Krabbe Disease

Newborn Screening Implementation Resources and Tools

MAY 2025

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 United States and to assist states with quality improvement initiatives. NewSTEPs is a comprehensive resource center for state NBS programs and partners.

How to Use This Resource

The NewSTEPs New Disorders Subcommittee developed this tool to aid state and territorial NBS programs in communication and education of key partners during the implementation of screening for Krabbe disease. NBS programs routinely consider the expansion of their state and territorial panels, a process that can be lengthy and complex. The intended audiences for this tool—as with previous tools developed by NewSTEPs—are 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 NBS in the US.

Mission

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

WHAT IS NEWBORN SCREENING?

Newborn screening (NBS)— recognized as one of the largest and most successful morbidity and mortality 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.*

Key points of NBS:

- **NBS is comprised of three different parts:** dried blood spot (DBS) screening, hearing screening and critical congenital heart disease screening† (see **Appendix**). This resource focuses on newborn DBS screening for identifying infantile Krabbe disease (IKD).
- **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. IKD is a time-critical disorder.‡
- **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).§
    - A state-by-state list of disorders,¶ updated in real time, is maintained by the Newborn Screening Technical assistance and Evaluation Program (NewSTEPs)** of the Association of Public Health Laboratories (APHL).††
    - 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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* APHL Newborn Screening & Genetics Program

† APHL, NewSTEPs Newborn Screening Educational Resource

‡ APHL, NewSTEPs Time Critical Conditions

§ HRSA, Advisory Committee on Heritable Disorders in Newborns and Children Recommended Uniform Screening Panel

¶ APHL Screened Conditions Report

** NewSTEPs website: www.newsteps.org

†† APHL website: www.aphl.org

WHAT IS INFANTILE KRABBE DISEASE AND WHY WAS IT CONSIDERED FOR NBS?

Krabbe disease is a lysosomal disorder (LD) caused by pathogenic variants in the galactosylceramidase (*GALC*) gene, also called galactocerebrosidase. The *GALC* gene encodes an enzyme (called galactosylceramidase or GALC) that breaks down galactosylceramide into galactose and ceramide. The GALC enzyme also breaks down galactosylsphingosine, otherwise known as psychosine. A deficiency of the GALC enzyme results in the accumulation of psychosine. Psychosine causes apoptosis of oligodendrocytes and Schwann cells, which are responsible for maintaining myelin in the central and peripheral nervous systems, respectively. The loss of these cells affects the myelination and re-myelination processes, affecting the nervous system of the individual.

There is a continuum of severity of Krabbe disease that differ in signs and symptoms and age of onset: IKD and later-onset forms of Krabbe disease (LOKD). Only IKD has been added to the RUSP. Babies born with IKD, the most severe form of Krabbe disease, develop normally for the first few months, then somewhere between two and 12 months will develop symptoms of disease. Initial signs and symptoms of IKD include extreme irritability, feeding difficulties, failure to thrive and spasticity. Affected babies rarely reach milestones beyond some head control and smiling, and they lose these abilities rapidly as the disease progresses.

Untreated children with IKD rarely survive beyond two years of age and treatment is only effective if provided before the onset of symptoms. Thus, NBS is needed to detect these affected babies in time for presymptomatic treatment.* Since neurological damage is occurring quickly in IKD and treatment is needed within 30 days, it is important to consider IKD a time-critical disease so that diagnosis and the complex treatment process can occur quickly. Treatment has been shown to be most effective when administered before 30 days of life (see **"Treatment for Krabbe Disease" on page 6**), making timely reporting of screen-positive results pivotal.

Understanding the above, IKD was added to the Federal RUSP. IKD is defined through NBS as having decreased GALC enzyme activity and a psychosine level of 10 nM or greater. Because of ongoing uncertainty around benefits of screening for LOKD, these forms of Krabbe disease (as defined by a psychosine level between 2 and 10 nM) was not added the RUSP at this time.

Genetics and Inheritance of Krabbe Disease

Krabbe disease is inherited when each parent passes down a nonworking *GALC* gene to their offspring. Only individuals with two non-working *GALC* genes–one from the biologic mother and one from the biologic father–will have Krabbe disease (**Figure 1**). Carriers of Krabbe disease do not have nor do they develop the disease. If two parents are carriers of a non-working copy of the *GALC* gene, they have a one in four or 25% chance in each pregnancy of having a child with Krabbe disease. There are different genetic changes or variants that result in a non-working copy of the *GALC* gene. The most common type of pathogenic variant in Krabbe disease and of Northern European ancestry is a 30 kb deletion within the *GALC* gene. This 30kb deletion accounts for 40-45% of IKD variants in this population.†

Some level of genotype-phenotype relationships exists in patients with Krabbe disease with the 30 kb deletion, nonsense variants, and frameshift variants most commonly being associated with IKD. There are also variants that are known to be associated with a later-onset phenotype, including p.Gly286Asp, p.Leu634Ser, p.Pro318Arg and p.Tyr319Cys.‡

Many of the LDs, including Krabbe disease, have variants that cause an individual to have a low enzyme level, but no clinical symptoms or signs of the disease (normal psychosine levels). This situation is often referred to as pseudodeficiency. Individuals with pseudodeficiency have 5–15% enzyme activity compared to the normal population, which is

* Kwon, et al. (2018). Consensus guidelines for newborn screening, diagnosis and treatment of infantile Krabbe disease.

