Mucopolysaccharidosis, Type II

New Disorder Resources and Tools

AUGUST 2022

NewSTEPs

A Program of the Association of Public Health Laboratories™

TABLE OF CONTENTS

ABOUT NewSTEPs

The Newborn Screening Technical assistance and Evaluation Program (NewSTEPs) is a program of the Association of Public Health Laboratories (APHL). It is a national newborn screening (NBS) program designed to provide data, technical assistance and training to NBS programs across the country and to assist states with quality improvement initiatives. NewSTEPs is a comprehensive resource center for state NBS programs and stakeholders.

HOW TO USE THIS RESOURCE

The NewSTEPs New Disorders Workgroup developed this tool to aid state NBS programs in communication and education of key stakeholders during the implementation of new disorders. NBS programs routinely consider the expansion of their state panels, a process that can be lengthy and complex. The intended audience for this tool is state NBS programs who can distribute it amongst key stakeholders such as specialists, advocacy groups, or legislators and governmental agencies seeking information on NBS disorder implementation.

NewSTEPs VISION

Dynamic NBS systems have access to and utilize accurate, relevant information to achieve and maintain excellence through continuous quality improvement.

NewSTEPs MISSION

To achieve the highest quality for NBS systems by providing relevant, accurate tools and resources and to facilitate collaboration between state programs and other NBS partners.

WHAT IS NEWBORN SCREENING?

Newborn screening (NBS)—recognized as the largest and most successful disorder prevention system in the United States—is the practice of testing every newborn for certain harmful or potentially fatal disorders that are not otherwise apparent at birth. NBS takes place before the newborn leaves the birth facility and identifies serious, life-threatening disorders before symptoms begin. Although such disorders are usually relatively rare, together they affect over 13,000 newborns each year in the US. Early detection is crucial to prevent death or a lifetime of severe health problems.[1]

Key points of NBS:

- **NBS is comprised of three different parts:** dried blood spot screening, hearing screening and critical congenital heart disease screening[2] (see **Appendix**) This resource is focused on dried blood spot NBS, as the method used for mucopolysaccharidosis type II (MPS II) screening.
- **NBS programs are essential public health programs that perform laboratory screening, conduct follow-up on actionable results and refer infants to clinical care for diagnosis and treatment as necessary.**
    - Successful programs require knowledge and coordination from multiple stakeholders who play critical roles in the screening process.
    - NBS programs test large numbers of dried blood spot specimens each day, and many of the disorders screened for are considered time-critical. Time-critical disorders are those that pose a significant health risk to newborns within days of birth.[3]
- **NBS programs are state-based.**
    - Variations between NBS programs exist from state-to-state, including the number of disorders screened and the number of routine specimens collected from each newborn.
    - While states determine which disorders to screen, federal guidance is provided by the US Department of Health and Human Services' (HHS) Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) and includes the Recommended Uniform Screening Panel (RUSP).[4]
    - A state-by-state list of disorders[5] updated in real time is maintained by the Newborn Screening Technical assistance and Evaluation Program (NewSTEPs)[6] of the Association of Public Health Laboratories (APHL).[7]
    - Occasionally, states may add disorders through legislative routes motivated by parents, disorder advocates and/or specialists, researchers and clinicians. These disorders can be unique to certain states' screening panels and may not necessarily be screened nationally.
- **NBS programs are opt-out programs.** In most states, parents can refuse NBS in writing based on their beliefs; otherwise, it is automatically conducted. This process is typically referred to as "opt out" as opposed to "consent."
- **NBS programs are designed to detect treatable disorders of the newborn.** Disorders on the NBS panel typically must meet certain criteria for screening (such as affecting newborns and not being clinically obvious), have an available screening modality or technologies (from dried blood spots) with acceptable sensitivity and specificity (not too many false-positive or false-negative results), and have effective pre-symptomatic treatments available.

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1 APHL. Newborn Screening & Genetics Program. Accessed 12 May 2021: www.aphl.org/programs/newborn_screening/Pages/program.aspx

2 NewSTEPs. Newborn Screening Educational Resource. July 2017. www.newsteps.org/sites/default/files/nbsmod3screenstabletop_educationalresource_july2017_ss.pdf

3 NewSTEPs. Time Critical Conditions. Accessed August 15, 2022: www.newsteps.org/sites/default/files/case-definitions/qi_source_document_time_critical_disorders_0.pdf

4 US Health Resources & Services Administration (HRSA). Advisory Committee on Heritable Disorders in Newborns and Children. Recommended Uniform Screening Panel. February 6, 2020. Available from: www.hrsa.gov/advisory-committees/heritable-disorders/rusp/index.html

5 APHL. Screened Conditions Report. Accessed 11 May 2021: www.newsteps.org/data-resources/reports/screened-conditions-report

6 NewSTEPs website: www.newsteps.org

7 APHL website: www.aphl.org

WHAT IS MPS II AND WHY WAS IT CONSIDERED FOR NBS?

Mucopolysaccharidosis type II (MPS II), also known as Hunter syndrome, is a lysosomal disorder (LD) caused by pathogenic variants in the iduronate 2-sulfatase (IDS) gene. The IDS gene encodes an enzyme that breaks down large sugar molecules called glycosaminoglycans or GAGs. A deficiency of the IDS enzyme results in the accumulation of GAGs in the lysosomes, causing the tissues and organs to enlarge and progressive respiratory and skeletal issues.[8]

MPS II is a relatively rare disorder with a reported birth prevalence of approximately 1 in 160,000 live male births in the United States. MPS II is more common in newborns of East Asian descent.[9]

Genetics and Inheritance of MPS II

MPS II is inherited in an X-linked recessive pattern, which means that the gene that causes MPS II is found on the X-chromosome. Because of this inheritance pattern, MPS II is typically inherited from the biological mother, who passes down a non-working IDS gene to their offspring (**Figure 1**). Biological females have two X-chromosomes, so if they inherit a non-working IDS gene on one X-chromosome, the other X-chromosome typically has a working copy of the IDS gene, and this one working copy is usually enough to prevent severe disease. Biological females with one non-working copy of the IDS gene are often called carriers, though in some cases they may have some symptoms of the disorder as well. Biological males, on the other hand, only have one X-chromosome. As a result, biological males that inherit a non-working copy of the IDS gene on their one X-chromosome will have MPS II.

Historically, the severity and onset of MPS II in males have prevented males from reproducing, which is why MPS II is typically inherited through the biological mother. However, with earlier treatment administration due to NBS, especially in less severe forms, inheritance through an affected biological father may become possible. In these cases (and assuming an unaffected biological mother), all biological female offspring of an affected biological father will be carriers for MPS II, and all biological male offspring will be unaffected (**Figure 1**).

MPS II may also be caused by a spontaneous event that results in the formation of a new variant in the egg or sperm cells. These variants are called de novo variants and are reported to occur in 10–33% of MPS II cases.[10,11]

Figure 1. X-linked Recessive Inheritance Pattern

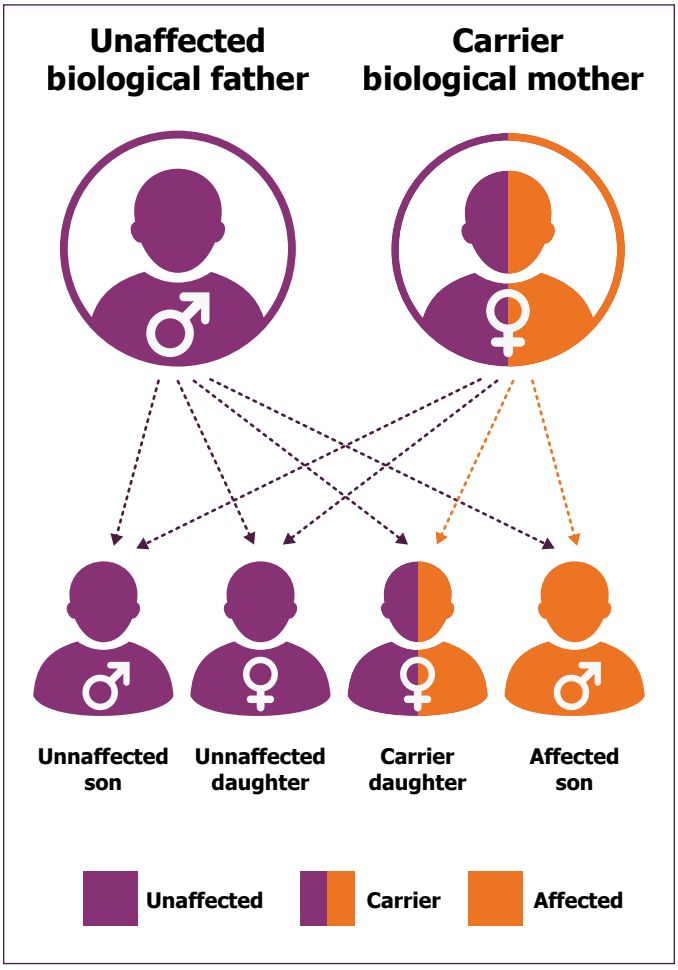

Diagram modified from NxGen MDx. Accessed January 3, 2022: nxgenmdx.com/genetic-screening/

