Guanidinoacetate Methyltransferase Deficiency

New Disorder Resources and Tools

AUGUST 2023

NewSTEPs

A Program of the Association of Public Health Laboratories™

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INTRODUCTION

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

APHL NEWBORN SCREENING

Vision

All babies have a healthier start through newborn screening in the US and globally.

Mission

Driving global NBS systems to excellence by shaping policy, promoting data- driven improvements, and pursuing innovations in public health lab practice.

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 screening 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 guanidinoacetate methyltransferase (GAMT) deficiency 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 partners 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- or territory-based.**
    - Variations between NBS programs exist from state-to-state (for the purposes of this report we will refer to states and territories as "states"), 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 that present in childhood.** 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.

1 APHL. Newborn Screening & Genetics Program. Available from: 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. Available from: www.newsteps.org/data-resources/reports/screened-conditions-report

6 NewSTEPs website: www.newsteps.org

7 APHL website: www.aphl.org

WHAT IS GAMT DEFICIENCY AND WHY WAS IT CONSIDERED FOR NBS?

GAMT deficiency is a creatine deficiency disease caused by pathogenic variants in the *GAMT* gene. The *GAMT* gene encodes an enzyme that assists in the production of creatine from the compound guanidinoacetate. Creatine is needed for the body to store and use energy properly. A deficiency of the GAMT enzyme results in the accumulation of guanidinoacetate (GUAC), which can have toxic effects on brain and muscle cells.

GAMT deficiency was first described in 1994 and is a rare disorder with a reported birth prevalence between 1 in 2,640,000 and 1 in 550,000 live births in the United States. There have only been around 120 affected individuals reported in the literature, most of whom are of Portuguese ancestry.[8]

Genetics and Inheritance of GAMT Deficiency

GAMT deficiency is inherited when each parent passes down a non-working *GAMT* gene to their offspring. Only individuals with two non-working *GAMT* genes—one from the biologic mother and one from the biologic father—will have GAMT deficiency (**Figure 1**). Carriers of *GAMT* do not have nor do they develop the disease. If two parents are carriers of a non-working copy of the *GAMT* gene, they have a 1 in 4 or 25% chance in each pregnancy of having a child with GAMT deficiency. There are different genetic changes or variants that result in a non-working copy of the *GAMT* gene.

To date, there have been 15 different types of variants (including nonsense, splice site, small deletions and small insertions) reported in the *GAMT* gene.[9] These variants span the entire gene and there are very few commonly recurring variants (e.g., c.59G>C in the Portuguese population[10] and a pan-ethnic variant, c.327G>A), such that full gene sequencing (rather than a targeted variant panel) is needed to determine the underlying variants in a patient with GAMT deficiency.