† Jain & De Jesus (2023). Krabbe Disease.

‡ Orsini, et al. (2018). Krabbe Disease.

sufficient to metabolize the substrates and prevent their accumulation and explains their asymptomatic health status. Prior to newborn screening for Krabbe disease, there were variants known to lower GALC enzyme activity, the most common are p.Arg184Cys, p.Asp248Cys and p.Ile562Thr. These variants lower GALC enzyme activity in DBS testing and are often associated with false-positive screen results. However, the diagnostic laboratories testing GALC enzyme activity in leukocytes will measure low activities, but not low enough to cause Krabbe disease.

Figure 1. Autosomal Recessive Inheritance Pattern

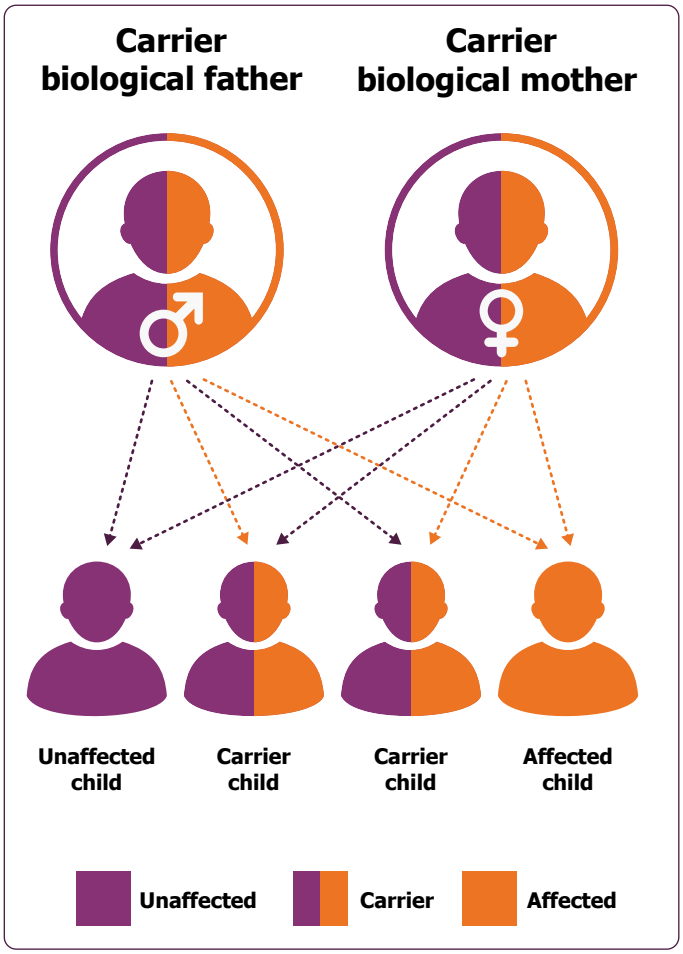


Diagram modified from NxGen MDx. Accessed January 3, 2022 from: nxgenmdx.com/genetic-screening/

Diagnosis and Clinical Manifestations of Krabbe Disease

Krabbe disease comprises a spectrum ranging from IKD (i.e., onset of extreme irritability, spasticity and developmental delay before age 12 months) to LOKD (i.e., onset of manifestations after age 12 months and as late as the seventh decade). Prior to NBS, 85%-90% of symptomatic individuals with Krabbe disease diagnosed by enzyme activity alone have IKD and 10%-15% have LOKD.*

IKD is characterized by normal development in the first few months followed by rapid severe neurologic deterioration; the average age of death is 24 months (range eight months to nine years). Patients with Late-infantile Krabbe disease (LIKD) present between 12–36 months old with a slightly milder phenotype than IKD; despite this, LIKD is relentless and fatal at a median age of seven years old. Juvenile and adult onset Krabbe disease are much more variable in their presentation and disease course. Clinical findings of IKD within the first year of life include, but are not limited to, excessive crying to extreme irritability, feeding difficulties with gastroesophageal reflux disease, spasticity of lower extremities and fisting. Later symptoms (>12 months) include slow development of motor milestones to loss of milestones, slurred speech, spasticity of extremities, truncal hypotonia and vision loss.

Treatment for Krabbe Disease

As of 2025, hematopoietic stem cell transplantation (HSCT) remains the gold standard for treatment of Krabbe disease. Treatment of newborns determined to have IKD should be administered as soon as possible, ideally within 30 days of life. This 30-day timeline must include the collection transport and screening of the DBS specimen to determine the risk of the disease, the referral of the family to specialists, confirmation of the disease, HSCT matching, an immune conditioning process required before transplant of approximately nine days, and the HSCT. 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.

* OMIM Entry - #245200: Krabbe Disease; KRB.