8 MedlinePlus. Mucopolysaccharidosis type II. Accessed April 17, 2022: medlineplus.gov/genetics/condition/mucopolysaccharidosis-type-ii/#frequency

9 D'Avanzo F, Rigon L, Zanetti A, Tomanin R. Mucopolysaccharidosis Type II: One Hundred Years of Research, Diagnosis, and Treatment. Int J Mol Sci. 2020;21(4):1258. doi:10.3390/ijms21041258

10 Amartino H, Ceci R, Masllorens F, et al. Identification of 17 novel mutations in 40 Argentinean unrelated families with mucopolysaccharidosis type II (Hunter syndrome). Mol Genet Metab Rep. 2014;1:401-406. doi:10.1016/j.ymgmr.2014.08.006

11 Filocamo M, Tomanin R, Bertola F, Morrone A. Biochemical and molecular analysis in mucopolysaccharidoses: what a paediatrician must know. Ital J Pediatr. 2018;44(Suppl 2):129. Published 2018 Nov 16. doi:10.1186/s13052-018-0553-2

When MPS II is caused by a de novo variant, there are no risks of MPS II in other family members, but there may still be a residual recurrence risk to future pregnancies in the immediate family. In general, recurrence risk for de novo variants of maternal origin are thought to be between 1–10%, but exact recurrence risk of variants in the IDS gene have not been published.

To date, there have been over 600 variants reported in the IDS gene. These variants span the entire gene and there are very few commonly recurring variants, such that full gene sequencing (rather than a targeted variant panel) is often needed to determine the underlying variant in a patient with MPS II. Because of the high genetic heterogeneity of MPS II, very few genotype-phenotype correlations are possible. However, an approximate relationship has been observed where missense variations appear to be associated with both severe and attenuated phenotypes, while nonsense variants, splicing transcriptional defects, gross rearrangements or deletions/insertions are more commonly associated with severe phenotypes.[12]

Diagnosis and Clinical Manifestations of MPS II

MPS II is often considered the most variable of the mucopolysaccharidoses with the widest range of symptoms. MPS II is usually classified into two main types: the attenuated or non-neuronopathic phenotype and the severe or neuronopathic phenotype.[13] However, it is important to note that there are overlapping symptoms between the two types, suggesting that the disease spectrum of MPS II is much broader than two distinct phenotypes.

Diagnosis of MPS II in biological males after a positive NBS depends on several key aspects. Often, the first steps will include both analysis of GAG levels in the urine (which will typically be elevated in MPS II) and determination of associated IDS enzyme activity (typically decreased). If molecular testing has not already been performed by the NBS program, gene sequencing may be performed to allow for the identification of the disease-causing genetic variant and for confirmation of the biochemical findings.

In biological females, diagnosis requires genetic analysis, as both GAG levels and IDS enzyme activity are often uninformative.

Distinguishing features in both attenuated and severe MPS II are light-colored skin papules, inguinal and umbilical hernias, and characteristic coarse facial features. Nearly two out of three MPS II patients will develop central nervous system issues, which usually present between two to four years of age. Additional clinical manifestations and their relative frequency are found in **Table 1**.

Table 1. Clinical Manifestations of MPS II*

Manifestation	Frequency
Cognitive impairment	100%
Upper respiratory issues	100%
Coarse facial features	95%
Skeletal manifestations	80%
Lower respiratory issues	80-90%
Joint stiffness	75-90%
Loss of hearing	70-95%
Umbilical hernia	70-95%
Inguinal hernia	70-95%
Poor growth	79%
Hepatosplenomegaly	60-90%
Diarrhea	60%
Seizures	60%
Valvular heart disease	50-60%
Kyphosis	34%
Behavioral disturbances	30-45%
Epidermal symptoms (thickened skin with pebble formation, persistent Mongolian spots)	13-17%
Odontoid hypoplasia	Rare
Corneal clouding	Rare

* Modified from Hampe, et. al 2021

12 Semyachkina AN, Voskoboeva EY, Nikolaeva EA, Zakharova EY. Analysis of long-term observations of the large group of Russian patients with Hunter syndrome (mucopolysaccharidosis type II). BMC Med Genomics. 2021;14(1):71. doi:10.1186/s12920-021-00922-1

13 Hampe CS, Yund BD, Orchard PJ, Lund TC, Wesley J, McIvor RS. Differences in MPS I and MPS II Disease Manifestations. International Journal of Molecular Sciences. 2021; 22(15):7888.

Treatments for MPS II

As of 2022, four primary treatment methods exist: Enzyme Replacement Therapy (ERT), Hematopoietic Stem Cell Transplantation (HSCT), substrate reduction therapy and gene therapy. With multiple treatments options available, patients with MPS II receiving treatments early in life often meet their motor milestones—including sitting and walking. The new options show potential improvements in treating central nervous system symptoms, which can improve overall quality of life for patients and their families. It is easier to prevent the onset of disease manifestations than it is to reverse them after they occur. Therefore, timely diagnosis and early treatment are essential for changing the course of affected patients. NBS is leading the way in achieving this goal.

Enzyme Replacement Therapy

In 2006, the US Food and Drug Administration (FDA) approved Elaprase® (Idursulfase) as an ERT treatment option for patients diagnosed with MPS II. While Elaprase® was shown to improve patients' ability to meet motor milestones (specifically walking), it has little to no beneficial effect on the neurocognitive aspects of the disease. This is due to the inability of infused enzyme to effectively pass through the blood-brain barrier. Current research is investigating intrathecal injections (IT) to circumvent the issue of diffusion into the brain, and modifications to the enzyme to facilitate more efficient penetration into the brain across the blood-brain barrier.[14,15]

Inevitably, ERT leads to a certain level of immunogenicity in patients who produce no endogenous enzyme. This has been most noticeably shown in Pompe disease, where patients with no cross-reactive immune material (CRIM) have a high rate of antibody (Ab) production to exogenously administered enzyme. In Pompe disease, there is clear evidence that the Ab response in CRIM-negative patients is associated with worse outcomes, whereas in MPS II, the focus has been on the association between biomarkers and Abs as they pertain to clinical efficacy of ERT. Thus, CRIM status is typically not evaluated prior to ERT in cases of MPS II.

Hematopoietic Stem Cell Transplantation

HSCT was originally utilized for MPS II in 1982. Donor-derived cells have the potential to enter the brain, providing the opportunity for some benefit to the neurologic aspects of the disease. However, concerns related to HSCT include the risk of death from the transplant process and treatment-related complications, including infections, graft-vs-host disease, and rejection of the donor cells. Determining which MPS II patients would benefit at a sufficiently early stage of the disease to justify the risks of transplantation has been challenging, but it is clear that early intervention is key to maximizing outcomes.

Substrate Reduction Therapy

Another avenue for treatment is substrate reduction therapy. This methodology focuses on the reduction of GAG synthesis rather than a decrease through the delivery of enzyme. Interventions using agents such as genistein have provided positive results, although whether it may be effective in the brain is unclear.

Gene Therapy

There is no currently FDA-approved gene therapy for MPS II. There are several promising candidates for treatment, including RGX-121 (REGENXBIO) using an adeno-associated virus that is injected into the spinal fluid. There is also interest in using a lentiviral system to express enzyme in the patient's own blood stem cells, rather than using donor cells from someone else to provide enzyme. Gene therapy may prove a superior method of treatment, as it provides higher levels of enzyme than can be achieved with ERT or HSCT; and, therefore, may be shown to provide better correction of the disease. However, it remains very early in the experience of using gene therapy for MPS II.[16]

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14 Wikman-Jorgensen PE, López Amorós A, Peris García J, et al. Enzyme replacement therapy for the treatment of Hunter disease: A systematic review with narrative synthesis and meta-analysis. Mol Genet Metab. 2020;131(1-2):206-210.

15 Parini R, Deodato F. Intravenous Enzyme Replacement Therapy in Mucopolysaccharidoses: Clinical Effectiveness and Limitations. Int J Mol Sci. 2020;21(8):2975.

16 REGENXBIO Inc. (Feb 9, 2022). REGENXBIO Presents Additional Positive Interim Data from Phase I/II Trial of RGX-121 for the Treatment of MPS II (Hunter Syndrome) at 18th Annual WORLDSymposium™ 2022.