Figure 1. Autosomal Recessive Inheritance Pattern

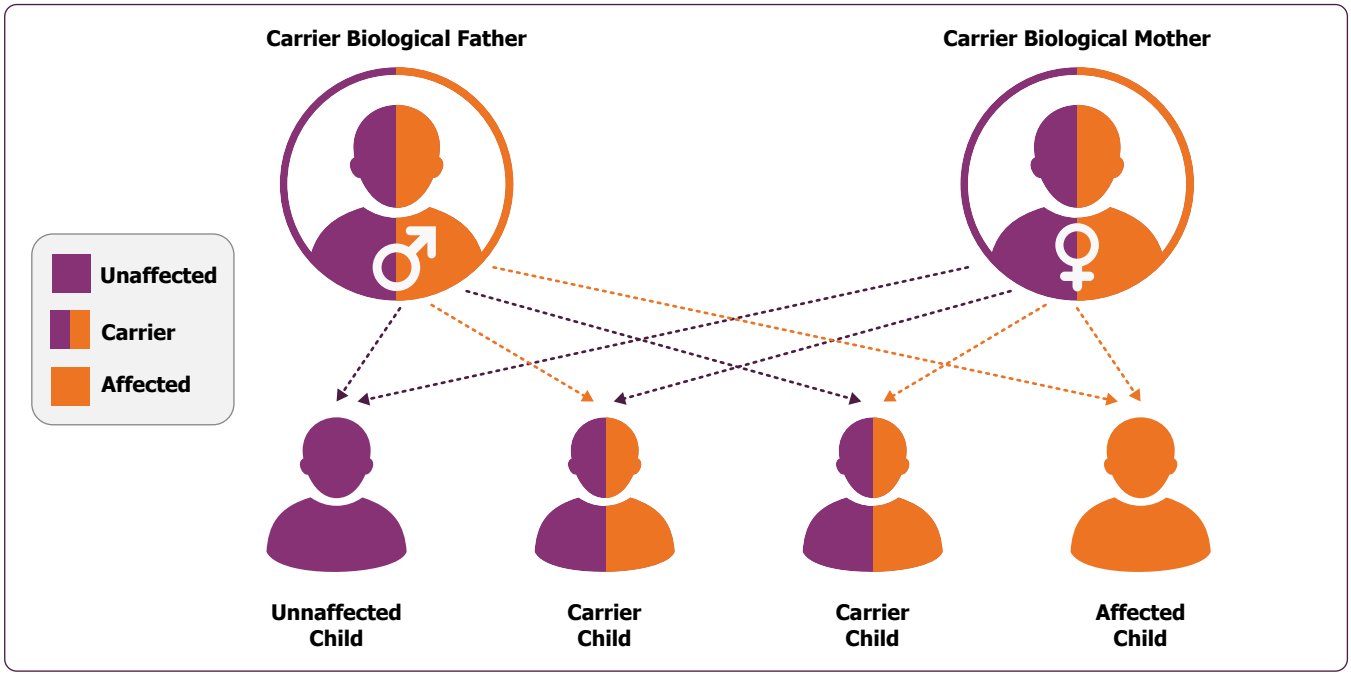


Diagram modified from NxGen MDx. Accessed January 3, 2022 from: nxgenmdx.com/genetic-screening/

8 MedlinePlus. Guanidinoacetate methyltransferase. Accessed February 1, 2023: https://medlineplus.gov/genetics/condition/guanidinoacetate-methyltransferase-deficiency/

9 Dhar SU, Scaglia F, Li F-Y, Smith L, Barshop BA, Eng CM, Haas RH, et al. "Expanded clinical and molecular spectrum of guanidinoacetate methyltransferase (GAMT) deficiency." Molecular genetics and metabolism 96, no. 1 (2009): 38-43.

10 Almeida, LS, Vilarinho L, Darmin PS, Rosenberg EH, Martinez-Munoz C, Jakobs C, Salomons GS. "A prevalent pathogenic GAMT mutation (c. 59G> C) in Portugal." Molecular genetics and metabolism 91, no. 1 (2007): 1-6.

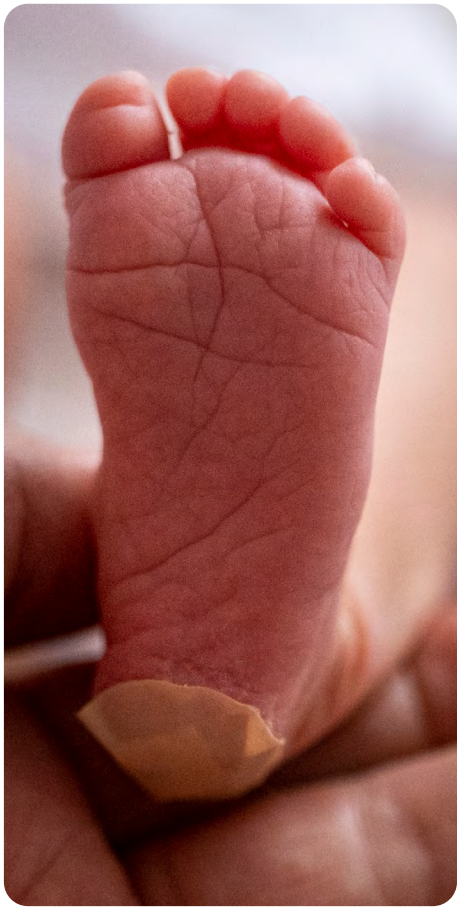
Diagnosis and Clinical Manifestations of GAMT Deficiency

The clinical features of GAMT deficiency are non-specific with variable ages of onset. Signs and symptoms may include global developmental delay, hypotonia, muscle weakness, intellectual disability, seizures/epilepsy, progressive movement disorders and autism-like behavior. Additional clinical manifestations and their relative frequencies are found in **Table 1**.[11] The most common clinical manifestations are intellectual disability and epilepsy with developmental delay often being the presenting clinical manifestation.[11] Onset typically ranges from early infancy (three to six months) to two years of age.

Table 1. Diagnostic Findings in GAMT Deficiency

Sign or Symptom	Frequency
Seizures	Very frequent
Developmental Delay	Frequent
Progressive Movement Disorder	Frequent
Poor Speech	Frequent
Intellectual Disability	Frequent
Behavior Abnormalities	Frequent
Involuntary Movements	Frequent
Hypotonia	Occasional

Diagnosis of GAMT deficiency after a positive NBS depends on several key aspects. Often, the first steps will include analysis of creatine in plasma and urine (which will typically be decreased in plasma and may be decreased or normal in urine in newborns with GAMT deficiency) as well as urine and plasma guanidinoacetate (typically increased in both plasma and urine). 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 variants and for confirmation of the biochemical findings.



Treatments for GAMT Deficiency

Recommendations for treatment of GAMT deficiency continue to evolve. As of 2023, there are two primary treatment approaches for GAMT deficiency: supplementation and dietary management. The goal of treatment of GAMT deficiency is to replete cerebral creatine levels and reduce guanidinoacetate concentrations.[12]

Because GAMT deficiency affects central nervous system development, early and pre-symptomatic treatment is critical.