THE NEWBORN SCREENING PROCESS

Screening vs. Diagnostic Tests

NBS allows for population-based screening of all newborns in a timely and affordable manner. Currently, all states screen for numerous disorders in which timely diagnosis and management improves overall outcome. NBS programs establish screening strategies and result decision algorithms with the goal of identifying all newborns with a specific disorder without burdening the system with 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**).*

Figure 2. Newborn Screening Process

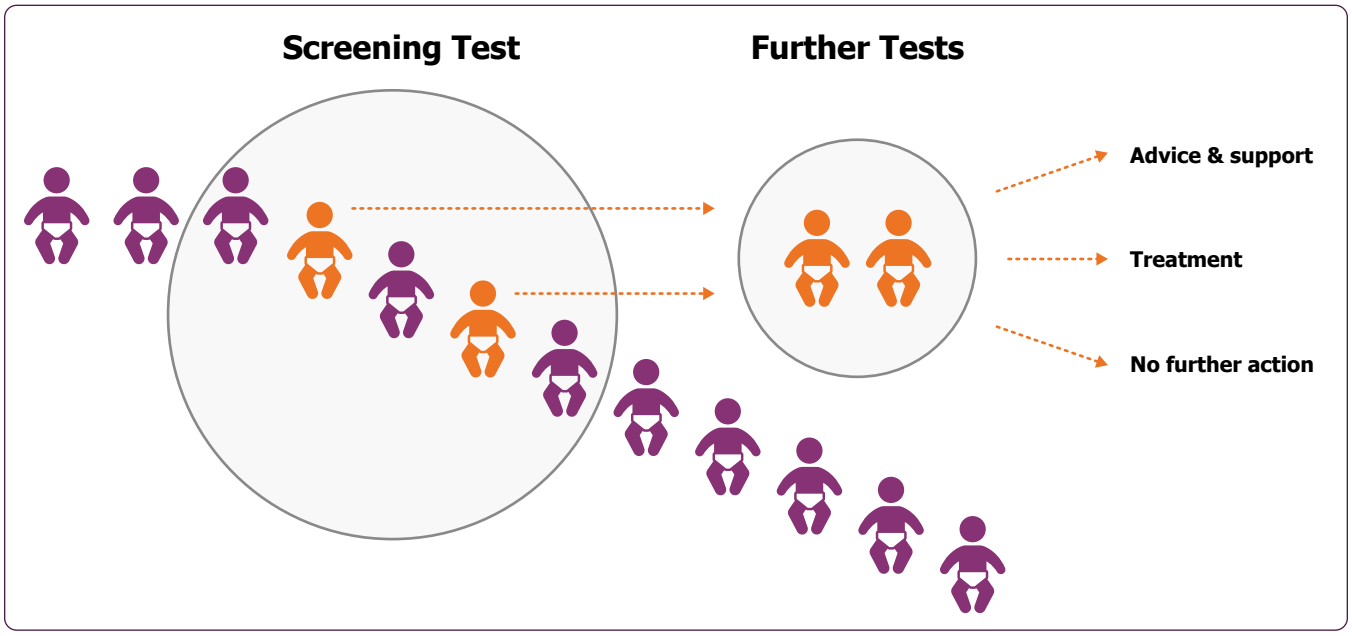


Table 2. Screen vs. Diagnostic Test

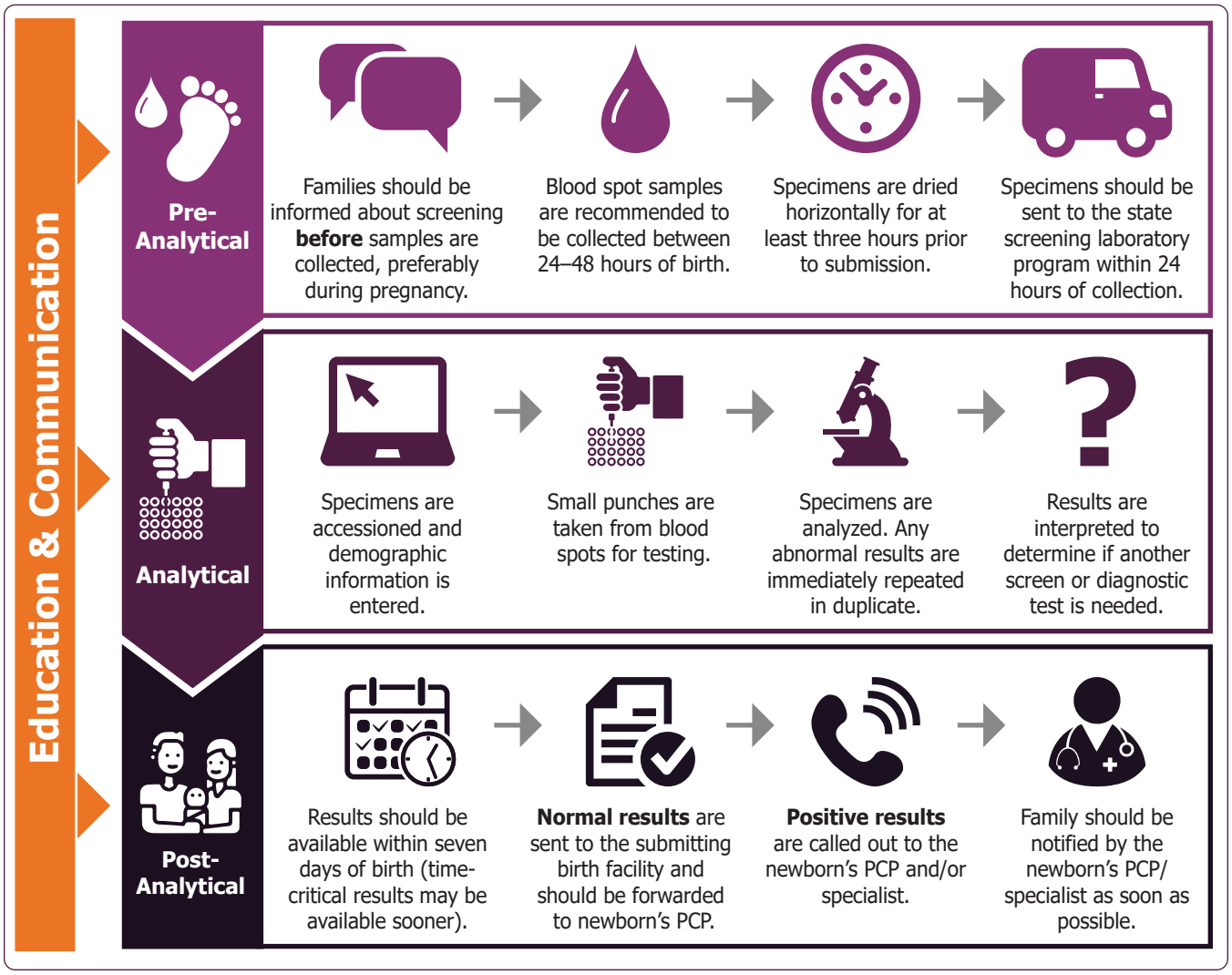
	Screen	Diagnostic Test(s)
Population (offered the test)	Those **without clear signs or symptoms** of disorder where early detection is essential.	• Those **with symptoms**. • Those undergoing further work-up **after a positive screen**.
Results	• Result is an **estimate of level of risk**. • Determines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