THE NEWBORN SCREENING PROCESS

Screening vs Diagnostic Tests

NBS allows for population-based screening of all newborns in a timely and affordable manner. Currently, most states screen for numerous disorders in which timely diagnosis and management improves overall outcome. NBS programs establish cutoffs and result decision algorithms to try to identify all newborns with a specific disorder without burdening the system with a high rate of false-positive results (**Figure 2**). Newborns identified to be at risk for a disorder through NBS will require additional diagnostic testing to confirm the screening and to make the diagnosis (**Table 2**).[17]

Figure 2. Newborn Screening Process

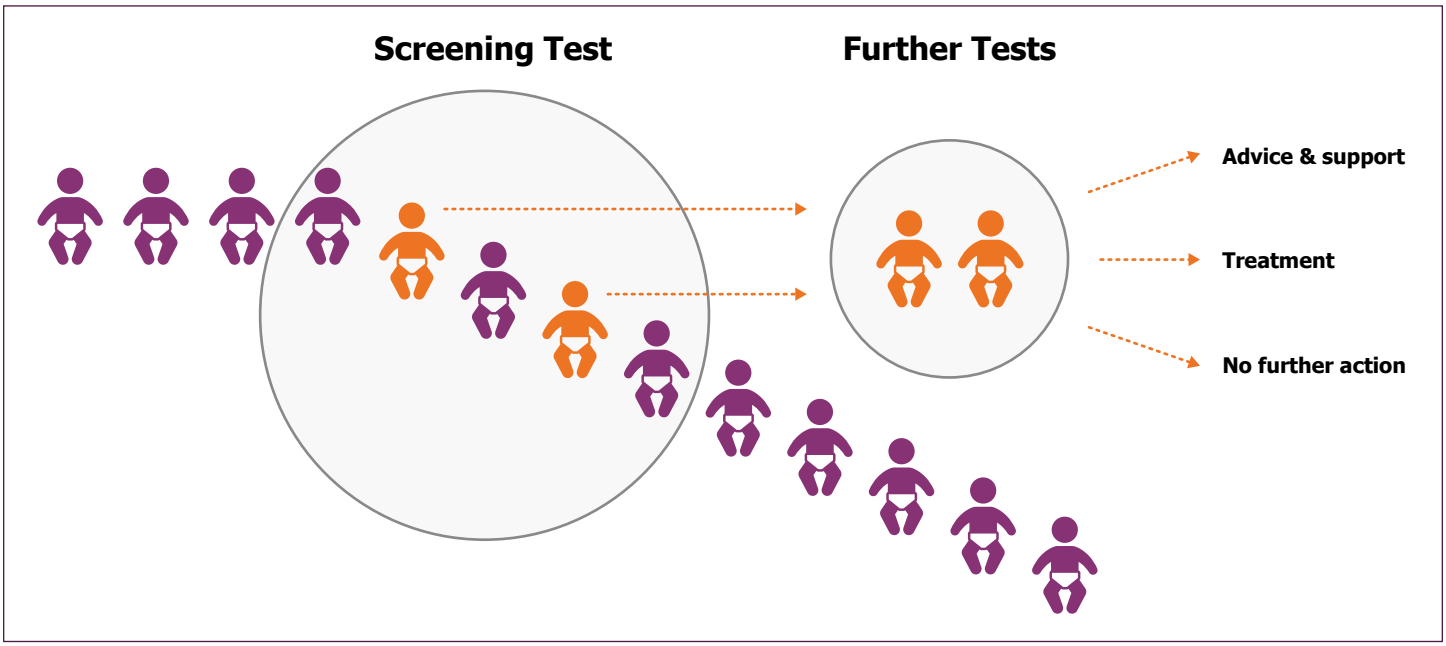


Table 2. Screen vs. Diagnostic Test

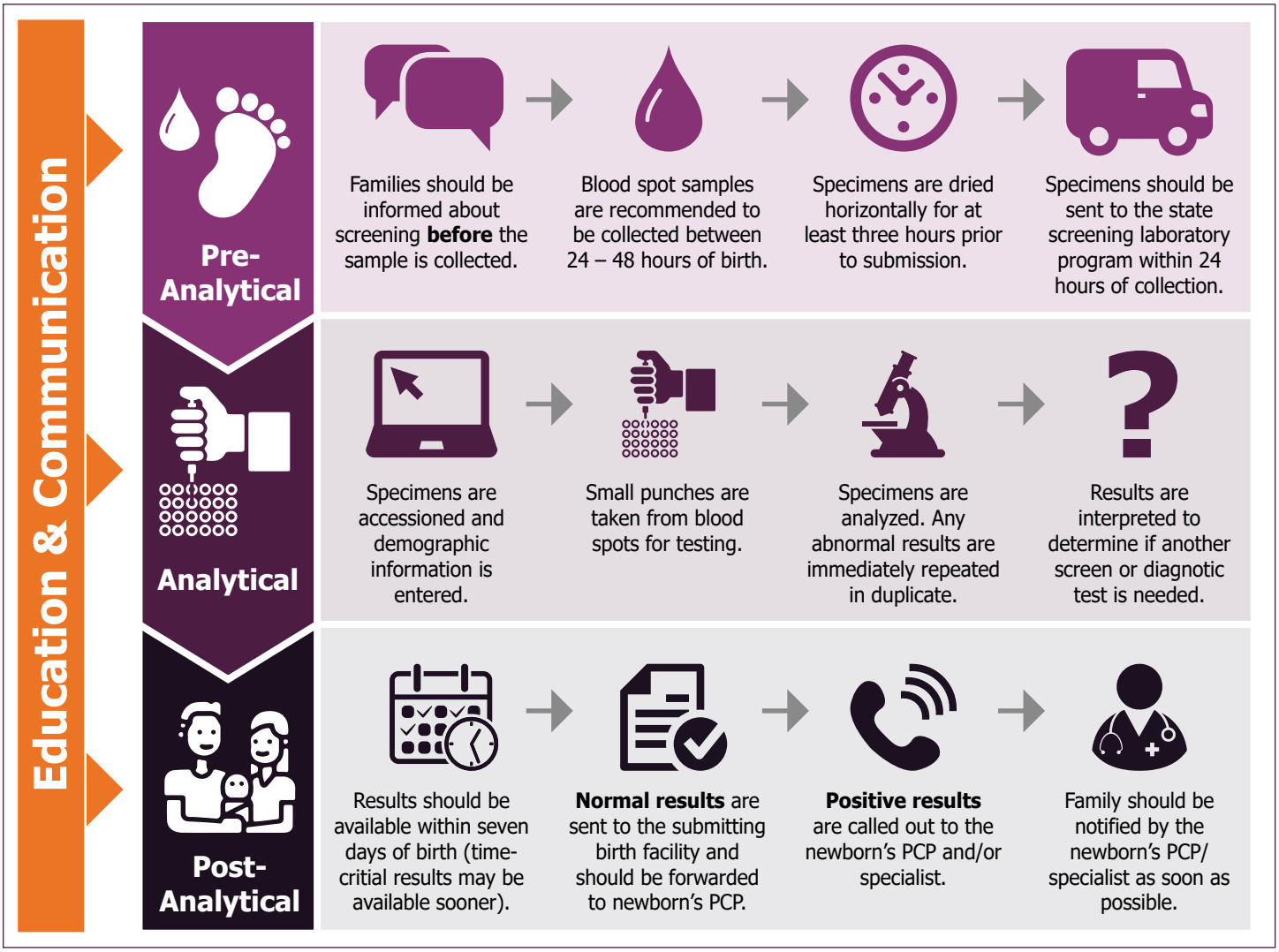
	Screen	Diagnostic Test
Population (offered the test)	Those **without clear signs or symptoms** of disorder where early detection is essential.	Those **with symptoms**. Those undergoing further work-up **after a positive screen**.
Results	Result is an **estimate of level of risk**. Determines whether a diagnostic test is warranted.	Result provides a **definitive diagnosis**.
Test Metrics	Cutoffs set towards high **sensitivity**. Acceptance of **false-positive results**.	Cutoffs set towards high **specificity**. Greater **precision and accuracy**.

17 APHL (March 2019). Overview of Cutoff Determinations and Risk Assessment Methods Used in Dried Blood Spot Newborn Screening- Role of Cutoffs and Other Methods of Data Analysis.

Components of the NBS Process

Newborn dried blood spot screening is a process that has three phases: pre-analytical, analytical and post-analytical (**Figure 3**).

Figure 3. Phases of the NBS Blot Spot Process



State-specific Algorithms

NBS programs are state-run public health programs and, therefore, work within the confines of their own state governments. Each state will determine its own testing algorithm and follow-up processes, often with input and guidance from stakeholders, specialists and other state and national partners. This algorithm may include the number of days of the week the specimens will be processed and analyzed, as well as which days of the week the results will be reported. Some states require a second screen to be conducted on all newborns, while other states may only require additional screening on their premature and/or ill newborn population.

Types of Results

A breakdown of the types of NBS results is found in **Table 3**.