Supplementation

Creatine is typically supplemented daily along with high or low-dose ornithine supplementation. Sodium benzoate may also be provided. In GAMT deficiency, creatine supplementation can restore brain creatine levels and improve neurological status.

Dietary Therapy

An arginine-restricted diet (with or without medical food) may also be utilized to aid in the reduction of cerebral guanidinoacetate levels. However, more recent data has shown a relatively small impact on guanidinoacetate levels.[13]

11 https://rarediseases.info.nih.gov/diseases/2578/guanidinoacetate-methyltransferase-deficiency

12 Viau, KS, et al. "Evidence-based treatment of guanidinoacetate methyltransferase (GAMT) deficiency." Molecular Genetics and Metabolism 110.3 (2013): 255-262.

13 Mercimek-Mahmutoglu S, Dunbar M, et al., "Evaluation of two year treatment outcome and limited impact of arginine restriction in a patient with GAMT deficiency, Mol. Genet. Metab. 105 (2012) 155–158.

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 analytical 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**).[14]

Figure 2. Newborn Screening Process

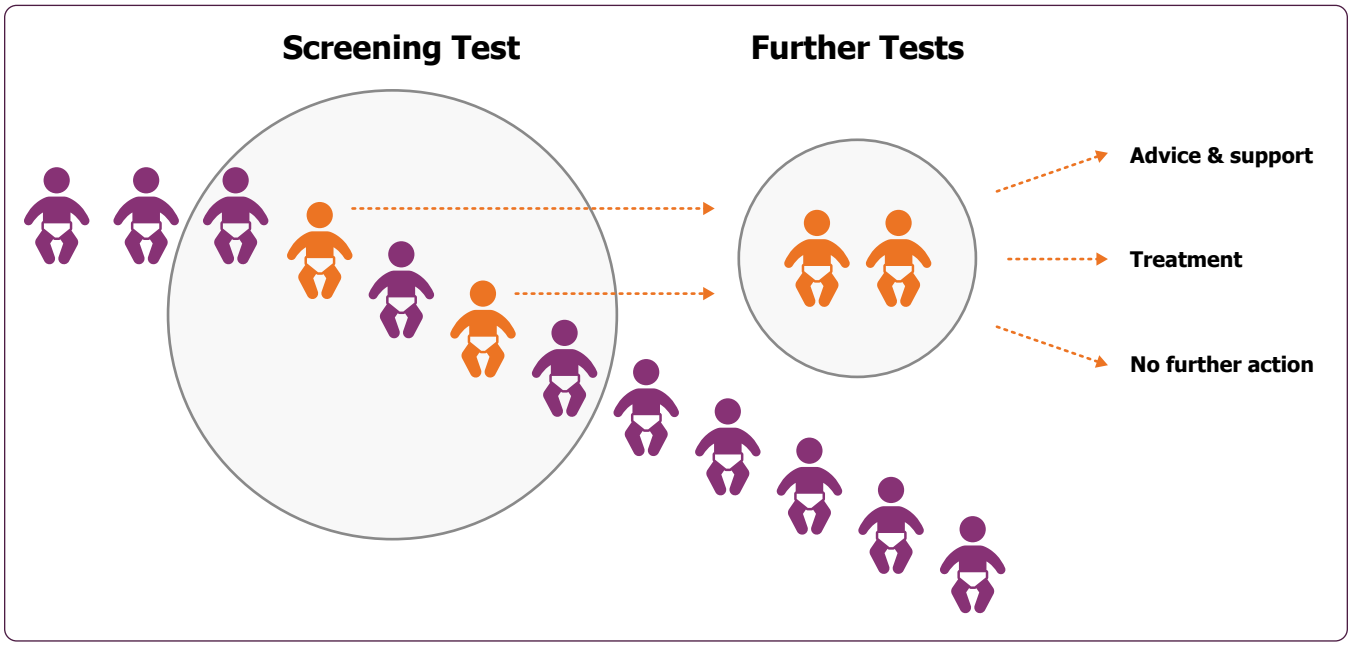


Table 2. Screen vs. Diagnostic Test

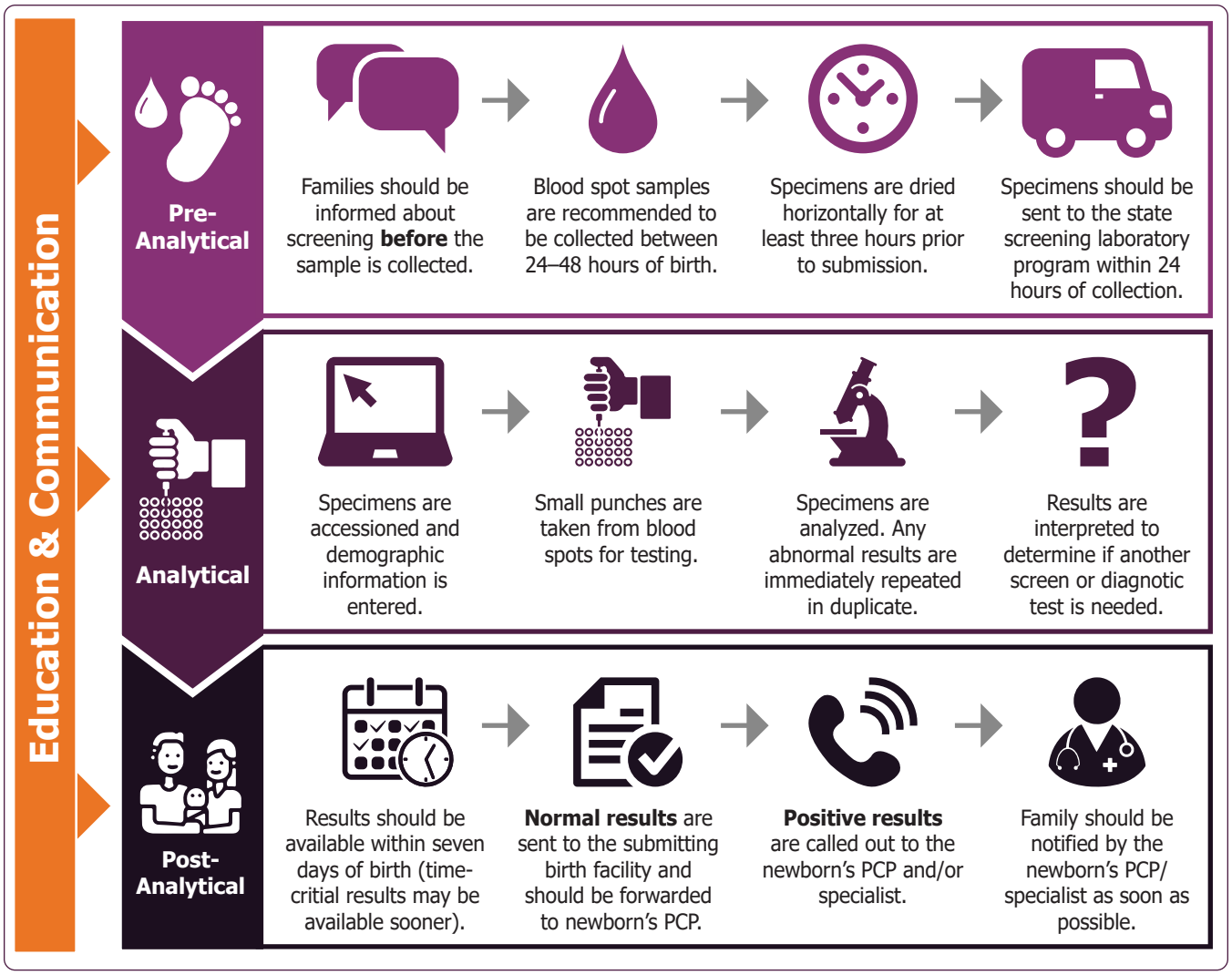
	Screen	Diagnostic Test
Population (offered the test)	Those **without clear signs or symptoms** of disorder where early detection is essential.	Those **with symptoms**. Those undergoing further work-up **after a positive screen**.
Results	Result is an **estimate of level of risk**. Determines whether a diagnostic test is warranted.	Result typically 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**.

14 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 Blood 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 community members, 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 for 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/ In-range	• 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.
Abnormal/Positive/ Out-of-Range	• The child is at moderate- to high-risk for having the disorder. • Clinical evaluation and specialty referral are advised.

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 that are confirmed to be affected with the disorder.

False Positives

Infants identified through screening that are confirmed to not be affected with the disorder. This category typically includes unaffected carriers and some completely unaffected individuals who may be 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.

Sensitivity

The test's ability to correctly identify those with the disorder (True Positive Rate).

Specificity

The test's ability to correctly identify those without the disorder (True Negative Rate).

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

Positive Predictive Value (PPV)

The proportion of true positives among all positive screens.

Negative Predictive Value (NPV)

The proportion of true negatives among all negative screens.

Accuracy

The 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[15] 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.