* 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 DBS 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 may determine its own testing algorithm and follow-up processes, often with input and guidance from the peer-reviewed literature, 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. Prior to initiating NBS for IKD, at least one transplant center should be identified that would accept screen positive newborns at any time for confirmation and treatment if indicated.

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/ Multiple LD Enzymes with Low Activity	• The specimen was deemed invalid for accurate screening. • Results cannot be accurately interpreted. • Repeat NBS is needed.
Abnormal/Positive/ Normal Psychosine (Under 2.0 nM)*	Low enzyme activity, negative screening result.
Abnormal/Positive/ Intermediate Psychosine (2.0 nM to under 10 nM)*	At risk for later onset disease, if late onset Krabbe disease is also a target of the screening program, the baby should be referred for follow-up diagnostic testing. Note: This designation may only be used by programs that choose to deviate from the RUSP and also call out results suggestive of LOKD.
Abnormal/Positive/ Elevated Psychosine (10 nM or greater)	• Presumptive positive • The newborn is at high-risk for having the disorder. • An emergent clinical evaluation is needed, preferably at the designated transplant center. • Notify transplant team of likely IKD newborn that will need timely 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 considered diagnostic; it will flag some infants who do not have the disorder with a false-positive result, and, on rare occasions, may be unable to detect truly affected infants, thus presenting a false-negative result (Figure 4). When implementing a new disorder, it is helpful for NBS programs and key partners to define goals, including metrics to measure successes and shortcomings. The goals should be clearly stated on informational brochures and public-facing websites. These metrics can define timeliness of screening, reporting, referral and initiation of treatments. Following implementation, evaluation and continuous quality improvement efforts should be outlined.

Following implementation of NBS for Krabbe disease, it is important that the NBS system partners continue to meet regularly to review metrics and evaluate both the successes and shortcomings of NBS.

Continuous quality improvement is an essential component of a NBS program.

* Exact reference ranges may differ by reference laboratory and programs should determine reporting and follow-up processes accordingly.

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 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.

Second-tier psychosine is strongly recommended in the screening process (see RUSP), as virtually all false positives are completely eliminated, and as such, are not reported as presumptive positive for Krabbe disease and will not need further testing.

False Negatives

Infants affected with the targeted (form of 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

Recommendations include time from:

- Birth to specimen collection: **24–48 hours**, or before a red blood cell transfusion
- Specimen collection to receipt by NBS program: As soon as possible; ideally within **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**
- Birth to notification and reporting of Krabbe disease (time critical condition): **Immediately after psychosine is tested and reported with elevated levels above 10**

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.

Figure 4. **NBS Test Results ***

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

Collaborators

There are many collaborators 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 professionals (PCPs)
- Clinical specialists
- Genetic counselors
- Transplant team
- NBS laboratory and follow-up
- Policy makers
- Researchers

* Adapted from Carvajal, Diana & Rowe (2010). Sensitivity, specificity, predictive values, and likelihood ratios.

Fiscal Constraints

The key factors to NBS are readiness to screen and feasibility of adding the screen to the screening program.* 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 Krabbe disease, 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. Using the tiered screening mechanism above should also result in few positive results, so an additional follow-up staff member may also not be necessary; though staffing needs to be assessed at the level of each individual program.

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.