Table 3. Types of Possible NBS Results

Result Interpretation	Result Meaning
Normal/Negative/ Within Normal Limits	• The child is at low-risk for having the disorder. • All values were within the expected range for unaffected newborns.
Unsatisfactory/Invalid	• The specimen was deemed invalid for accurate screening. • Results cannot be accurately interpreted. • Repeat NBS is needed.
Borderline/Inconclusive	• The child is at low- to medium-risk for having the disorder. • A repeat screen is usually requested and often (but not always) resolves the result.
Pseudodeficiency	• A known pseudodeficiency variant was found. • Clinical evaluation may still be recommended.
Abnormal/Positive/ Out-of-Range	• The child is at moderate- to high-risk for having the disorder. • Clinical evaluation and specialty referral are advised.
Presumptive Positive	• High probability that the infant is affected. • Clinical evaluation is needed.

Many of the LS disorders, including MPS II, have known pseudodeficiency variants that cause an individual to have a low enzyme level, but normal urinary GAG levels and no clinical symptoms or signs of the disease. This situation is known as pseudodeficiency. Individuals with pseudodeficiency have 5–15% enzyme activity compared to the normal population, which is sufficient to metabolize the substrates and prevent their accumulation and explains their asymptomatic health status. Such persons must be recognized promptly to avoid misdiagnosis and unnecessary treatment.

Performance Metrics and Continuous Quality Improvement

NBS is intended to flag infants that may be at risk for the screened disorder. Screening is not diagnostic; it will flag some infants who do not have the disorder (a false-positive result), and, on rare occasions, may be unable to detect truly affected infants (a false-negative result). When implementing a new disorder, it is helpful for NBS programs and key stakeholders to define goals, including metrics to measure successes and shortcomings. These metrics can define timeliness of screening, reporting, referral and initiation of treatments. Following implementation, evaluation and continuous quality improvement efforts should be outlined. The performance of NBS, which needs to be continually monitored, is measured through the following indicators:

True Positives

Infants identified through screening who are confirmed to be affected with the disorder.

False Positives

Infants identified through screening who are confirmed to not be affected with the disorder. This category typically includes unaffected carriers, individuals with pseudodeficiency, and some completely unaffected individuals who may get flagged on the screening test but prove to be negative upon further diagnostic testing.

False Negatives

Infants affected with a disorder that are not identified through NBS. Most screens are designed to minimize false negatives (maximizing sensitivity).

True Negatives

Infants with in-range NBS results who are not affected with the disorder.

It is rare for a screening test to ever have 100% sensitivity or specificity.

Sensitivity

The ability of correctly identifying those with the disorder (True Positive Rate).

Specificity

The ability of correctly identifying those without the disorder (True Negative Rate).

Predictive Value Positive (PPV)

The proportion of true positives among all positive screens.

Negative Predictive Value (NPV)

The proportion of true negatives among all negative screens.

Accuracy

Proportion of patients correctly identified (true positives plus true negatives divided by all screens).

Birth Prevalence/Incidence/Detection Rate

The number of true positives per number of births. This is typically calculated on an annual basis; however, disorders that are very rare may need to be calculated over an average of several years, depending on the state's birth rate.

Timeliness

Federal recommendations[18] include time from:

- Birth to specimen collection: **< 48 hours**
- Specimen collection to receipt by NBS program: **24 hours**
- Birth to notification and reporting of screen-positive results (time critical conditions): **5 days**
- Birth to notification and reporting of all other results: **7 days**

Programs should also consider ensuring timely diagnosis and administration of intervention or treatment to ensure the best possible health outcomes for affected children. Disorder-specific guidelines around time to diagnosis and intervention may be available.

Following implementation of NBS for MPS II, it is important that the stakeholders continue to meet regularly to review metrics and evaluate both the successes and shortcomings of NBS. Continuous quality improvement is an essential component to a NBS program.

18 HRSA (2017) NBS Timeliness Goals.

Figure 4. **NBS Outcomes**

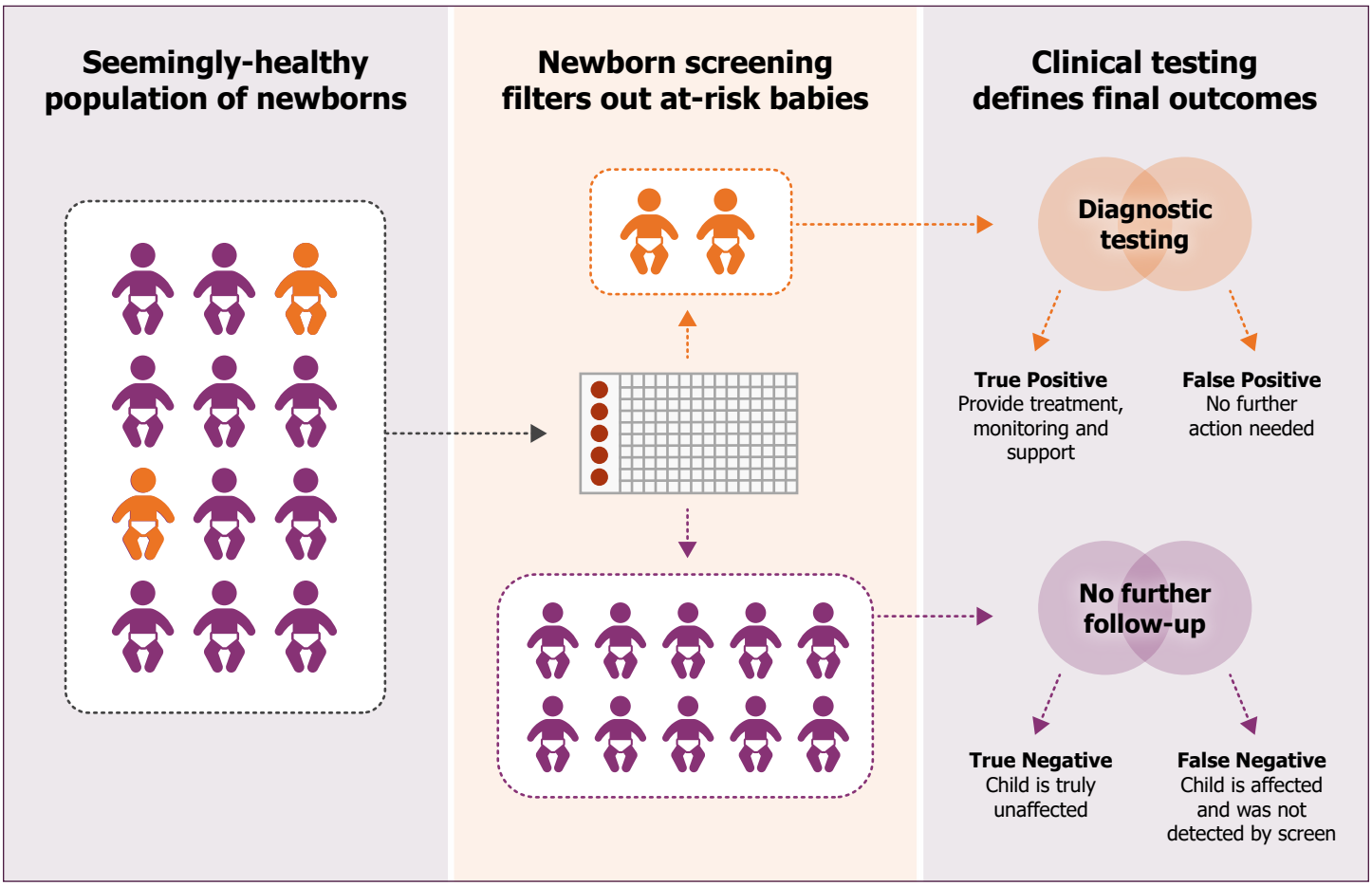


Figure 5. **NBS Test Results** [19]

	Disorder	No Disorder
Positive Test Result	**True Positive (TP)**	**False Positive (FP)**
Negative Test Result	**False Negative (FN)**	**True Negative (TN)**

Sensitivity	Specificity	PPV	NPV
$\frac{TP}{(TP+FN)}$	$\frac{TN}{(TN+FP)}$	$\frac{TP}{(TP+FP)}$	$\frac{TN}{(TN+FN)}$

19 Carvajal, Diana & Rowe, Peter. (2010). Sensitivity, specificity, predictive values, and likelihood ratios. Pediatrics in review / American Academy of Pediatrics. 31. 511-3. 10.1542/pir.31-12-511.

