1st Edition

NBS07

Newborn Blood Spot Screening for Pompe Disease by Lysosomal Acid α-Glucosidase Activity Assays

This report discusses the detection of Pompe disease (PD) by population-based newborn screening using dried blood spot specimens to measure acid α-glucosidase enzyme activity. Classic infantile-onset PD is a lethal disorder that is not evident at birth, and therapy effectiveness is improved by presymptomatic detection.

A CLSI report for global application.

Newborn Blood Spot Screening for Pompe Disease by Lysosomal Acid α-Glucosidase Activity Assays

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Abstract

Clinical and Laboratory Standards Institute report NBS07—*Newborn Blood Spot Screening for Pompe Disease by Lysosomal Acid α-Glucosidase Activity Assays* describes the currently available laboratory tests used to measure acid α-glucosidase (GAA) enzyme activity in dried blood spot (DBS) specimens. Pompe disease (PD) is a congenital clinical disorder not evident at birth that is due to GAA deficiency. Early detection is critical, because without treatment, most babies with classic infantile-onset PD will die from cardiorespiratory failure. A laboratory operations overview detailing the physical layout, instrumentation, assay protocols, automated methodologies, and the potential for future expansion is included. Steps for implementing PD newborn DBS screening, including validating the laboratory test, conducting pilot studies, and transitioning to routine screening, are discussed.

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Suggested Citation

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

Reaffirmed:
June 2022

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Foreword

In 2001, it was demonstrated that enzyme function assays using fluorescent substrates could be applied to dried blood spot (DBS) specimens to identify mucopolysaccharidosis type I (Hurler syndrome) and Fabry disease,[1] opening the door to newborn DBS screening for lysosomal storage disorders (LSDs). Since then, multiple approaches to newborn DBS screening for LSDs have been reported, each with varied measurand panels and methodologies. Newborn screening (NBS) for Pompe disease (PD) was first initiated using a fluorescence assay in 2005.[2]

In 2004, a tandem mass spectrometry (MS/MS) method that enabled multiplexed screening for up to five LSDs (PD, Krabbe disease, Gaucher disease, Fabry disease, and Niemann-Pick disease type A/B) was published.[3] By that time, MS/MS methods for detecting biomarkers had been widely implemented by public health laboratories to expand NBS for identifying amino acidemias, organic acidemias, and fatty acid oxidation disorders. Unlike the MS/MS assays used to detect acylcarnitines and amino acids, compounds already present in the blood, the MS/MS and fluorescence methods used to screen for LSDs detect enzymatic products that are produced from *in vitro* reactions of lysosomal enzymes extracted from DBS specimens. However, different synthetic substrates need to be added for each enzyme in the test panel. Many approaches have been published to simplify MS/MS assays for multiple LSDs, such as introducing online sample clean-up and combining the enzyme reactions so less DBS sample and fewer individual reaction conditions are needed to perform MS/MS screening.

A microfluidic fluorometry platform for NBS has also been introduced to identify newborns with enzyme deficiencies for PD and other LSDs.[4]

This report provides guidance on assay platform considerations for PD newborn DBS screening. Technology selection may be complicated by regulatory considerations, reagent availability, and other LSDs that may be candidates for screening. On a practical level, assay platform choice depends on factors such as funding, internal capabilities and expertise, differences in conditions included or added to NBS programs, and current and future test methods. Once a decision has been made, this report provides the user with the information essential for implementing PD newborn DBS screening.

NOTE: The content of this report is supported by the CLSI consensus process, and does not necessarily reflect the views of any single individual or organization.

Key Words

Acid α-glucosidase, dried blood spots, fluorometry, lysosomal storage disorders, newborn blood spot screening, Pompe disease, tandem mass spectrometry

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Chapter 1:   Introduction

This chapter includes:

- Report's scope and applicable exclusions

- Background information pertinent to the report's content

- Standard precautions information

- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions

- Terms and definitions used in the report

- Abbreviations and acronyms used in the report

1.1   Scope

This report discusses the detection of Pompe disease (PD), also known as glycogen storage disease type II, by population-based newborn dried blood spot (DBS) screening, and focuses on enzyme activity assays for detecting PD. It is intended to provide information for incorporating PD newborn DBS screening into the routine operations of existing newborn screening (NBS) programs.

