guiding criteria to be addressed in the assessment of adding or removing conditions from the NBS programs that include elements of linked evidence. Because there is limited 'direct from test to health outcomes' evidence for MPS II, a linked evidence approach will also be used during the assessment (Figure 1).

Figure 1 Assessment framework showing the links from the test population to health outcomes

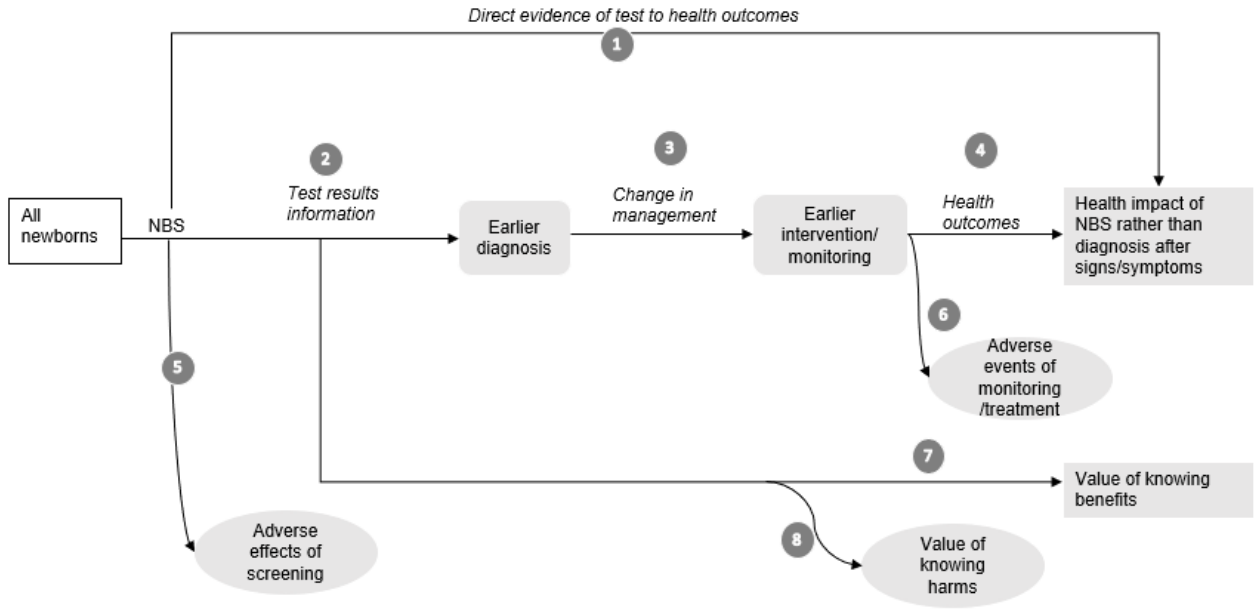


Figure notes: 1: direct from test to health outcomes evidence; 2: test performance; 3: change in diagnosis/treatment/management; 4: influence of the change in management on health outcomes; 5: adverse events due to screening; 6: adverse events due to treatment/monitoring 7: benefits of knowing; 8: harms of knowing.

The assessment questions related to the HTA assessment framework are:

1. Is there direct from screening to health outcomes evidence to support the claim that NBS leads to improved health outcomes?
2. Test accuracy: When compared with diagnostic testing of symptomatic children as the reference standard, what is the accuracy of NBS screening for identifying patients with MPS II? What are the implications of discordances among the test results?
   What proportion of patients are diagnosed with MPS II prior to symptom development due to NBS or no NBS?
3. How does the NBS test result impact the clinical management of the individual (in either the timing or the type of monitoring/treatments used), compared with diagnosis after symptom onset (or due to family history)?
4. Does the change in the clinical management (monitoring and early treatment with ERT/HSCT) improve health outcomes (morbidity, mortality, QoL)?
5. What are the adverse events associated with NBS for detection of MPS II, when compared to the current practice of no screening and diagnosis after symptom onset? *(no incremental safety issues expected for the baby as bloodspots already collected, but any additional false positive results may result in psychological harms to the parents)*
6. What are the adverse events associated with the monitoring and treatment of individuals diagnosed with MPS II through NBS?
7. What value of knowing is there for patients with an MPS II diagnosis, diagnosed early due to NBS? *(this may be relevant for attenuated MPS II not otherwise diagnosed prior to symptom onset)*
8. What harms come from the knowledge of MPS II status? *(this may be relevant for attenuated MPS II prior to symptom onset)*

Other relevant considerations

Proposals considered by MSAC can have aspects that are unique to the proposed technology, circumstances of use or funding arrangement, such that MSAC is unlikely to have considered the factors previously in the same context. Other relevant considerations should be explored in section 5 of the assessment report.

Additional information relevant to decision-making that is not captured elsewhere in the assessment is anticipated to include:

- Are there any additional implementation issues, such as:
    - The costs of purchasing equipment and training additional personnel, that are required for screening for this condition.

PASC noted that implementation of MPS II NBS will require additional funding for specialised equipment, test consumables and staff. PASC noted that direct funding is being provided by the Commonwealth to states and territories to support expansion of Australia's NBS programs and support consistency in screening across Australia. States and territories can determine how to allocate this funding within their jurisdiction to best support implementation in line with the terms of the Federation Funding Agreement (FFA) Schedule.

    - Additional resources and personnel for managing and counselling babies identified pre-symptomatically via the NBS and who may not develop symptom until adulthood.

PASC acknowledged that families of newborns identified as having MPS II via NBS would require access to specialist medical information, clinical support, and counselling.

PASC noted that access to clinical trials may provide alternative or new therapeutics as they have the potential to provide superior treatments compared to the current enzyme therapy, however clinical trial access and future therapies are out of scope for the HTA.

PASC considered that as there would be no LSDP treatment for some patients detected as having MPS II through NBS, NBS would create "patients in waiting", with extensive ethical implications.

PASC noted the assessment group reported a lack of registry data, and raised whether better registry support could be provided after a condition is added to NBS, to better inform treatment and other analyses in the future. PASC noted the clinical expert co-applicants agreed better resourcing for publicly held registries would be valuable, as the registry environment is supported by industry at present.

Clinical management algorithms

Current (Figure 2) and proposed (Figure 3) clinical management algorithms have been developed for MPS II. Children presenting with symptoms consistent with MPS II undergo the same diagnostic testing and treatment pathways as children identified via NBS (as described below). The key difference is the time of confirmed diagnosis.