Stakeholders

There are many stakeholders in the NBS process. These stakeholders may include:

- Families
- Advocacy groups
- Birthing providers (e.g., doctors, nurses, midwives)
- Hospitals and birthing centers
- Couriers for timely transport of specimens
- Primary care providers
- Clinical specialists
- Genetic counselors
- NBS laboratory
- NBS follow-up
- Policy makers
- Researchers

Fiscal Constraints

The key factors to NBS are readiness to screen and feasibility of adding the screen to the screening program.[20] Almost all state programs charge a fee for the screen, and some states receive additional support for screening through state funding. The addition of a new disorder to the NBS panel can be costly; therefore, funding can be a major hurdle in the overall implementation process. Obtaining additional staff can be very difficult for some programs as well. However, for MPS II, the screening may be fully or partially "multiplexed" with other LDs currently screened by NBS programs, so an additional laboratory staff member may not be necessary.

NBS COST CONSIDERATIONS

- Adding additional laboratory and/or follow-up staff. Creating new positions within state government can be difficult during poor state revenue, hiring freezes and other fiscal scenarios.
- Laboratory equipment needed to screen.
- Physical capacity of laboratory, how much additional lab space is required.
- Testing materials and reagents needed to screen.
- Startup costs for development and validation. Sometimes the NBS fee cannot be increased until after the program has gone live with testing and reporting.
- Creating and distributing education materials.
- Revisions to or added information technology (IT) components.
- Medical specialist contracts.

State programs are often asked to demonstrate cost effectiveness of NBS when implementing screening for a new disorder. These cost analyses are not always readily available, can be difficult to perform and vary from state-to-state. Lastly, many of the treatments for rare diseases are costly, and there may not be a specialized treatment center close to the family's home or even within the state.

Timeline Hurdles

- Obtaining appropriate approval for the disorder's official addition to state panels, including fee increases and revision of rules/regulations as needed.
- Working through all the possible considerations above (see NBS Cost Considerations box).
- Completing pilot testing (if necessary) and finalizing screening cutoffs and decision algorithms.
- Education of stakeholders regarding MPS II, the plan for screening and available treatment options within the state.

Note: Several of the timeline hurdles may have been addressed previously if the state program has already begun screening for Pompe disease and/or MPS I. This situation may greatly impact their ability to implement or add screening for MPS II. States that have not yet begun any LD screening will have more hurdles to overcome when adding MPS II.

20 APHL (2020). NewSTEPs 2019 Annual Report.

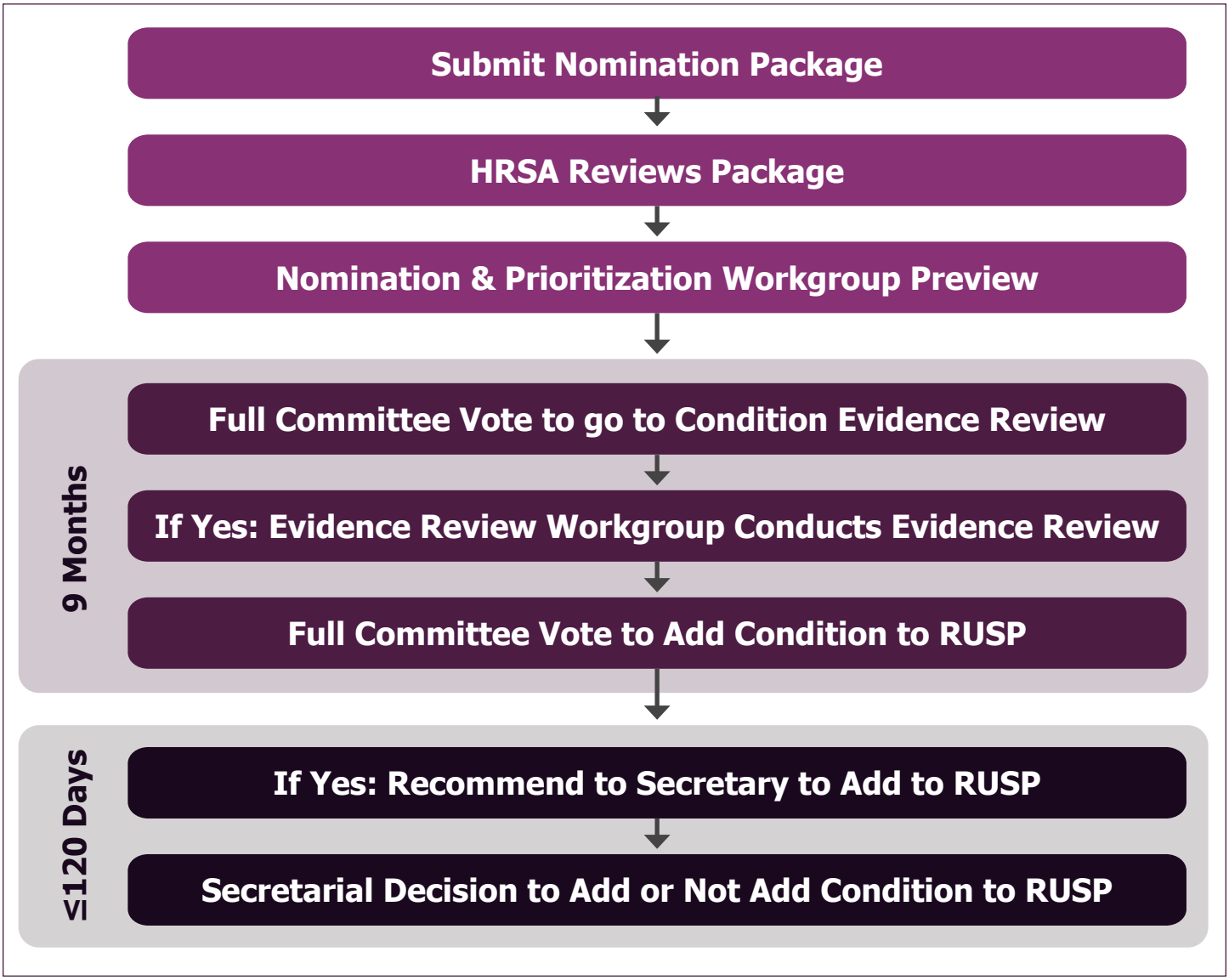
GETTING READY TO SCREEN FOR A NEW DISORDER

Before a program can implement statewide screening for a new disorder, many things need to happen. In many states, there is a well-established process to get approval to add a disorder to the state NBS panel.

In some states, the addition of new disorders is achieved through legislative action, relying on the efforts of advocates and legislators. In other states, the process includes changes to rules and regulations that govern the NBS program through actions by the state board of health or the NBS advisory committee. Some states rely on national guidance through ACHDNC, while still utilizing their own process of adding disorders to their state panels. The RUSP is a list of disorders that have passed scientific evidence review and are recommended for universal screening in the US. The RUSP was based on a report authored by the American College of Medical Genetics and Genomics (ACMG) and endorsed by the US Secretary of Health and Human Services in 2010.[21]

The RUSP was created in response to a recommendation from the American Academy of Pediatrics Newborn Screening Task Force to create uniformity in NBS throughout the US as well as a process for government, professionals and consumers to nominate a disorder to be considered by all state NBS programs. Although the RUSP provides recommendations and not requirements, many states look to it when determining whether to screen for a disorder.

Figure 6. **How Disorders Are Added to the RUSP**



21 Watson M, Lloyd-Puryear M, Mann M et al. (2006). Main Report. Genet Med 8, 12–252. doi:10.1097/01.gim.0000223467.60151.02

Approval to Screen

If legislation has mandated that a state begin screening for a new disorder, the processes and time frame for activities required by the legislation will dictate the course of events to add the disorder.

If a state is considering adding a disorder to its NBS panel, the NBS program may need to gain approval and authority to screen for the disorder. Each state NBS system follows its own processes, but below is an example of the possible steps that will need to be taken.

Most state NBS programs conduct implementation pilots to build and/or assess the state capacity to screen for the disorder and to validate testing methodology, evaluate follow-up processes, and ensure all NBS system components are operating as designed. NBS implementation pilots may require separate or additional approvals.

Support for Disorder Implementation

Understanding that successful disorder implementation requires numerous resources, states may seek assistance from organizations like the US Centers for Disease Control and Prevention (CDC), the US Health Resources & Services Administration (HRSA) and APHL when working towards implementation of disorders. CDC, HRSA and APHL provide financial resources through grants as well as technical assistance and testing materials that can aid in successful implementation.

In addition to services provided by national organizations, states may also seek guidance and assistance from their peers. For example, Missouri serves as an APHL-funded Peer Network Resource Center for implementation of MPS II, other LDs and spinal muscular atrophy that provides educational materials, standard operating procedures, follow-up guidelines, a virtual training session for digital microfluidics, and samples for method familiarization and validation (regardless of laboratory method chosen).