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. Can the new disorder be multiplexed with current equipment?**
- **Physical capacity of laboratory. How much additional lab space is required?**
- **Testing materials and reagents needed to screen. FDA kit versus LDT?**
- **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.**

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 (see **NBS Cost Considerations** box).
- Completing pilot testing (if necessary) and finalizing screening cutoffs and decision algorithms.
- Education of partners regarding Krabbe disease, the plan for screening and available treatment options within the state.
- Setting up testing or contracting out second-tier psychosine testing.
- Develop an emergency response plan to ensure timely referral of likely IKD patients to other clinical specialists and transplant centers. Note that there are only a select number of centers in the US capable of transplanting children with Krabbe disease. Plans and evolving requirements associated with the need for out-of-state travel and care should be considered prior to starting universal screening in order to ensure identified families can receive treatment within the recommended timeline of 30 days.

* APHL (2023). NewSTEPs 2022 Annual Report.

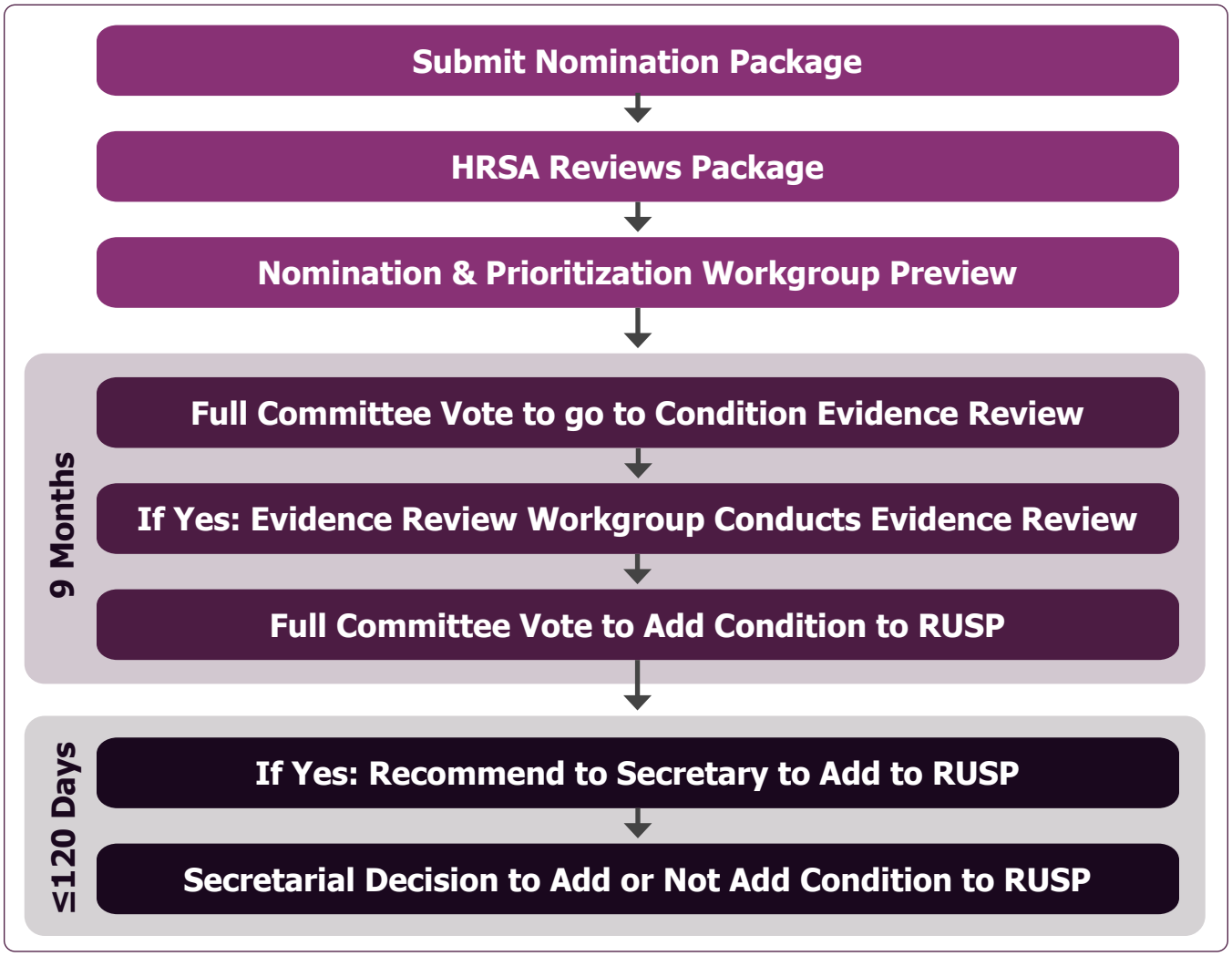
GETTING READY TO SCREEN FOR A NEW DISORDER

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.*

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**



* Main Report (2006) Genetics in Medicine

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.