PASC noted that not all patients found through NBS to have MPS II will be eligible to access ERT on the LSDP and considered that the clinical management algorithms should include two-tier NBS and best supportive care for individuals with MPS II who are not suitable for or decline HSCT or ERT.

Current and proposed cascade testing algorithms are presented in Figure 4 and Figure 5.

Current clinical management algorithm for MPS II (without universal NBS)

Management following either presentation with symptoms consistent with MPS II would be referral to a metabolic disorders' clinic for clinical assessment and diagnostic testing including endogenous GAG fragment analysis in urine, and genetic analysis to identify the causative *IDS* gene variant. Testing for I2S enzyme activity is required to confirm I2S deficiency to meet the eligibility criteria for the LSDP for idursulfase treatment. Genetic testing is not essential for a confirmed diagnosis of MPS II but is required to facilitate cascade testing. Due to genetic heterogeneity and the need for expert interpretation due to the presence of the *IDSP1* pseudogene, genetic testing is used for diagnosis of MPS II in the context of results from clinical and metabolic assessment. Genetic testing can identify an *IDS* variant of uncertain significance (VUS) which may be less informative for diagnosis.

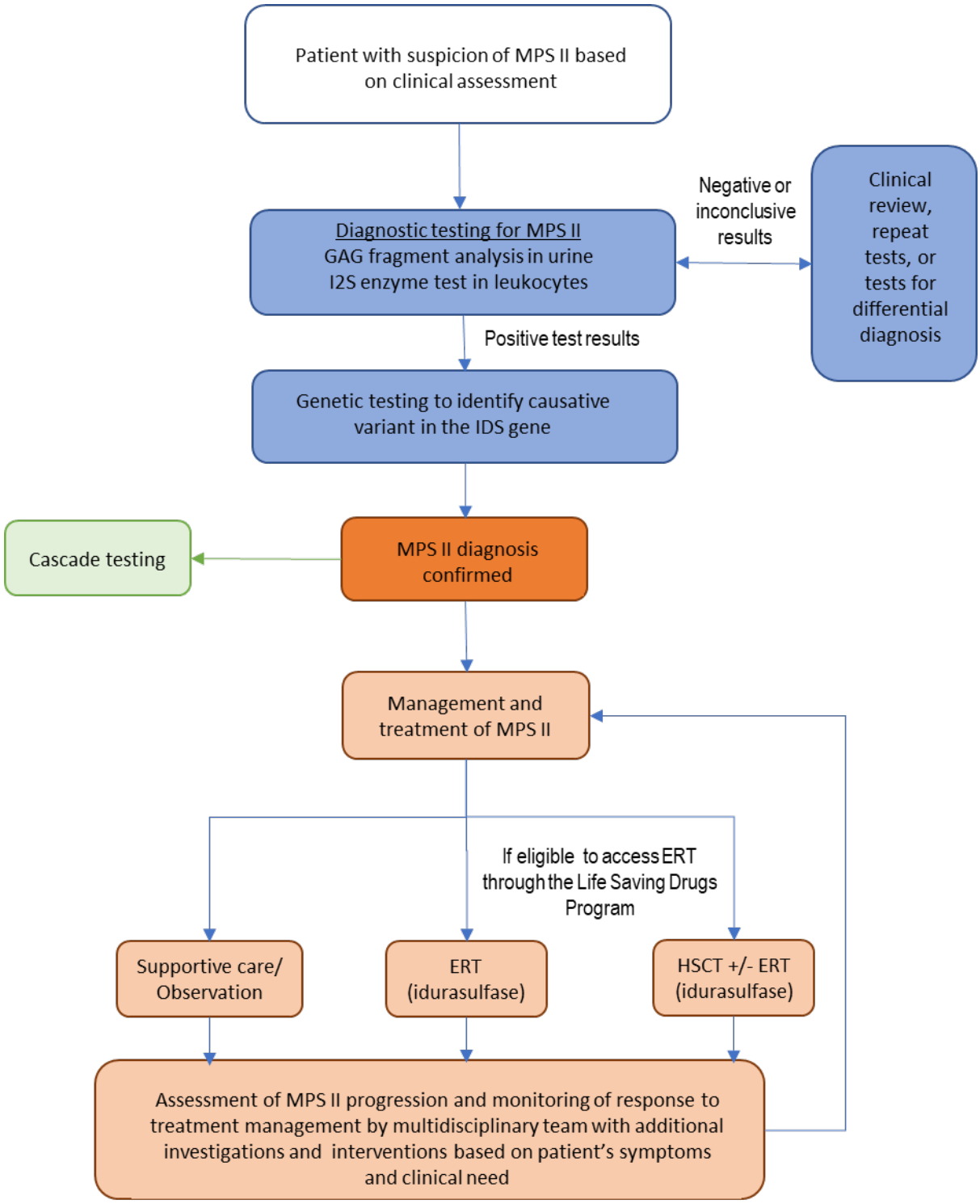
Where a patient's diagnosis is inconclusive after diagnostic testing, the patient may require further clinical assessments, investigations, or tests to exclude other potential diagnoses (e.g., for other metabolic disorders). After GAG fragment testing, this may no longer be a significant issue, however, the main challenge is the prediction of disease severity.

In cases where a MPS II diagnosis is confirmed, the patient and their family would discuss their MPS II diagnosis, treatment options and the option of cascade testing for family members with a metabolic disease specialist and/or clinical geneticist.

A multi-disciplinary team provides specialized care because of the complexity of the disease. Treatments currently available for MPS II are not curative. Eligible patients with MPS II can be treated with ERT (idursulfase; ELAPRASE®) in Australia via the LSDP. A very limited number of patients in Australia have received HSCT with or without additional ERT. It is currently considered as experimental and funding is not available via the Commonwealth.

Additional treatments for recognized manifestations of MPS II are frequently required throughout the course of the disease. Common interventions required as the symptoms of MPS II become apparent during disease progression include developmental, occupational, and physical therapy; shunting for hydrocephalus; tonsillectomy and adenoidectomy; continuous positive pressure ventilation (or tracheostomy); carpal tunnel release; cardiac valve replacement; spinal fusion, inguinal hernia repair; and hip replacement (Joseph, DiCesare & Miller 2018).

Figure 2 Current management of MPS II in the absence of MPS II screening as part of the universal NBS program



ART=assisted reproductive technology; ERT=enzyme replacement therapy; GAG=glycosaminoglycans; HSCT=haematopoietic stem cell transplant; I2S=iduronate-2-sulfatase; *IDS*=iduronate-2-sulfatase gene; LSDP= Life Saving Drugs Program; MPS=mucopolysaccharidosis.