STEPS FOR APPROVAL / AUTHORITY TO SCREEN

- Obtain approval to screen for the disorder from the NBS Advisory Committee.
- Obtain approval to screen for the disorder from approval by Board of Health, Commissioner/other leaders.
- Develop a budget to show costs for developing the NBS program's capacity to screen, and then for costs of statewide screening—including laboratory testing, follow-up, IT, etc.
- Obtain approval by NBS Advisory Committee for funding, including funds necessary to build the NBS program's infrastructure and capacity to screen.
- Obtain approval by the State Budget Authority for funding, including funds necessary to build the NBS program's infrastructure and capacity to screen.
- Approval for fee increase, if required.

Laboratory Readiness to Screen for MPS II

The factors influencing laboratory readiness to screen are broad reaching and can vary from state to state and one disorder to another. As stated above, readiness may be greatly enhanced and expedited if the state program is already screening for other LDs, such as Pompe disease and/or MPS I.

The key aspects NBS laboratories need to consider well in advance of routine screening for MPS II are:

Readiness Steps for NBS Laboratory Screening

- **Identify which screening method to use**; some disorders have up to four laboratory methods available to use for screening.
- **Have needed equipment for testing.** Contract for purchasing or renting the testing equipment may take up to a year to become available to the laboratory.
- **Have space needed for testing equipment.** Some test equipment requires major retrofitting, ventilation and electrical changes, have a large footprint and/or need multiple platforms depending on the birthrate of the state.
- **Ensure testing method performance validations and verifications to meet regulatory requirements** for the NBS laboratory.
- **Ensure testing cutoffs and decision schemes meet specificity/sensitivity and other performance targets** to meet the goals of the NBS program. Second- or third-tier testing may need to be added as well.
- **Define true and false positives** for measurement of the screen's performance metrics once full population screening begins.
- **Obtain adequate staffing for full population screening.** May require approval for additional staff to be hired and/or require time for some current staff cross-training.
- **Integrate MPS II testing workflow** with all other NBS workflows.
- **Establish communication algorithm** with short term follow-up program (phone, IT, messaging).

Considerations for Testing Methodology

- What are pros/cons of possible testing methods?
- What equipment is needed?
- Purchasing versus reagent rental?
- Is more/different facility space needed?
- Is additional power/construction needed?
- Will the program utilize a tiered testing algorithm?
- Will the program contract out for tiered testing?
- How does the proposed algorithm affect timeliness metrics?

Considerations for Testing Validation

- Prospective (current specimens) versus retrospective (stored specimens)?
- Identified, de-identified, or anonymized specimens?
- If identified, how will results be confirmed? Who will call out abnormal results?
- What are the availabilities of positive specimens and quality assurance (QA), reference and proficiency testing materials?

Considerations for Program Staff Needs

- Are new hires needed? At what level?
- Is training and education needed for existing and new staff? Including testing and clinical considerations?
- Will additional staff be needed on weekends?
- Will new specialist contracts be needed?

After Screening Starts: Heterogeneity of Disorder/Spectrum of Findings

- Will family members be detected?
- What else is being detected?
- What is the distribution/prevalence of mild versus severe patients and is that different than what was expected?
- How is the screen performing?

Laboratory Methodology

Bench Fluorometric Assay

- Extraction of the iduronate-2-sulfatase (IDS) enzyme from the dried blood spot samples is conducted by adding sample extraction solution, covering the plates with a clear adhesive sealer to prevent evaporation and then placing on a plate shaker for 30 minutes.
- Substrate solution, which includes an IDS enzymatic substrate with an additional 4-methylumbelliferone (4-MU) fluorescent probe, is added to the appropriate wells of a black flat bottom, half-area 96-microwell plate.
- Extract from each sample is transferred to the black 96-well plate.
- The plate is covered with an aluminum plate sealer and placed in an incubator at 37°C for two (2) hours. During the incubation, a multi-step reaction involving endogenous IDS enzyme from the DBS punch liberates free 4-MU from the substrate.
- Following incubation, stop solution is added to each reaction well.
- The plate is then re-sealed and mixed on the plate shaker for two (2) minutes before centrifugation for two (2) minutes.
- Lastly, solution from each tube of the 4-MU dilution set is added to the appropriate wells of the assay plate for calibration measurements.

The fluorescence observed from the samples in each plate is read in a microtiter plate reader and is measured as relative fluorescence units (RFUs). Lower RFU values correspond with lower IDS enzyme activity in the DBS, indicating babies that may be at risk for MPS II. The detailed procedure has been previously published.[22]

Future multiplexing of MPS II with other LS disorders may be possible; the metabolic pathways within the lysosome are related as enzyme mediated chain reactions, so the product of one enzymatic reaction may be the substrate of another. As an example, the product of the iduronate sulfatase reaction (related to MPS II) is the substrate of the a-L-iduronidase reaction (related to MPS I). For this reason, assays of multiple enzymes within the same metabolic pathway can be challenging in a classically-multiplexed reaction scheme. Digital microfluidics uses “spatial multiplexing” to create a separate reaction for each assay for each sample, which allows for absolute specificity between each reaction. Feasibility of a multiplexed LD assay including MPS II from a single DBS punch has been previously described.[23] Baebies is active in the research and development process with the full intent of adding MPS II to the current SEEKER® product offering, pending addition to the RUSP.

Tandem Mass Spectrometry

There are two approaches currently in use to evaluate newborn dried blood spots for MPS II that are based on tandem mass spectrometry (MS/MS). One is to evaluate the level of the IDS enzyme activity in the blood spot and the other is to evaluate the buildup of materials in situations where the IDS enzyme is not functioning properly. In the latter situation, the substrate for IDS, glycosaminoglycans (GAGs), will accumulate to toxic levels.

While determination of elevated GAGs may suggest a case of MPS II, this situation is not specific for MPS II and may be caused by other, related disorders. Consequently, NBS laboratories have implemented direct evaluation of IDS enzyme activity as the primary newborn screen for MPS II. As an alternative to the bench fluorometric assay, IDS enzyme activity can be evaluated by MS/MS. An MS/MS assay for MPS II would be similar to MS/MS assays used for NBS of the other two lysosomal disorders currently on the RUSP, mucopolysaccharidosis type I (MPS I) and Pompe disease.[24]

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1 Bilyeu H, Washburn J, Vermette L, Klug T. Validation and Implementation of a Highly Sensitive and Efficient Newborn Screening Assay for Mucopolysaccharidosis Type II. International Journal of Neonatal Screening. 2020; 6(4):79

23 Sista RS, Wang T, Wu N, et al. Multiplex newborn screening for Pompe, Fabry, Hunter, Gaucher, and Hurler diseases using a digital microfluidic platform. Clin Chim Acta. 2013; 424:12-18.

24 CLSI. Newborn Blood Spot Screening for Pompe Disease by Lysosomal Acid α-Glucosidase Activity Assays. 1st ed. CLSI report NBS07. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

While these two LDs can be evaluated in a multiplex assay utilizing a single dried blood spot punch incubated in a suitable buffer with appropriate substrates and internal standards, there are several issues to consider when extending this to a three-plex assay (or an even higher order assay in states already screening for LDs in addition to Pompe and MPS I) beyond merely adding substrate and internal standard for IDS. One concern is that the product of IDS enzyme is the substrate for the enzyme associated with MPS I, alpha-L-iduronidase (IDUA). While this situation might suggest that assays for MPS I and MPS II cannot be multiplexed, differences in how these enzymes respond to the concentrations of their substrates can be taken advantage of in designing the reaction mixture.

Of greater concern, however, is the fact that some of the unreacted substrate for the IDS enzyme will be broken down to product by the electrospray source after injection into the MS/MS by a process termed "in-source fragmentation." The product derived by in-source fragmentation will be indistinguishable from that produced by IDS enzyme, resulting in an overestimate of the amount of IDS enzyme present in the specimen and the potential for missing a case of MPS II. Another issue is the ability to design a reaction buffer so that multiple enzymes will each function adequately, if not optimally.

One solution to these concerns is to use a separate 3.2 mm punch along with a separate incubation for the IDS assay. In this way, the buffer can be optimized, and if recombinant IDUA enzyme is added to this buffer, all products formed by IDS enzyme activity will be converted to IDUA enzyme product, which is not subject to in-source fragmentation. Following incubation, pre-injection and processing to remove materials that would otherwise contaminate the mass spectrometer, the products of this separate reaction can be added to the products of a reaction for other LDs before a single injection into the MS/MS. A downside to this strategy, however, is that the products of the MPS II assay cannot simply be combined with the products of a reaction assaying for MPS I, since the same product will be present in both cases. Consequently, laboratories already performing analysis for MPS I (with or without additional LDs) would have to use a separate MS/MS injection to evaluate MPS II assays of this design. Unfortunately, such a constraint often translates into additional MS/MS instruments with their attendant costs and operational requirements.