15 HRSA (2017) NBS Timeliness Goals.

Figure 4. **NBS Outcomes**

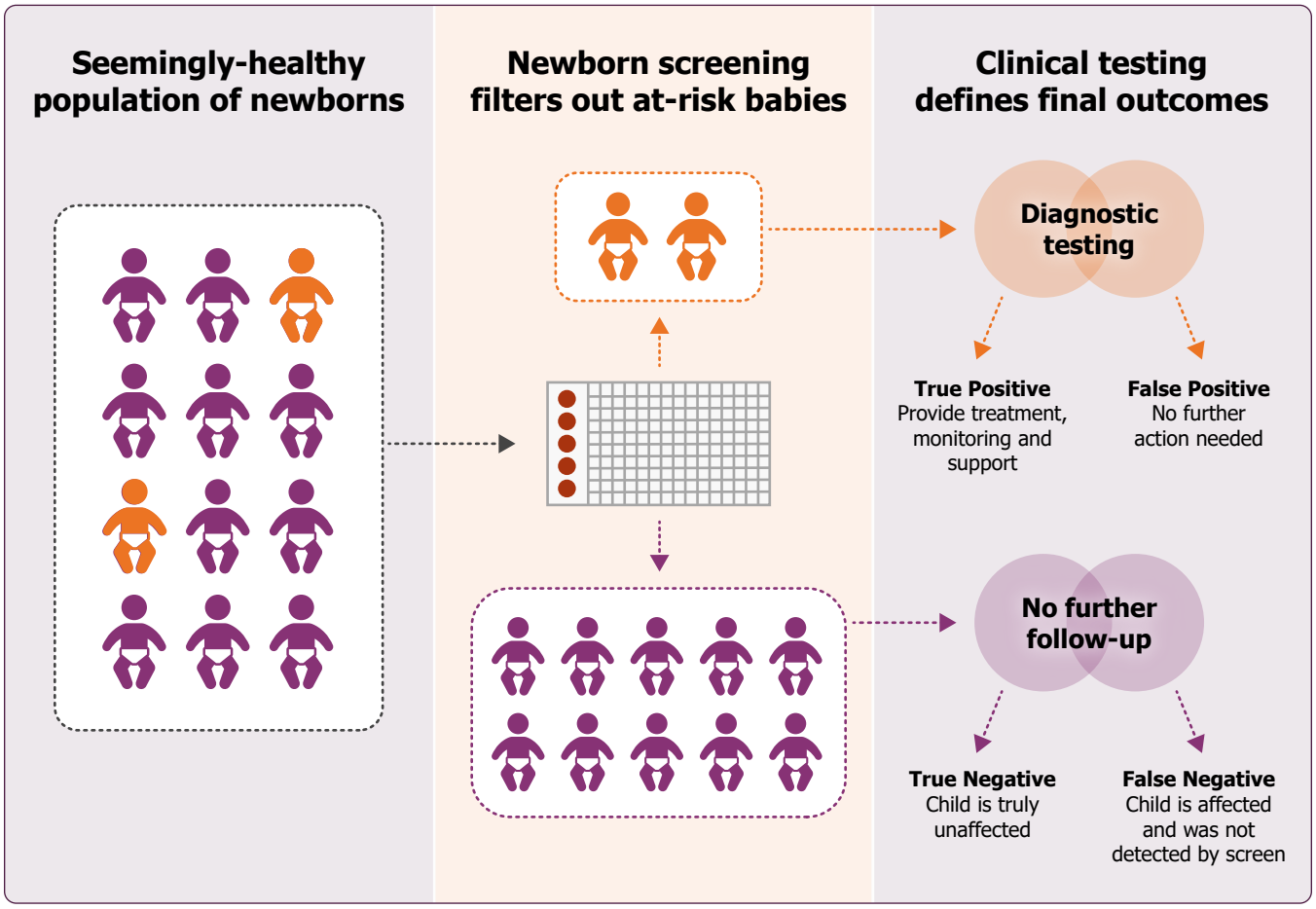


Figure 5. **NBS Test Results** [16]

	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
$\frac{TP}{(TP+FN)}$	$\frac{TN}{(TN+FP)}$	$\frac{TP}{(TP+FP)}$	$\frac{TN}{(TN+FN)}$

16 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.

Partners

There are many partners in the NBS process; they 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 (PCPs)
- 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.[17] 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.

State programs are often asked to demonstrate the 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 partners regarding GAMT deficiency, the plan for screening and available treatment options within the state.