Proposed clinical management algorithm for MPS II with universal NBS

All newborns in Australia that participate in NBS would be screened for MPS II. Screening for MPS II will utilise the NBS DBS samples. As discussed in the 'Intervention' section, the most likely screening protocol would be a two-tier protocol (I2S enzyme activity level followed by the endogenous biomarker method for

measuring small non-reducing end GAG fragment analysis on LC-MS/MS), although a single-tier protocol using the GAG fragment analysis on LC-MS/MS may also be possible.

Newborns receiving a positive or indeterminate screening result for MPS II will be referred to a metabolic clinic for clinical assessment and confirmatory diagnostic testing using the same diagnostic tests currently utilised for diagnosis of patients presenting with clinical signs and symptoms consistent with MPS II (GAG fragments or levels in urine and genetic analysis), as discussed above. The I2S enzyme activity testing may be required to meet criteria for treatment under the LSDP.

With NBS, more individuals will receive confirmatory diagnostic testing than with the comparator. With the comparator health technology only children presenting with signs and symptoms of MPS II would be tested, although some of these may not have MPS II as the symptoms of MPS II are heterogenous and are common to other childhood syndromes and common conditions.

In cases where a MPS II diagnosis is confirmed, the patient and their family will discuss their MPS II diagnosis, treatment options and cascade testing for family members (biological mother, female siblings, male siblings) with a metabolic disease specialist and clinical geneticist. Cascade testing for family members will remain the same but occur at an earlier time point than if NBS for MPS II was not available (comparator).

The difference between the current and proposed management algorithms is that individuals with MPS II are identified via NBS and diagnosed shortly after birth when they appear asymptomatic rather than many years later when they have manifestations of MPS II due to accumulation of excess GAGs in the lysosomes and associated tissue/organ damage.

Earlier diagnosis may permit earlier treatment. Those who have a confirmed diagnosis of MPS II may be eligible for treatment with either ERT (and/or HSCT as an experimental therapy in Australia, if appropriate, after consultation with the patient's family). Current treatment options for MPS II remain the same and are not curative. Current treatment options reduce MPS II symptoms, delay disease progression and extend life expectancy. Patients receiving treatment would be regularly monitored to assess adverse events and treatment effectiveness.

If treatment is declined or not possible, expert advice has indicated that regular clinical surveillance of the baby would likely be carried out during childhood (3 monthly for the first 1-2 years and then maybe 6 monthly until 6 and then 1-2 yearly if deemed to have an attenuated form) to monitor for symptoms consistent with development of the early onset severe form of MPS II. Many patients with the severe form of MPS II will develop potentially life-threatening manifestations by the second decade of life. If symptoms do not become apparent during childhood, the individual may have the later onset slowly progressing attenuated form of MPS II. Further clinical assessment may only occur in these cases at the point that a patient presents with symptoms of MPS II.

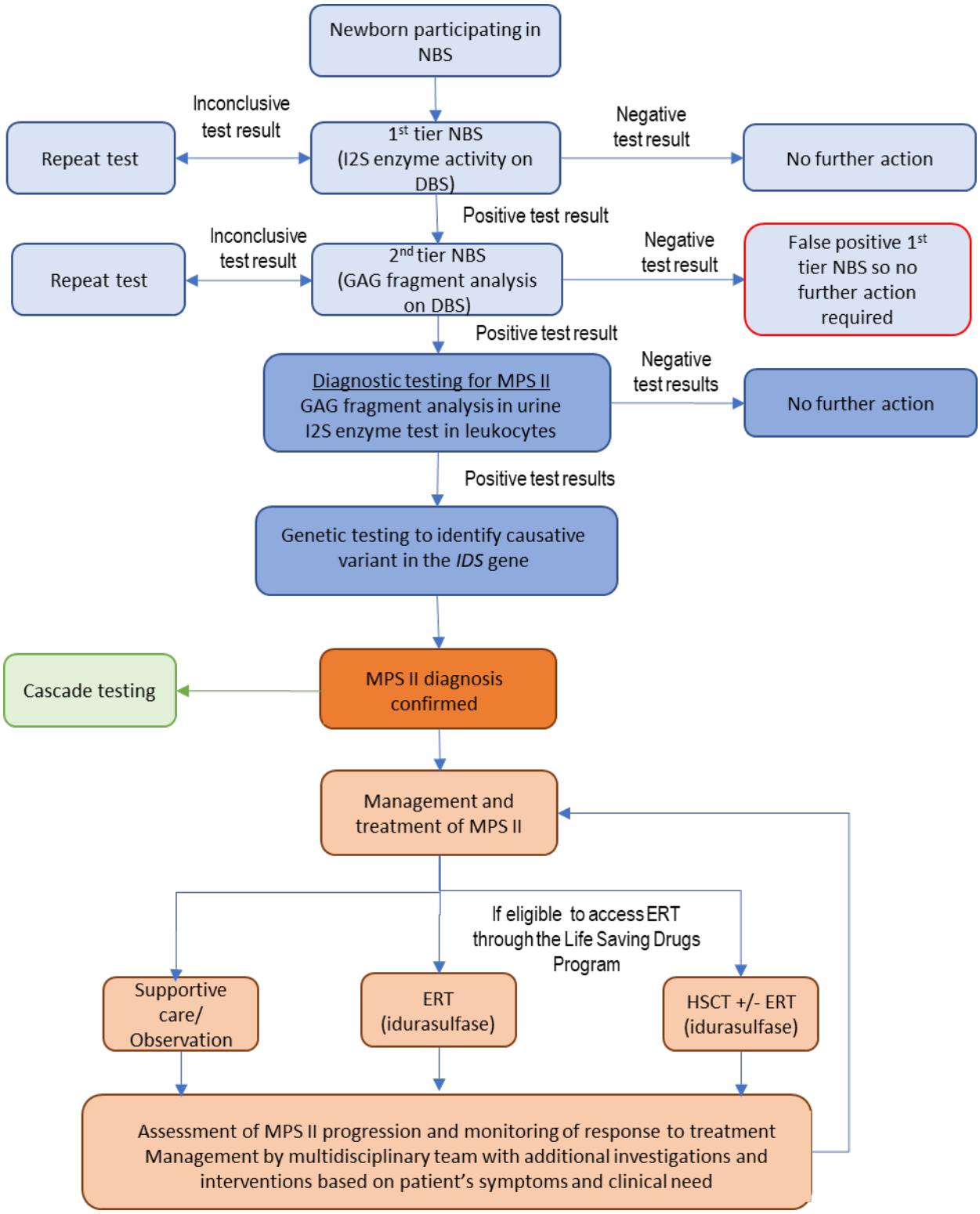
As MPS II progresses, further treatment and interventions for somatic or neuronopathic manifestations may be required and are managed by a multidisciplinary team.