A more novel approach to this problem would be to configure or isotopically label the substrate for IDS in such a way that upon conversion by recombinant IDUA, its product can be distinguished from the product generated by IDUA in the separate reaction mixture utilizing a differently configured substrate. Taken a step further, substrates for IDS and IDUA could be designed in such a way that their reactions can occur simultaneously in a multiplex reaction, and their respective products could then be resolved by mass differences. And, while it may be possible to design a substrate for IDS that is not susceptible to in-source fragmentation, a substrate has been designed that can be retained by solid-phase extraction during post-incubation processing and thereby separated from its product prior to exposure to the electrospray source.[25]

Another solution, which addresses all the concerns previously mentioned while offering certain advantages, involves abandoning the traditional method of directly injecting material into the MS/MS, which is called flow injection analysis (FIA) and utilizes a medium-pressure pump to introduce material into the mass spectrometer as a continuous stream. Instead, a high-pressure liquid chromatography column would be used ahead of the mass spectrometer in an arrangement termed LC-MS/MS. In the case of MPS II analysis, the main advantage of LC-MS/MS over FIA-MS/MS is that unreacted substrate will be separated from enzyme-derived product prior to passage into the electrospray source. Consequently, any in-source fragmentation of substrate will not contaminate the enzyme-derived product and confound its quantitation.

Another advantage of LC-MS/MS analysis is the elimination of steps required to clean up reactions prior to injection into the mass spectrometer, which are usually necessary to avoid excessive contamination of the electrospray ionization source. Instead, the LC column will separate unwanted material during the injection-to-injection cycle and divert it by a flow line valve to waste rather than to the mass spectrometer. This process also results in reduced maintenance of the electrospray ionization source and less downtime for the instrument. It has been shown that a single-punch multiplex reaction followed by LC-MS/MS can be performed for the NBS of MPS I and MPS II (along with three other mucopolysaccharidoses).[26] This method incorporates all the advantages outlined above.

25 Wang D, et al., Tandem Mass Spectrometry for the Direct Assay of Enzymes in Dried Blood Spots: Application to Newborn Screening for Mucopolysaccharidosis II (Hunter Disease), Clinical Chemistry, Volume 53, Issue 1, 1 January 2007, Pages 137–140.

26 Oguni T, Tomatsu S, Tanaka M, et al. Validation of Liquid Chromatography-Tandem Mass Spectrometry-Based 5-Plex Assay for Mucopolysaccharidoses. Int J Mol Sci. 2020;21(6):2025. Published 2020 Mar 16. doi:10.3390/ijms21062025

A final feature of LC-MS/MS is the ability of the column to concentrate analytes into small volumes, which improves sensitivity of the mass spectrometer and allows for detection of low-abundance analytes that would not otherwise be detected by FIA-MS/MS. Although the IDS enzyme products are present in sufficient quantities to be quantitated by standard instrumentation and FIA-MS/MS, some other related LDs are associated with enzymes that do not generate enough product for accurate quantitation without concentrating the components of the enzyme reaction or utilizing a much more sensitive (i.e., expensive) MS/MS than that generally found in a NBS laboratory. While this feature of LC-MS/MS may not figure prominently in deciding what sort of assay to implement for MPS II NBS, laboratories in the US and elsewhere have shown that multiple LDs (including MPS II) can be multiplexed by using one or two reactions each with a single dried blood spot punch, then combining everything for a single injection using LC-MS/MS. Such flexibility may prove very advantageous in coming years as more disorders are recommended for addition to screening panels.

GAG Analysis

Analysis of GAGs can supplement enzyme analysis and help rule out many false positives such as normal outliers, pseudodeficiencies and several variants of unknown significance, resulting in improved screening performance for severe MPS II. Elevations of dermatan (DS) and/or heparan sulfate (HS) are the specific GAGs most commonly seen in MPS II. These biochemical markers, if elevated, greatly increase the child's risk for being affected with severe MPS II and can expedite follow-up decisions and actions if high risk, while also greatly reducing the impact from false-positive screening results on the NBS follow-up system and resulting alarm to families.

GAG analysis in a dried blood spot matrix (using LC-MS/MS) is offered by a few contract laboratories as second-tier analyses for NBS.[27] Because MPS II is not considered a time-critical disorder, programs can wait until this second-tier analysis is completed before issuing a final interpretation.[28] In general, turn-around times for second-tier GAG analysis have been reported to be between two and seven days.

GAG analysis is currently not considered viable for first-tier NBS for MPS II as the sample run time is too long and GAG analysis is not specific to MPS II as both DS and HS are elevated in numerous mucopolysaccharidoses, including MPS I.

Molecular Sequencing

For the purpose of NBS for MPS II, molecular sequencing will be used largely for additional information for the treating clinicians and families. Molecular sequencing can aid in determining known MPS II genotypes, variants of unknown significance, pseudodeficiencies or completely non-affected individuals. Molecular testing may also be helpful from a familial perspective given X-linked inheritance.

NBS programs may differ as to what their goals are for their screening process and whether they wish to provide molecular sequencing as a second-tier test conducted on the dried blood spot, or rather pursue molecular testing as part of the follow-up confirmatory process by way of their specialists after seeing the child. States may choose to conduct second-tier sequencing at the outset of the screening implementation to provide genotype information on all their presumptive-positives screens and thereby collect detailed feedback on their screening cutoffs going forward. They may even choose to provide both second-tier biochemical and molecular testing for a period of time in order to correlate those results and decide on the benefits to their follow-up specialists and other stakeholders. However, such extensive testing could be cost prohibitive for a NBS program to provide long term.

27 Stapleton M, Kubaski F, Mason RW, et al. Newborn screening for mucopolysaccharidoses: Measurement of glycosaminoglycans by LC-MS/MS. Mol Genet Metab Rep. 2020;22:100563.

28 NewSTEPs Time Critical Disorders.

Follow-Up Readiness

Follow-up is an essential component of the NBS process and therefore vital for successful implementation of a new disorder. NBS follow-up can include communication of screen-positive results to primary care providers and families, coordination of confirmatory testing, and connecting identified babies to appropriate specialists and/or treatment centers. For MPS II, follow-up staff will need to work closely with local genetics/metabolic specialists and treatment centers to determine a plan of communication including information to be shared with primary care providers (PCPs) and families.

Follow-up staff should understand potential geographical, financial or cultural barriers that may arise and hamper timely follow-up, diagnosis and treatment. Additionally, it is important to recognize that families receiving news of a positive NBS result for MPS II may need added support in accepting the potential of a very serious disorder in their seemingly healthy newborn.

Some NBS programs might consider a script or outline for initial notifications when implementing a new disorder. Follow-up staff can also work with the specialists to identify timeliness metrics for initial results, confirmatory testing and referral to specialists for initial evaluation. Follow-up can often identify delays in the process, barriers to confirmatory testing, and access to care issues including gaps in management and treatment.

Key components of follow-up readiness for MPS II screening include:

- Integration of MPS II follow-up workflow with other follow-up workflows.
- Identification and communication with medical specialists and/or treatment centers for infants with actionable MPS II NBS results.
- Development of action plan templates and fact sheets for PCP and families, including any confirmatory testing needed.
- Development of a communication plan for follow-up coordinator and family/PCP.
- Development of a procedure for referral from NBS program to genetics or metabolic specialist.
- Communication to third-party payers of MPS II screening and understanding of the need for coverage for treatments/therapies.
- Development of clinical data elements to be collected to determine diagnostic outcome (true positive vs. false positive) and severity of disorder (attenuated vs. severe).

Long-term follow-up is also a beneficial component of NBS, as health departments may track key indicators for an extended time once an infant is confirmed to have a disorder. These activities can include care coordination, assuring access to both care and treatment, mode of treatment and periodic assessment of outcomes in patients. These additional data can be valuable when assessing the success of implementation. The data collected will inform the NBS program and can be beneficial for continuing quality improvement.

Information Technology (IT) Readiness

NBS programs process tens of thousands of specimens a year and require robust information management systems, inclusive of laboratory information management systems (LIMS) and case management systems (CMS) used for follow-up. These systems may be developed by the state program or purchased from a vendor. Each time a disorder is added or changes are made to the NBS program, these systems must be modified for the analyte cutoffs, analyte reporting logic, new reports, assay quality control definitions, follow-up logic, parent letters and result reports, and diagnostic criteria and case definitions. Some programs include long-term follow-up in their systems. Fields need to be queryable for continued evaluation of implementation and quality improvement efforts. NBS reports must be securely distributed to birthing facilities, midwives, primary care physicians and/or other medical providers through a web-based portal, electronic messaging, or paper copies by fax or mail. It is important to have stakeholder input when revising these reports so that the results are easy to understand and appropriate guidance is provided when there is a positive result or a need for a repeat specimen.

Any changes to a NBS program's systems takes time (i.e., specification gathering, extensive testing, user acceptance), expertise, stakeholder involvement and funding.

Key components of IT readiness include:

- **Integration of disorder into LIMS Testing & Reporting** (i.e., web portals, state health information exchange (HIE) and other reporting entities).
- **Integration of disorder into CMS Reporting System** (i.e., web portals, state HIE and other reporting entities)
- **Integration of disorder into Electronic Orders and Results Protocol.** Determine vocabulary and message standards, and coordinate changes with each partner.