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.**

17 APHL (2020). NewSTEPs 2019 Annual Report.

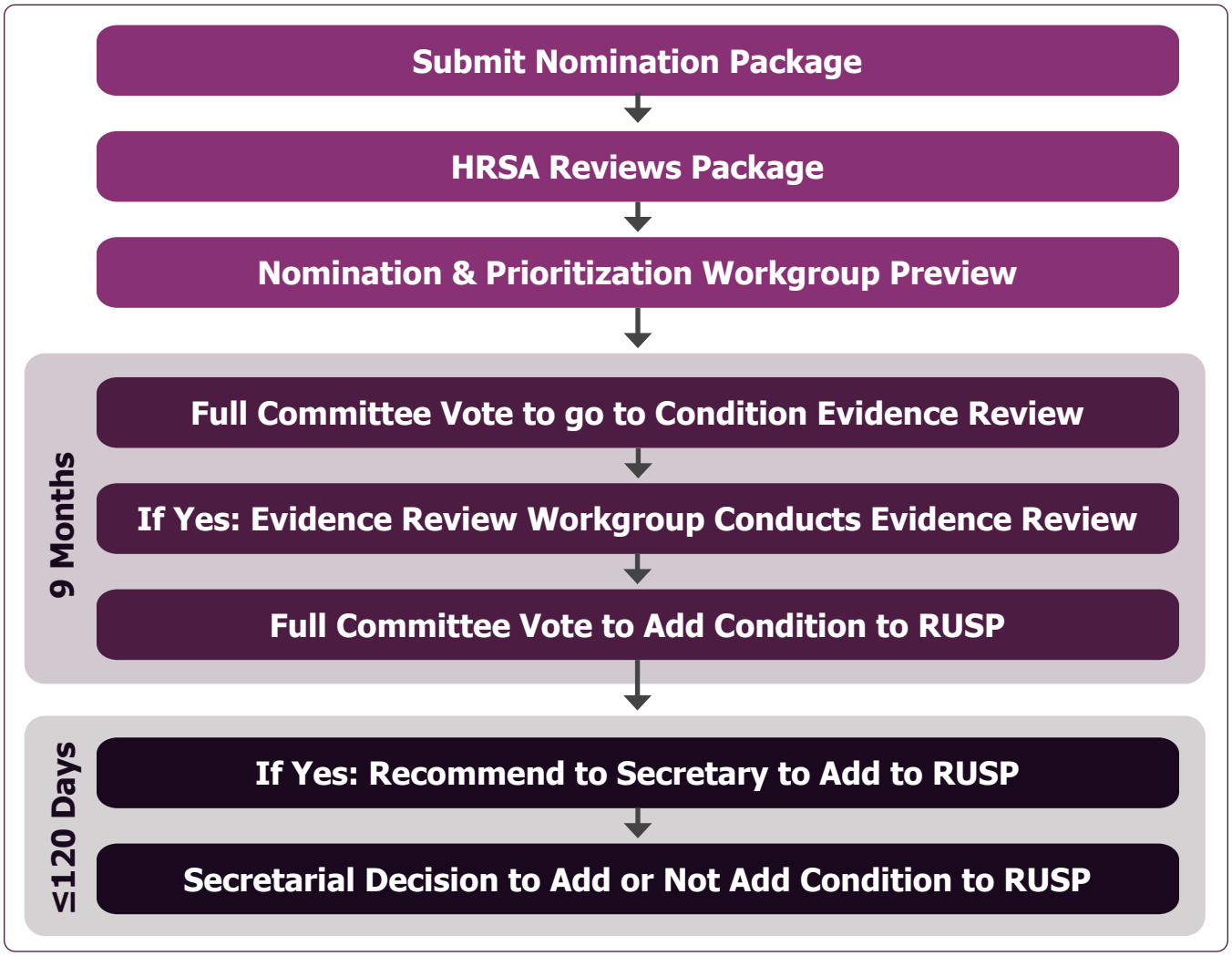
GETTING READY TO SCREEN FOR A NEW DISORDER

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.[18]

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



18 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 disease, 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.

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 the Board of Health, Commissioner or 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 GAMT Deficiency

The factors influencing laboratory readiness to screen are broad reaching and can vary from state to state and one disorder to another. The following are key aspects NBS laboratories need to consider well in advance of routine screening for GAMT deficiency:

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 GAMT 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?
- Can GAMT deficiency testing be multiplexed with the current MS/MS panel?
- 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 the results be confirmed? Who will call out out-of-range 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 from what was expected?
- How is the screen performing?

Laboratory Methodology

Michigan

Michigan began screening for GAMT deficiency in September 2022 using a commercially available non-derivatized kit for amino acids and acylcarnitines on the Waters TQD instruments. The kit was validated with the further addition of specific internal standards for GUAC and creatine. External quality control materials are provided by the CDC Newborn Screening Quality Assurance Program. During the validation, Michigan discovered using older, less sensitive, instrumentation is possible; however, due to a lack of sensitivity compared to newer tandem mass spectrometry (MS/MS) instruments, cleanliness is a crucial component to successfully screen for GAMT deficiency. The solution was to add a preventative maintenance agreement to the service contract for a total of three per year. Michigan has added additional GUAC alternative product ions to be analyzed in the case of an isobaric interferant, but they are not part of the reporting algorithm. Both the GUAC analyte level and the ratio of GUAC to creatine level (GUAC×1000÷CRE) must be elevated for a specimen to be screen positive. Michigan noticed a decrease in the GUAC and creatine values in infants 72 hours of age and older which led to having an age-related cutoff for both GUAC and the ratio. The screen-positive cutoffs are at 0−71 hours of age GUAC ≥ 6.50 and the ratio ≥ 25.0, for ages greater than 72 hours of age GUAC ≥ 6.00 and the ratio ≥ 30.0. Screen positive infants will be sent to a metabolic coordinating center for diagnostic testing.