The impact of earlier diagnosis on utilisation of the current treatment options is unknown. It is currently not possible to determine at the point of NBS and confirmatory diagnostic testing whether a newborn with MPS II will develop the severe or attenuated form of MPS II. Only a few genetic variants have confirmed phenotype/genotype associations due to the large number of novel or private variants associated with MPS II. Newborns with the attenuated form of MPS II could potentially be treated for many decades as

these patients often have normal life expectancy; whereas currently these patients would not receive treatment until they became symptomatic.

Treatment-related healthcare resources used will be affected by time of treatment initiation, type of treatment, benefits and risks associated with earlier treatment including reduction in somatic manifestations in patients treated at an early stage prior to these manifestations becoming apparent. Because there are limited data about the benefits and risks of either idursulfase or HSCT in very young children or very long-term treatment with idursulfase, it is not possible to predict the ongoing impact on healthcare resources. As evidence suggests that earlier treatment of MPS II is superior in terms of health outcomes, quality of life and life expectancy compared to treatment initiated after a patient develops somatic manifestations of MPS II, it might be reasonable to predict that patients may use fewer health resources across the course of the disease. However, as idursulfase would be initiated at an earlier stage, treatment costs and associated monitoring of treatment response and adverse events are likely to be higher due to treatment for a longer period.

Figure 3 Proposed management of MPS after addition of MPS II screening to the universal NBS program



ART=assisted reproductive technology; ERT=enzyme replacement therapy; GAG=glycosaminoglycans; HSCT= haematopoietic stem cell transplant; I2S=iduronate-2-sulfatase; *IDS*=iduronate-2-sulfatase gene; LSDP=Life Saving Drugs Program; MPS=mucopolysaccharidosis; NBS=Newborn bloodspot screening.

Current cascade testing algorithm for family members of a proband

The individual with an MPS II phenotype requires a confirmed diagnosis for MPS II that includes identification of a pathogenic variant in the *IDS* gene to enable cascade testing of family members. Some patients will have *IDS* variants immediately identifiable as pathogenic or likely pathogenic, but others will have a VUS. This would be mainly due to most *IDS* variants being private. Thus, clinical expert advice was that a VUS identified in a patient who had been biochemically diagnosed with MPS II may be able to be reclassified as being pathogenic or likely pathogenic.

Cascade testing is currently carried out for the biological mother of the proband, male sibling(s) with the same biological mother, and female siblings with the same biological mother (once old enough to provide informed consent and before reaching reproductive age). Cascade testing would require referral from a clinician following a confirmed genetic diagnosis. Cascade testing of maternal relatives including the maternal grandfather may be indicated to evaluate a variant.

The intervention for the biological mother of a newborn diagnosed with MPS II through NBS is genetic testing for the specific pathogenic *IDS* variant identified in the newborn and genetic counselling for family planning. Cascade testing to determine the presence of specific pathogenic variants is usually conducted using targeted sequencing methods. Where required, the method utilised should allow for detection of variants that are large gene rearrangements or deletions caused by the presence of the *IDS* pseudogene.

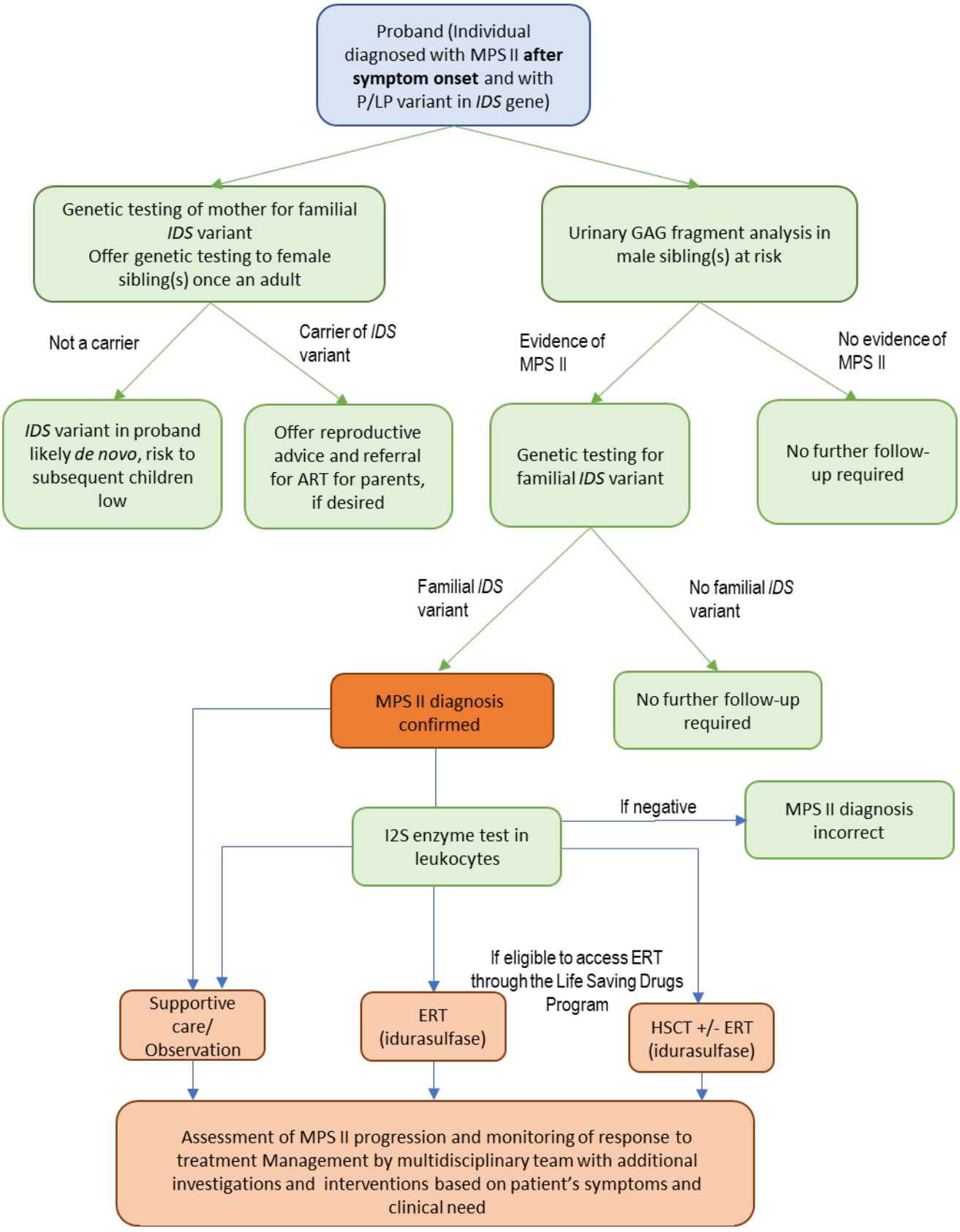
Male siblings are offered biochemical testing (initially urine GAG analysis) to assess their risk of having MPS II. Genetic testing would only occur if the biochemical tests were positive for MPS II. Affected older male siblings would subsequently require referral for appropriate management and treatment. The clinical expert advised that clinicians should be given the option of urine GAG analysis or genetic testing. In most cases, the clinicians will choose to conduct urine GAG analysis due to quick turnaround time.