Notify submitters of report changes, such as:

- How will the NBS report change?
- What are reference ranges? Possible results?
- What are the relevant vocabulary standards (e.g., Logical Observation Identifiers Names and Codes (LOINCs))?

Establishing Relationships with Specialists

It is important for state NBS programs to establish partnerships and strong relationships with specialists. Relationships start during consideration and implementation of a new disorder. It is beneficial for state programs to form a task force/subcommittee with all the specialists across the state. The work groups should include laboratory, follow-up, specialists and parent advocates. As the process evolves, these task forces/subcommittees can begin discussing contracts, continuous quality improvement during and following implementation, development of educational materials, technical assistance and content expertise.

IDENTIFYING & MEETING WITH SPECIALISTS

- Are "new to NBS" sub-specialists involved?
- What clinical coverage does the state have for evaluation and treatment?
- Will testing need to occur on weekends for this condition?
- Who should be notified of screen-positive results? How urgently?
- After which tier should specialists be notified?
- What is appointment availability for positive NBS in their clinic?
- What barriers might there be to follow-up testing?
- Who can treat which individuals? On which insurances?
- What are monitoring protocols?
- What are associated risks?

EDUCATIONAL TOOLS FOR MPS II

Education of providers, hospitals/birthing facilities and families is a key component of successful implementation. Since providers are often the first to discuss positive NBS results with families, educational tools and resources should be provided to them to facilitate this initial communication and ensure that accurate information is shared with the family. State programs can work with their specialists, disease specific support groups and families to develop educational material. It is important to review existing educational material for the specific disorder, since the current tools developed for clinically diagnosed patients may not be suitable for patients identified by NBS. Educational materials are often shared between state programs or materials are developed for national use through Expecting Health or the National MPS Society.

When a state is in the process of implementing a new disorder, it is beneficial to work with the communications group of the health department to develop a press release announcing the new disorder and benefits of screening. NBS programs may even consider working with stakeholders to develop a news story highlighting the implementation.

With MPS II, older educational materials sometimes show patients that are significantly impacted by MPS II and may not reflect patients that were identified shortly after birth and treated early.

EDUCATIONAL READINESS TASKS

- Develop educational and support materials for PCPs, hospitals and families
- Translate educational materials for families into appropriate languages
- Develop script for PCPs to use with families
- Establish a communication plan between NBS program, specialists and PCP

PILOT STUDIES vs. FULL STATEWIDE IMPLEMENTATION

Most state NBS programs conduct implementation pilots to build the state's capacity to screen for the disorder, validate testing methodology, evaluate follow-up processes and ensure all NBS system components are operating as designed. Pilots may last a year or more in order to properly screen a representative sample of newborns, particularly if the disorder is very new to NBS nationally.

Some states use a consented pilot, meaning that consent will be obtained from the parents of those newborns participating in the pilot screening process. A consented pilot may be conducted on a subset of newborns in the state or on all newborns born in the state. This is most common when NBS programs want to use blood spot specimens from newborns known to have MPS II so they may validate their testing methodology to obtain a certain result.

Some states will use an "opt-in" process—parents have to agree to the screening for MPS II—until the disorder is added to the state NBS panel and MPS II screening is implemented statewide. States often need to include their health department's Institutional Review Board (IRB) for approval of the pilot process.

During an implementation pilot, normal (negative) NBS results are not usually reported on the laboratory report. If the NBS for MPS II should return a positive result, the laboratory will notify the follow-up program staff, who will notify the newborn's PCP after consultation with the NBS program's clinical specialist so that affected babies can benefit from the pilot.

Other state NBS programs that have already implemented a new disorder may be willing to share their implementation process and experiences with states that are planning their own implementation.

Prior to testing specimens during a pilot, the NBS program and the clinical specialists should determine a plan of action for reporting identified cases of MPS II during this time so that these babies and their families can benefit from the pilot.

CONCLUSION

The intent of this MPS II resource has been to provide an overview of information regarding the many aspects that are involved in the addition of a new disorder to a state NBS panel, with specific focus on MPS II. Please direct any questions regarding implementation or technical assistance needs to NewSTEPs at newsteps@aphl.org.

Learn more about MPS II on HRSA's website: newbornscreening.hrsa.gov/conditions/mucopolysaccharidosis-type-ii

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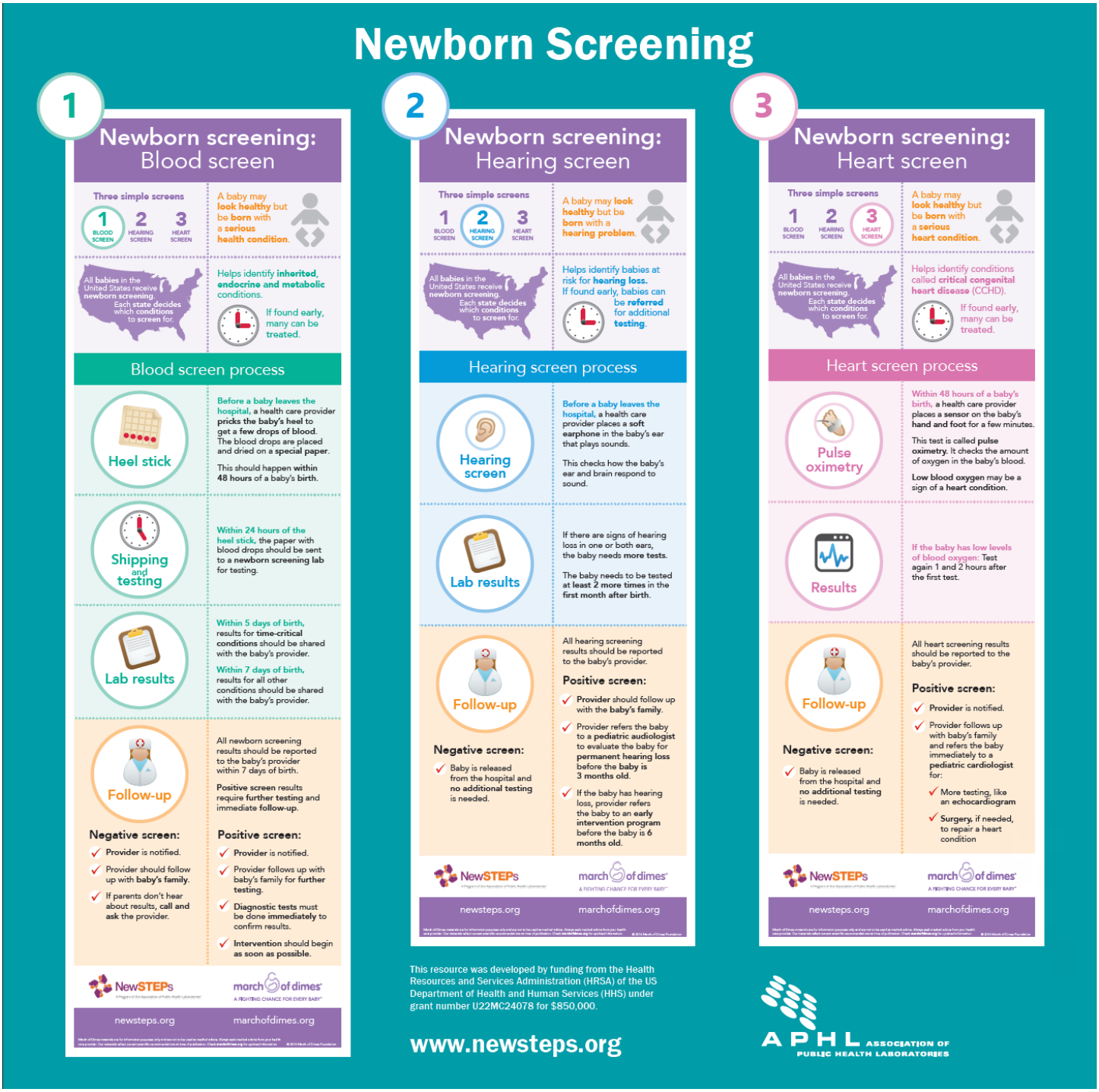
APPENDIX

NBS is comprised of three different parts: dried blood spot, hearing and critical congenital heart disease.



NewSTEPs Newborn Screening Educational Reference

Newborn Screening Technical Assistance and Evaluation Project

The Newborn Screening Technical assistance and Evaluation Project (NewSTEPs) is a national newborn screening project designed to provide data, technical assistance, quality improvement resources and training to newborn screening programs. NewSTEPs functions with the goal of improving outcomes for newborns by facilitating newborn screening initiatives and programmatic outcomes, thus improving the overall quality of the newborn screening system.

Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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