Contact for Michigan Program GAMT NBS: Shawn Moloney (moloneys1@michigan.gov)

New York

New York began screening for GAMT deficiency in 2018 using a three-tiered approach. Since then, the method has been modified to have a two-tiered approach by using a different ion of interest to eliminate the isobaric interferant that required the use of a second-tier high-performance liquid chromatography (HPLC) method. In March 2020, the method of analysis for GUAC was modified to use the alternative product ion. For a comparable six-month period, the modified method reduced the number of samples requiring second tier testing by 98%, reduced the number of borderline results requiring a repeat sample by 87.5%, and reduced the number of referrals to specialty care centers by 85%. As of June 2023, there is no separate extraction for GAMT analysis; it is run with daily samples with derivatized amino acids and acylcarnitines. Ratios of GUAC to creatine levels are evaluated (GUAC×1000÷CRE) and samples with a value greater than or equal to 12.0 and a GUAC level greater than 5.00 are considered a screen-positive, referral-level specimen. Screen-positive samples are sent to the DNA team for sequencing of the *GAMT* gene. Quality controls for this assay are separate from amino acids/acylcarnitines controls and are made in-house.

New York's full method details can be found in the manuscripts below:

- Hart, Kim, et al. "Prospective identification by neonatal screening of patients with guanidinoacetate methyltransferase deficiency." Molecular Genetics and Metabolism 134.1-2 (2021): 60-64.
- Wojcik, Matthew et al. "Method modification to reduce false positives for newborn screening of guanidinoacetate methyltransferase deficiency." Molecular genetics and metabolism vol. 135, 3 (2022): 186-192.

Contact for New York Program GAMT NBS: Tara Whispell (Tara.Whispell@health.ny.gov)

Utah

Utah began screening for GAMT deficiency in 2015 using a two-tiered approach. Screening initially utilized a derivatized MS/MS method (2015 to mid-2019), but this was later changed to a non-derivatized method (mid-2019 to present). Mean GUAC and CRE levels were similar with the two methods. Utah requires two screens (one collected around 24–48 hours and one collected between seven and 16 days) and has not seen any significant concentration differences between the two screens for GUAC, however, CRE concentration does appear to decrease by almost 50% in specimens collected >seven days of age in both methods.

Utah's full method details can be found in the manuscripts below:

- Hart, Kim, et al. "Prospective identification by neonatal screening of patients with guan-idinoacetate methyltrans-ferase deficiency." Molecular Genetics and Metabolism 134.1-2 (2021): 60-64.
- Pasquali, Marzia, et al. "Feasibility of newborn screening for guanidinoacetate methyl-transferase (GAMT) defi-ciency." Journal of inherited metabolic disease 37 (2014): 231-236.

Contact for Utah Program GAMT NBS: Kim Hart (kimhart@utah.gov)

Other Countries

Other countries have explored and/or implemented newborn screening for GAMT deficiency. Their methodologies have also been published and can be found in the manuscripts below:

- **British Columbia, Canada:**
  Sinclair, Graham B et al. "A three-tier algorithm for guanidinoacetate methyltransferase (GAMT) deficiency new-born screening." Molecular genetics and metabolism vol. 118, 3 (2016): 173-177.
- **Victoria, Australia:**
  Pitt, James J et al. "Newborn screening for guanidinoacetate methyltransferase deficiency." Molecular genetics and metabolism vol. 111, 3 (2014): 303-304

Molecular Sequencing

For the purpose of NBS for GAMT deficiency, molecular sequencing may be used largely for additional information for the treating clinicians and families.

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 sequencing as part of their algorithm 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.

In some programs, the *GAMT* gene is sequenced in infants who screen positive for GAMT deficiency. DNA is extracted from one 3-mm dried blood spot punch. The six coding exons are PCR amplified using primers described by Sinclair with modifications and Sanger sequenced.[19] Infants positive for GAMT deficiency are referred for follow-up regardless of molecular results.

19 Sinclair GB, van Karnebeek CDM, Ester M, Boyd F, Nelson T, Stockler-Ipsiroglu S, Vallance H. A three-tier algorithm for guanidinoacetate methyltransferase (GAMT) deficiency newborn screening. Mol Genet Metab. 2016 Jul; 118(3):173-177. doi: 10.1016/j.ymgme.2016.05.002. Epub 2016 May 7. PMID: 27233226

Follow-Up Readiness

Follow-up is essential to the NBS process and is 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 GAMT deficiency, 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 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 GAMT deficiency 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.

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.

KEY COMPONENTS OF READINESS

Key components of follow-up readiness for GAMT screening include:

- **Integration of GAMT follow-up workflow with other follow-up workflows.**
- **Identification and communication with medical specialists and/or treatment centers for infants with actionable GAMT NBS results.**
- **Development of action plan templates and fact sheets for PCPs 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 GAMT 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).**

Information Technology 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 able to query 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 partner 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 the 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 GAMT DEFICIENCY

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 materials. 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 Association for Creatine Deficiencies. When a state is implementing a new disorder, it is beneficial to work with the agency's communications group to develop a press release announcing the new disorder and benefits of screening. NBS programs may even consider working with partners to develop a news story highlighting the implementation.