Cascade testing requires input from genetic counsellors, clinical geneticists, and laboratory scientists/geneticist with appropriate training and qualifications.

Cascade testing of mothers and female siblings provides the value of knowing and helps to inform future reproductive decision-making. It may also support earlier diagnosis and management of disease in MPS II affected older male siblings, who have not presented clinically.

Where the proband has a *de novo* P/LP *IDS* variant (~10–30%), cascade testing can exclude family members as being affected by or carriers of MPS II.

Figure 4 Current cascade testing algorithm for family members of proband with MPS II



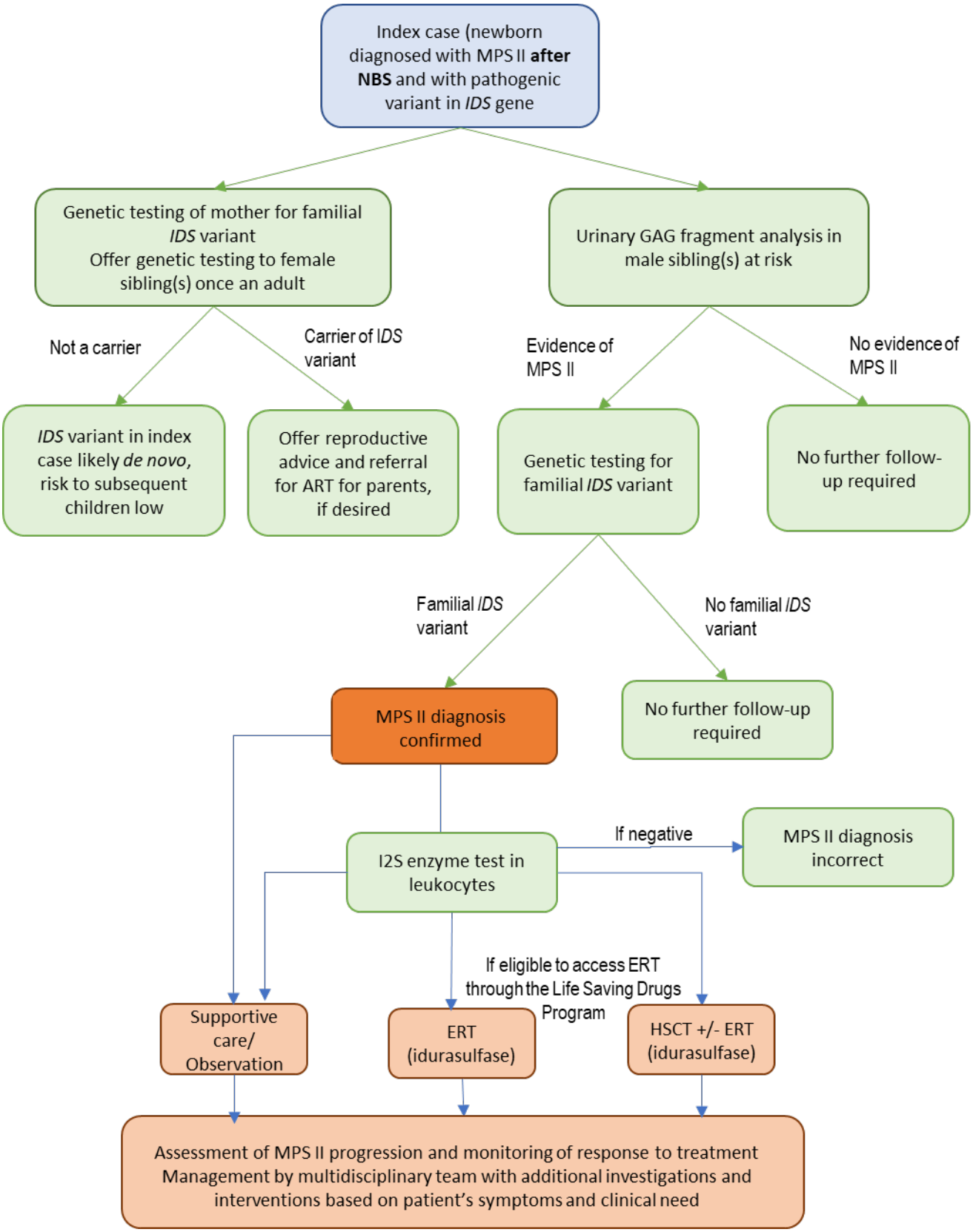
ART=assisted reproductive technology; ERT=enzyme replacement therapy; GAG=glycosaminoglycans; HSCT= haematopoietic stem cell transplant; I2S=iduronate-2-sulfatase; *IDS*=iduronate-2-sulfatase gene; MPS=mucopolysaccharidosis; P/LP = pathogenic or likely pathogenic.

Proposed cascade testing algorithm for family members of an index case

The proposed cascade testing algorithm is the same as the current cascade testing algorithm except that the index case would have been identified by NBS for MPS II. Additionally, cascade testing and any subsequent treatment or referrals for assisted reproductive therapy would occur at an early stage (i.e. shortly after MPS II NBS and confirmatory diagnostic testing of the newborn index case).