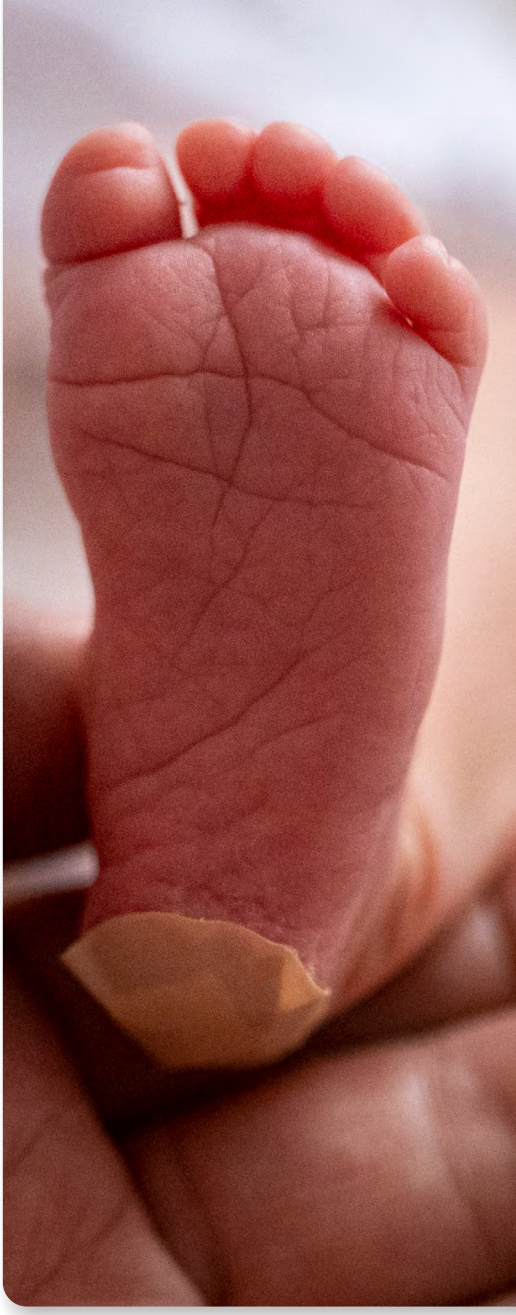
Most state NBS programs conduct implementation pilots to build and/or assess the state capacity to screen for the disorder and to validate testing methodology, evaluate follow-up processes and ensure all NBS system components are operating as designed.

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), 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/territories may also seek guidance and assistance from their peers and subject matter experts. For example, APHL offers subject matter expert support for specific projects related to screening implementation, laboratory and follow-up practices, educational activities, continuity of operations planning (COOP), information technology systems, workforce development, program administration, policy support and quality improvement activities.



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 Krabbe Disease

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.

NBS laboratories need to consider the following well in advance of routine screening for Krabbe disease:

Readiness Steps for NBS Laboratory Screening

- **Identify which screening method to use, including higher-tier testing methods.** For Krabbe disease, programs need to consider both GALC ensyme activity and second-tier psychosine, as only IKD was added to the RUSP. There may be instances where programs choose to vary from the strategy outlined in the RUSP, which recommends screening for "Infantile Krabbe Disease (characterized by low [GALC] galactocerebrosidase levels and psychosine ≥ 10nM)."
- **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.
- **Setup contract with laboratory performing second-tier psychosine testing if test will not be performed in house.**
- **Ensure testing cutoffs and decision schemes meet specificity/sensitivity and other performance targets** to meet the stated goals of the NBS program. For Krabbe disease, this will likely include at least a second-tier test.
- **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 Krabbe disease testing workflow** with all other NBS workflows.
- **Establish communication algorithm** with short-term follow-up program (phone, IT, messaging).

Considerations for Testing Methodology and Higher-tiered Testing

- 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 contract-out for tiered testing? Has this contract been established?
- 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?

* For NBS programs already screening for other LSs by MS/MS, only the addition of in-house second-tier testing may require additional equipment and space.

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 Examples

Bench Fluorometric Assay (Missouri's Laboratory Developed Test Method)

- Punch 3.2mm samples from the NBS specimen cards into microtiter plates leaving the first well of each row empty (A1–H1)
- Extract samples (100 µL of extraction solution – 30 minutes)
- Add 10 µL of GALC substrate to each well of a new plate
- Transfer 10 µL of sample extract to the new plate with GALC substrate
- Seal plates and incubate at 37°C (17 hours)
- The next morning at the 17 hour point, add stop buffer (50 µL /well)
- Add calibrants (70 µL in wells A1–H1)
- Read plates in fluorometer (BioTek Synergy HTX)

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 GALC enzyme activity in the DBS, indicating babies that may be at risk for Krabbe disease.

The biggest advantage of this assay is its speed as it takes less than 24 hours to obtain results. Results are available on the next calendar day in the morning from when the testing was started. The instruments are easy to use and robust.

The biggest disadvantage of this assay is that it requires its own workflow as it is not multiplexed with other screening markers.

Tandem Mass Spectrometry Assay (NeoLSD FDA-cleared Kit)

Day 1

1. Prepare Incubation Cocktail as described. Preheat the incubator shaker unit to 37°C.
2. Punch out controls and 3.2mm samples into the wells of a U-bottom microplate. At the beginning, each plate should contain two blank wells that have no punch (A1 and A2). Place singlicates of the DBS controls C1, C2 and C3 after the blank wells and singlicates of the DBS controls at the end of the plate after the patient samples. Check that all wells except the 2 blanks have a filter paper disk.
3. Pipette 30 µL of Incubation Cocktail into each well that is to be tested, including the two blank wells.
4. Cover the microplate(s) tightly with an adhesive aluminum foil microplate cover. Place the microplate in the pre-heated shaking incubator.
5. Incubate for 18 ± 2 hours in the Incubator Shaker unit at 37°C and 400 RPM.