With GAMT deficiency, older educational materials sometimes show patients that are significantly impacted by GAMT deficiency 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 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 GAMT deficiency so they may validate their testing methodology to obtain a certain result.

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 GAMT deficiency during this time so that these babies and their families can benefit from the pilot.

Some states will use an "opt-in" process—parents have to agree to the screening for GAMT deficiency—until the disorder is added to the state NBS panel and GAMT deficiency 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 GAMT deficiency 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.

CONCLUSION

This resource provides an overview of the many aspects involved in the addition of a new disorder to a state NBS panel, with specific focus on screening for GAMT deficiency. Please direct any questions regarding implementation or technical assistance needs to NewSTEPs at newsteps@aphl.org.

Learn more about GAMT deficiency on HRSA's website:
newbornscreening.hrsa.gov/conditions/guanidinoacetate-methyltransferase-deficiency

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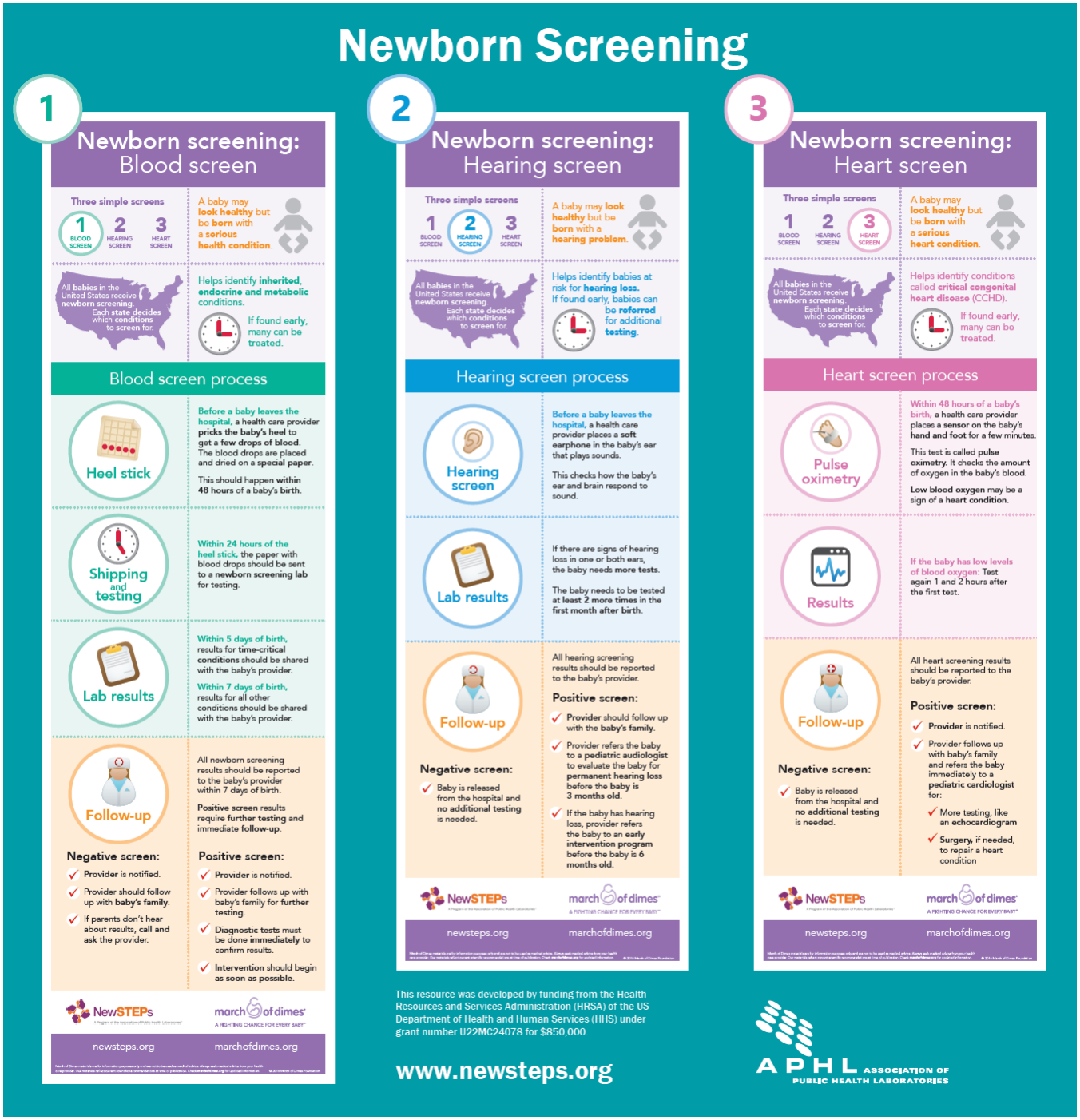
APPENDIX

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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