Older male siblings, with the same mother as the index case, born prior to the implementation of NBS for MPS II may not have presented with symptoms and potentially would have remain undetected until presentation with symptoms consistent with an MPS II phenotype. These siblings may therefore receive an earlier diagnosis of MPS II than if the index case was not identified *via* NBS.

Figure 5 Proposed cascade testing algorithm for family members of an index case with MPS II identified via MPS II newborn bloodspot screening



ART=assisted reproductive technology; ERT=enzyme replacement therapy; GAG=glycosaminoglycans; HSCT= haematopoietic stem cell transplant; I2S=iduronate-2-sulfatase; *IDS*=iduronate-2-sulfatase gene; MPS=mucopolysaccharidosis; NBS=Newborn bloodspot screening; P/LP = pathogenic or likely pathogenic.

Proposed economic evaluation

The expectation is that the evidence on NBS for MPS II will demonstrate superior effectiveness and non-inferior safety, compared to no universal newborn screening for MPS II. The appropriate form of health economic evaluation is therefore a cost-utility analysis or cost-effectiveness analysis (Table 7).

The evidence for cascade testing of family members for MPS II may either demonstrate superior effectiveness and non-inferior safety, or non-inferior effectiveness and safety compared to cascade testing of family members for MPS II after a family member has been diagnosed with MPS II in the absence of newborn screening for MPS II. The appropriate form of health economic evaluation is therefore a cost-minimisation analysis, a cost-utility analysis or cost-effectiveness analysis.

PASC noted that the costs vary depending upon the screening protocol chosen. In the chosen protocol, the economic evaluation is based on first tier I2S enzyme activity screening, followed by a second tier GAG fragment analysis.

PASC also requested a cost analysis of single-tier screening with GAG fragment analysis.

Table 7 Classification of comparative effectiveness and safety of the proposed intervention, compared with its main comparator, and guide to the suitable type of economic evaluation

Comparative safety	Comparative effectiveness			
	Inferior	Uncertain[a]	Noninferior[b]	Superior
Inferior	Health forgone: need other supportive factors	Health forgone possible: need other supportive factors	Health forgone: need other supportive factors	? Likely CUA
Uncertain[a]	Health forgone possible: need other supportive factors	?	?	? Likely CEA/CUA
Noninferior[b]	Health forgone: need other supportive factors	?	CMA	CEA/CUA
Superior	? Likely CUA	? Likely CEA/CUA	CEA/CUA	CEA/CUA

CEA=cost-effectiveness analysis; CMA=cost-minimisation analysis; CUA=cost-utility analysis
? = reflect uncertainties and any identified health trade-offs in the economic evaluation, as a minimum in a cost-consequences analysis
a 'Uncertainty' covers concepts such as inadequate minimisation of important sources of bias, lack of statistical significance in an underpowered trial, detecting clinically unimportant therapeutic differences, inconsistent results across trials, and trade-offs within the comparative effectiveness and/or the comparative safety considerations
b An adequate assessment of 'noninferiority' is the preferred basis for demonstrating equivalence

Although there are three separate PICO confirmations for the three LSDs being considered for inclusion in the NBS programs for independent consideration of each condition (GSD II, MPS I and MPS II), there are potential efficiencies to be considered if all three are added to NBS programs at the same time.

Some kits can detect multiple lysosomal storage disorders, including other forms of MPS in addition to MPS II.

PASC noted the potential for multiplexing NBS for multiple conditions. PASC considered that the assessment should allocate the whole cost of the first-tier screening to the assessment of the first condition, as per standard practice to assess the incremental cost (with sensitivity analyses adjusting the proportion of first-tier costs allocated to an individual condition if MSAC supports multiple conditions that can be multiplexed

being introduced to NBS programs). Other costs will remain specific to each condition assessment, e.g. monitoring and treatment costs.

Proposal for public funding

Funding of NBS for MPS II (PICO Set 1)

Australian NBS programs are funded and delivered through public hospital services in all Australian jurisdictions. Patients and families can choose to utilise services through the private system at their own cost for postpartum care and any necessary ongoing intervention for rare diseases. However, all NBS samples are tested by the newborn screening laboratories which are managed and funded within the public system.

Each jurisdiction has unique arrangements for the funding and delivery of NBS services to align with specific local health system structures. Funding for the Australian NBS programs comes from a mix of jurisdictional and national funds. The Australian government contributes funds for public hospital services, including typical sample collection, testing and downstream care in the NBS programs, under the 2020-25 National Health Reform Agreement (NHRA). The NHRA recognises the states and territories as system managers of public hospitals. In addition to these standard funding mechanisms, the Australian government is directly contributing $25.3 million to states and territories to support the expansion of the NBS programs through a schedule to the Health Federation Funding Agreement. This funding can be used by jurisdictions at their discretion.

There are no Medicare Benefits Schedule (MBS) items specifically for the delivery of NBS services. MBS items may be used in the delivery of downstream medical care, although biochemical testing for MPS is not funded by the MBS.

Funding for the ongoing delivery of ERT for MPS II is provided by the Australian Government via the LSDP. The LSDP covers medicines for ultra-rare conditions (1 case per 50,000 or fewer) that could not be listed on the Pharmaceutical Benefits Scheme (PBS) on grounds of cost effectiveness but have been determined as being clinically effective by the PBAC, where the sponsor has applied for LSDP listing and the medicine has been assessed as meeting LSDP eligibility criteria. Medical services included in monitoring and treatment of newborns detected through NBS may also use other Commonwealth funding sources such as the MBS and PBS, and State/Territory funding.

Funding of cascade testing (PICO Set 2)

New MBS items for cascade testing in relation to this application are not proposed. The Department's advice was that cascade testing will continue to be funded by existing arrangements. Cascade testing is available on the MBS for other conditions, such monogenic conditions (73361), familial hypercholesterolaemia (73353) and mitochondrial disease (73462).

The MBS fee for genetic testing a close biological relative of a child with a known pathogenic or likely pathogenic disease variant for all three of these conditions is $400.00 (Benefit: 75% = $300.00 85% = $340.00) and indeed this is the fee for almost all cascade testing MBS items, except for 73423 for neuromuscular disorders which has a fee of $500.00 (Benefit: 75% = $375.00 85% = $425.00). For NBS of sickle cell disease, PASC previously advised that the cost for a laboratory to conduct genetic testing of the *HBB* gene or the *HBB* and *HBD* genes (for confirmatory testing for the newborn and for cascade testing of

family members) was approximately $500 (1737 PICO, pg 32; 1737 and 1737.1 PSDs). Thus, the cost of cascade testing for close relatives (biological mother and male siblings) of a newborn diagnosed with MPS II would likely be the same. *PASC advised it would be appropriate for the assessment to use a cost of $500 for cascade testing.*

The total cost for this service would be small as the estimated incidence of MPS II was 0.57 per 100,000 male live births in a recent Australian publication (Chin & Fuller 2022). It should be noted that male siblings would receive biochemical testing for GAGs in urine prior to genetic testing. For these individuals there will be an additional cost associated with the urine GAG test.