Day 2

Note: *Perform steps 6-11 in a chemical hood to limit exposure to organic solvent fumes.*

6. Prepare the Quench Solution as described.
7. Remove the plate(s) from the incubator and record the time the incubation was stopped. Remove the adhesive aluminum foil cover carefully avoiding spillover.
8. Quench the reaction by adding 100 µL of the Quench Solution to each assay well. Mix the contents of each well by pipetting up and down 10 times.

   Note: *Turn off the Incubator Shaker unit, as it will need to cool to ambient temperature for an upcoming step.*

   Note: *The quench step should be performed within ½ hour after removing the plate from the Incubator Shaker.*

9. Transfer all of the liquid from each well into the corresponding well of a deep-well plate, leaving the DBS punch behind. Note: Ensure that the liquid is deposited at the bottom of each well of the deep-well plate.
10. Add 400 µL of the NeoLSD Extraction Solution to each well.
11. Add 200 µL of water (CLRW, CLSI) to each well. Mix the contents of each well by pipetting up and down 20 times. Cover each deep-well plate with an adhesive aluminum foil cover.
12. Centrifuge the covered deep-well plate(s) for 5 minutes at 700 x g, ±10%.

Note: *Perform steps 13-15 in a chemical hood to limit exposure to organic solvent fumes.*

13. The contents of the plate wells will have separated into an upper organic layer and a lower aqueous layer. Transfer 50 µL of the organic top layer into the corresponding wells of a U-bottom microplate. Be careful not to touch the aqueous layer.
14. Dry the contents of the plate(s) on the microplate evaporator using streams of clean, dry air or nitrogen at 40°C, about 5 minutes until all wells are completely dry.
15. Add 100 µL of Flow Solvent to each well. Cover each plate with an adhesive microplate cover.
16. Load plate(s) into the Incubator Shaker unit; shake at ambient temperature and 400 RPM for 10 minutes.
17. Remove the plate(s) from the Incubator Shaker unit and load them onto the MS/MS screening system, keeping the adhesive cover(s) in place. Run the MS/MS analysis method. The MS/MS analysis must be done within 24 hours after shaking (Step 16).

The biggest advantage of this assay is that Krabbe disease can be multiplexed from the same DBS punch with other LDs that some laboratories may already be screening for, such as Pompe and MPS I. However, due to the inherent kinetics of the GALC enzyme, longer incubation times (i.e., 18 hours vs. three hours) will be necessary when screening for Krabbe disease rather than the aforementioned LDs. This situation can, therefore, affect workflow and overall turnaround times.

Second-Tier Psychosine Testing on the DBS

Sample: While second-tier testing generally provides advantages by improving specificity of the newborn screen and reducing false-positive call-outs, in the case of Krabbe disease it is virtually essential that psychosine testing be performed on the DBS before calling out screening results. The ability to reflex specimens that screen positive for Krabbe with low GALC enzyme results to second-tier psychosine testing has been monumental to the NBS process to screen for Krabbe disease. Babies that are truly affected or at risk for Krabbe disease will have an elevated (≥10 nM) psychosine level, revealing the buildup of this product in their system due to Krabbe disease. If the psychosine concentration is ≥10 nM, the baby should emergently be referred to a follow-up center that can fast track the baby to a trans- plant center that can handle an IKD referral. Additionally, now that it is available on the DBS, psychosine measurements can be used to eliminate false-positive GALC screens and, thereby, greatly reduce the harm of needlessly

alarming families in the screening process. The vast majority of positive GALC screens are due to the detection of carriers, pseudodeficiencies, normal outliers for low GALC and compromised DBS samples. These false-positive results can cause much undue harm to parents that would have to be referred to specialists and very quickly go through the scary diagnostic process to rule out Krabbe disease. Second-tier psychosine testing on the DBS sample can be done in-house, but it requires a tandem mass spectrometer with greater sensitivity than those usually employed by state NBS laboratories. Alternatively, a part of the initial DBS specimen can be sent out by state programs to qualified reference laboratories (e.g., the Mayo Biochemical Genetics Laboratory, Greenwood Genetic Center, Revvity) for testing with a two-day turnaround time.

Follow-up Readiness

Follow-up is essential to the NBS process and is therefore vital for successful implementation of a new disorder. NBS follow-up can include communication of screen-positive results to PCPs and families, coordination of confirmatory testing, and connecting identified babies to appropriate specialists and/or treatment centers. For IKD, 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 PCPs and families—and to ensure the baby is rapidly referred to a transplant center to increase the chance that the baby is transplanted as close to 30 days of age as possible. Because there are only a few centers in the US with the experience and capability to transplant young children with Krabbe disease, follow-up staff should prepare for the potential need for a family to quickly travel out-of-state for their treatment and care in order to meet the recommended timelines for intervention.

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 may need added support in accepting the potential of a very serious disorder in their seemingly healthy newborn.

Key Components of Readiness

Key components of follow-up readiness for Krabbe disease include:

- **Integration of Krabbe disease follow-up workflow with other follow-up workflows.**
- **Identification and communication with medical specialists and/or treatment centers for infants with actionable Krabbe disease 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 Krabbe disease 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).**

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.

Follow-up Resources

- **ACT Sheets: IKD and LOKD**
- **Algorithm**
- **Knowledge Nugget**

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 or is at risk for LOKD. 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.

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 able to query for continued evaluation of implementation and quality improvement efforts. Additionally, there may be a need to order second-tier testing and/or interface with reference laboratories to obtain and integrate these high-tiered testing results. 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.