PASC noted that the Minister for Health and Aged Care has announced additional funding for NBS programs, to incorporate screening for additional conditions.

PASC noted access to ERT for MPS II occurs via the LSDP, but that current LSDP criteria would not allow access to treatment for all individuals diagnosed with MPS II through NBS. PASC therefore expressed concern that the application had not proposed corresponding modifications to the LSDP treatment. PASC considered that where changes to NBS warrant corresponding changes to a therapy (such as to LSDP criteria), this would benefit from coordinated consideration. PASC advised that in future, where NBS applications involve a corresponding change to a therapy, a codependent application is required.

Summary of public consultation input

PASC noted and welcomed consultation input from 9 organisations and 2 individuals, 1 consumer and 1 health professional. The 9 organisations that submitted input were:

- Western Australian Newborn Bloodspot Screening Program (WA NBS)
- Australasian Society of Inborn Errors of Metabolism (ASIEM) [special interest group of Human Genetics Society of Australasia (HGSA)]
- Rare Voices Australia (RVA)
- Genetic Alliance Australia (GAA)
- Royal College of Pathologists of Australasia (RCPA)
- Australian Genomics
- Childhood Dementia Initiative (CDI)
- Statewide Biochemical Genetics Service within SA Pathology (SA Pathology)
- Sanofi-Aventis Australia

The consultation feedback received was both supportive of public funding for Newborn bloodspot screening for MPS II but also raised some concerns, mostly in relation to the test method, concerns around overdiagnosis and 'medicalisation' of newborns who may never develop MPS II.

PASC noted that Australia would need to develop standard monitoring and treatment pathways for MPS II.

Clinical need and public health significance

The main benefits of public funding received in the consultation feedback included earlier diagnosis of MPS II, avoiding a diagnostic odyssey (and associated stress on families and patients), and potentially improved outcomes from early treatment with ERT and HSCT.

The main disadvantages of public funding received in the consultation feedback included limitations in the proposed test methods (high false positives), screening may identify newborns who may have only have mild symptoms or never have symptoms with consequent ethical implications, and testing may not identify

patients who will develop neuronopathic or non-neuronopathic forms of MPS II. Several respondents stated that the *IDS* gene shows a large number of private variants (variant unique to patient and potentially their family). This will affect the prevalence of variants of unknown significance affecting equity of service delivery in non-Caucasian populations (including Aboriginal and Torres Strait Islander populations). Ethical issues were raised about screening newborns for a condition that may not present until adulthood.

Other services identified in the consultation feedback as being needed to be delivered before or after the intervention included resourcing of NBS labs (for staffing, equipment, facilities), confirmatory diagnostic testing, genetic counselling, specialised multidisciplinary care from metabolic service, cascade testing, prenatal testing, and support for families (education material, navigating the health system). Consultation feedback stated that people with MPS II need care from a range of professionals including GPs, paediatric specialists, genetic counsellors, pathology, clinical geneticists, neurology, cardiology, respiratory, surgery and anaesthetists, social workers and psychologists. The consultation input also noted limitations in access to treatment, stating that there are no metabolic services in NT, ACT and Tasmania, no HSCT provision in these regions as well as in SA, and that First Nations Australians in isolated geographical regions will need to have access to services.

Indication(s) for the proposed medical service and clinical claim

The consultation feedback ranged from agreeing to strongly agreeing with the proposed population.

The consultation feedback agreed with the proposed comparator.

The consultation feedback was mixed regarding the clinical claim. The main points of disagreement were that the proposed two-tier methodology was unnecessary and that measurement of specific oligosaccharides that are the substrate for the enzyme deficiency in newborn blood spot samples provide a 100% accurate result with zero false and is the best technology to be employed.

The consultation feedback was mixed regarding the proposed service fee. Some feedback suggested that using the correct technology will not be as expensive as stipulated. However, Australian Genomics provided several reasons why genetic testing would be more costly than expected.

Cost information for the proposed medical service

The consultation feedback was mixed regarding the proposed service descriptor. The health professional who strongly disagreed stated that the proposed technology for screening is expensive, unnecessary and will lead to false positives.

The consultation feedback was mixed regarding the proposed service fee. The main points of disagreement were that there are less costly testing options, the service fee does not capture the full costs of establishing screening or downstream costs (with and without screening) and that genetic testing may be more complex and costly due to the large number of pathogenic (and private) variants.

Additional comments

Additional consultation feedback stated that that all MPS could be included in the proposed intervention as the optimal screening method will work for all MPS with no additional cost or time. The assay is usually multiplexed and using mass spectrometry enables measurement of many MPS enzymes and/or substrates in the same sample at the same time. Additional consultation feedback supported subsidising cascade testing to prevent inequity, genomic newborn screening, genetic registries to improve variant classification

(and to improve equity for non-Caucasian people underrepresented in databases). Australia's lack of participation in a registry collecting MPS II data from 29 countries was considered a disadvantage. Sanofi (sponsor of ERT idursulfase) stated it has the capacity to support the provision of treatment for newly diagnosed patients who meet the eligibility criteria for the supply of this ERT through the LSDP pathway.

Consumer Feedback

Consumer feedback stated that early diagnosis enables access to the best care, allows families to connect to patient and peer support groups, reducing isolation and is particularly important for rare diseases where there can be uncertainties in clinical knowledge. They outlined how their child's diagnosis enabled diagnosis in another family member, and may explain the unexplained illness and premature death of another family member.