Key Components of IT Readiness

Key components of IT readiness for Krabbe disease 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.**
- **Review the NewSTEPs New Disorder LIMS Implementation Checklist for more information.**

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))?**

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

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. If necessary, consider including colleagues and specialists from other programs and states. 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?**
- **Set up partnerships with transplant centers.**
- **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 KRABBE DISEASE

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 materials 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, though organizations such as Expecting Health, Hunter's Hope, KrabbeConnect and the American College of Medical Genetics and Genomics that develop materials for national use. Hunter's Hope supports a Krabbe Council, which is a group chaired by experts in Krabbe disease, genetics, follow-up, difficult case reviews and transplant.

When a state is in the process of 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 Krabbe disease, older educational materials sometimes show patients that are significantly impacted by Krabbe disease 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 DBS specimens from newborns known to have Krabbe disease 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—until the disorder is added to the state NBS panel and Krabbe disease 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 Krabbe disease 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 Krabbe disease during this time so that these babies and their families can benefit from the pilot.

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 Krabbe disease. Please direct any questions regarding implementation or technical assistance needs to NewSTEPs at newsteps@aphl.org.

Learn more about Krabbe disease on HRSA's website: newbornscreening.hrsa.gov/conditions/krabbe-disease

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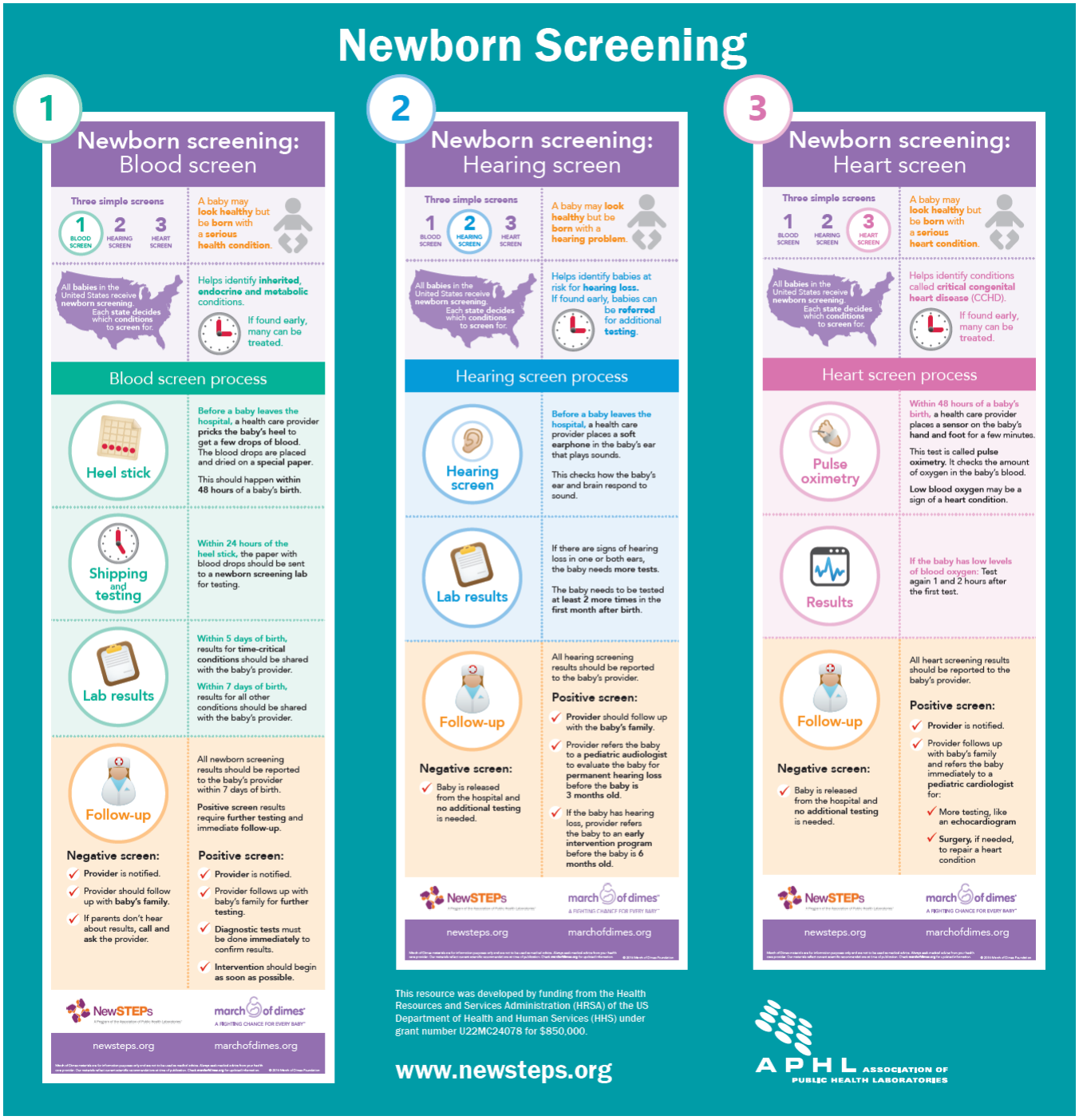
APPENDIX: THREE PARTS OF NBS

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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