PASC noted in some cases there would be no treatment available to patients who were screened and found to have MPS II. PASC considered that to inform a more balanced perspective it would also be beneficial to have consumer input regarding the acceptability of screening for a condition for which there may be no treatment. PASC noted out of session that consumer views on the acceptability of this could depend on a number of factors such as why the screening information is being gathered, how invasive the screening methodology is, how the data will be held and by whom, and whether consumers' details will be held on a list to be contacted if treatment is developed in the future. Out of session, PASC considered that NBS for this condition would be no more invasive than is NBS for other conditions (at most it might require a few more drops of blood), that the National Pathology Accreditation Advisory Council (NPAAC) requires the original report for genetic testing to be held for 100 years, and that consumers' details will not be held on a list to be contacted if a treatment is developed in the future. PASC noted out of session that other factors that may require consideration include whether consumers would be supported to connect with other parents with children who have also tested positive for this condition and likely push for action or treatment. PASC considered (out of session) that this was a likely outcome of the counselling and support that must form part of NBS programs and should be costed. PASC considered (out of session) that psychological counselling and support will likely be very important to ensure that consumers can make an informed decision.

Next steps

PASC noted that a Department Contracted Assessment Report will be performed.

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Appendix A NBS National Policy Framework (NBS NPF) Criteria

NBS National Policy Framework Criteria
The condition
1. The condition should be a serious health problem that leads to significant morbidity or mortality. 1.1 What data are there on the incidence of the condition, including in the Australian population? How is this incidence determined—through screening studies, international programs, cases identified clinically, modelled estimates based on data from variant databases or some other means? Are there any known differences in incidence in Australian sub-populations? 1.2 What is the burden of disease associated with the condition, including morbidity and mortality? Does the burden of disease vary between individuals?
2. There should be a benefit to conducting screening in the newborn period. ○ While the benefit to the baby must always be the first consideration, for some conditions a benefit for the family and/or community, as well as the benefit to the baby, may also be important and warrant consideration. This might include benefits to the family for conditions where there is currently no intervention and which will be likely to lead to early mortality but where a definitive diagnosis might be aided by a screening test. 2.1 What are the known health benefits from early detection that exist, or can be achieved, through screening for the condition? This may include early intervention, prevention of symptoms or reduction in condition severity. 2.2 Why is screening for this condition during the newborn period the most beneficial method of early detection? 2.3 Does detection of this condition provide families with actionable information that assists them in making informed choices about reproduction in the future? 2.4 What emotional or social benefits does early detection provide? 2.5 What harms may arise from screening for the condition in the newborn period?
3. The natural history of the condition, including development from latent to declared disease, should be adequately understood. 3.1 What information is known on the natural history of the condition in Australia or comparable international populations?

NBS National Policy Framework Criteria
3.2 When would the condition usually be detected clinically? 3.3 Explore the current knowledge of penetrance of the condition. Are there known benign or milder late-onset forms?
4. There should be a suitable test protocol to identify the presence of the condition. 4.1 What test protocols could be used to identify the presence of the condition? Is there consensus on the most appropriate test protocol? 4.2 When considering the test protocol, what is the clinical and analytic validity based on a consideration of: ○ Sensitivity; ○ Specificity; ○ False positive rate; ○ False negative rate; ○ Positive predictive value; ○ Negative predicative value. 4.3 Is the test protocol simple and reliable? 4.4 Can the test protocol be performed on the available dried bloodspot? 4.5 Can the test be multiplexed within existing newborn bloodspot screening panels? 4.6 What is the cost of the test protocol? 4.7 Will genetic testing be used as part of the test protocol? If genetic testing is needed: ○ Will this be by common mutations or sequencing? ○ Which mutations would be tested? ○ What is the penetrance of the mutations? ○ Are there variants of uncertain significance?
5. The test protocol should, on balance, be socially and ethically acceptable to health professionals and the public. 5.1 Can the test protocol detect other conditions of clinical or unknown significance and/or carriers and, if so, what are the implications? 5.2 What are the potential benefits and harms associated with the preferred test protocol(s)?

NBS National Policy Framework Criteria
The Intervention
6. Health care services for diagnosis and management should be available so that these services can be offered if there is an abnormal screening result. 6.1 What health care services are currently involved in the diagnosis and ongoing management of the condition? 6.2 What impact would screening for the condition have on the health care services that would be required to support diagnosis and management following an abnormal screening result? 6.3 Is diagnostic testing readily available and reliable? 6.4 Do current health care services have capacity to support the diagnosis and ongoing management of the condition? 6.5 Are current health care services of sufficient quality to support the diagnosis and ongoing management of this condition? 6.6 Is there equitable access to these health care services for families, including those from rural and remote areas?
7. There should be an accepted intervention for those diagnosed with the condition. 7.1 What accepted intervention(s) is (are) available for newborns that receive an early diagnosis through screening? 7.2 How well is the intervention and treatment pathway understood? Is there agreement on when intervention is required? 7.3 How effective is the intervention? Does it alleviate the symptoms of the condition or slow or halt its progression? What influence does the intervention have on quality and length of life? 7.4 How urgent is the intervention? Does the intervention need to be initiated before symptoms of the condition present? 7.5 Is the intervention readily available and accessible? 7.6 What are the potential harms associated with the intervention, and to what extent can these harms be mitigated or managed? 7.7 What is the cost of the intervention? What costs will be incurred for the diagnosis, management, and treatment of conditions, including the costs for false positives? 7.8 Is there equitable access to the intervention for families, including those from rural and remote areas?

NBS National Policy Framework Criteria
Additional considerations
8. The benefit of screening a condition must be weighed against its impact on the program as a whole. 8.1 Can screening for this condition be achieved within the current screening pathway? 8.2 Is the addition of this condition likely to require ethical considerations that may warrant a separate consent process? 8.3 Would it be likely that screening for the condition would impact negatively upon other elements of the program? For example, could it be anticipated that participation rates might fall? 8.4 Are there any additional costs, such as the purchasing of new technology or training, which are associated with screening for this condition? 8.5 What is the economic impact of excluding/including the condition? Do benefits exceed costs? Is it cost-effective to screen? It may be necessary for a detailed economic evaluation to consider this these questions and other relevant economic issues.
9. What other information relevant to decision making should be considered that has not been captured elsewhere?

Additional considerations
10. The benefit of screening a condition must be weighed against its impact on the program as a whole. 10.1 Can screening for this condition be achieved within the current screening pathway? 10.2 Is the addition of this condition likely to require ethical considerations that may warrant a separate consent process? 10.3 Would it be likely that screening for the condition would impact negatively upon other elements of the program? For example, could it be anticipated that participation rates might fall? 10.4 Are there any additional costs, such as the purchasing of new technology or training, which are associated with screening for this condition? 10.5 What is the economic impact of excluding/including the condition? Do benefits exceed costs? Is it cost-effective to screen? It may be necessary for a detailed economic evaluation to consider this these questions and other relevant economic issues.
11. What other information relevant to decision making should be considered that has not been captured elsewhere?