This report includes background information on the biological and clinical features of PD, a lysosomal storage disorder (LSD). It provides descriptions of the different enzyme activity assays for PD and discusses preanalytical, analytical, and postanalytical issues for laboratory practices (see Subchapter 1.4.1). Also, the report includes a discussion of short-term and long-term follow-up (LTFU) procedures, including case tracking, as well as the diagnostic tests needed to confirm a PD diagnosis. It contains limited discussion of LSDs other than PD for which DBS-based enzyme activity assays exist, including Gaucher disease, Fabry disease, Krabbe disease, and Niemann-Pick disease type A/B.

The intended users of this report are NBS laboratory personnel, public health program personnel, follow-up programs, health care professionals, those involved with oversight of NBS testing, and manufacturers of NBS instruments, reagents, and related products.

This report does not cover:

- DBS specimen collection for PD newborn DBS screening (see CLSI document NBS01[5])
- Enzyme activity assays for newborn DBS screening of LSDs other than PD
- Immunoreactive assays for LSD detection,[6] because the reagents are not generally available
- Method performance comparisons of assays currently used for PD newborn DBS screening

1.2   Background

In recent years, there have been significant advances in both the laboratory detection and clinical treatment of LSDs, primarily PD. Treatment of this disorder has benefitted from the development of recombinant acid α-glucosidase (GAA) as replacement therapy for the genetically altered GAA that is deficient in individuals with PD. Clinical studies have confirmed the beneficial effects of recombinant α-glucosidase therapy for both infants with PD and older affected persons presenting with progressive muscle weakness. The treatment of 16 patients diagnosed clinically and placed on enzyme replacement therapy (ERT) within the first six months of life has been reported.[7] Historically, based on natural history, this patient group would be expected to succumb by 2 years of age to cardiac failure or pulmonary insufficiency. However, after three years of therapy, ERT reduced the risk of ventilation or death by 87% and reduced overall mortality by 95% when compared to an untreated historical control group. In addition, cardiomyopathy improved over time, and the majority of patients learned and sustained substantial motor skills.[7] This successful introduction of ERT has stimulated the development of laboratory technology for early detection of individuals at risk for PD. This need for detection before onset of significant clinical symptoms led to consideration of the use of NBS for early recognition.

The first large-scale PD newborn DBS screening program was undertaken in Taiwan. Beginning in 2005, it was demonstrated that a fluorometric assay can be used to identify newborns at risk for developing PD. It was reported that newborns at high risk for developing infantile-onset Pompe disease (IOPD) could be diagnosed within the first month of life.[2] Previously, these newborns would not be clinically recognized until they presented with cardiac or pulmonary failure at 3 to 6 months of life. The Taiwan program documented that, as of December 2015, all newborns identified and confirmed through NBS to have IOPD and who received recombinant ERT soon after diagnosis did not need ventilator support and were alive three to five years after diagnosis. Five newborns had evidence of cardiomyopathy and, with therapy, showed normalization of cardiac status. Each of the affected newborns showed normal physical growth and age-appropriate gains in motor development.[8]

Beginning in 2013, Missouri was the first state in the United States to test for PD in all newborns in an unblinded study.[4] The incidence of PD in the United States, based on pilot studies in Missouri and Washington state, is estimated to be between 1/5000 to 1/28 000 live births.[4,9] Although the emphasis on NBS for PD is to detect IOPD (ie, children who are clinically symptomatic before 1 year of age), it is estimated that there are three to four times as many patients with late-onset Pompe disease (LOPD) compared with children with IOPD. Patients with LOPD may actually benefit more from early recognition and clinical therapy than those with IOPD.

The evidence accumulated to date for identifying PD through NBS has been reviewed,[10] and PD was recently accepted as a primary target condition on the Recommended Uniform Screening Panel (RUSP).[11] In addition, mucopolysaccharidosis type I (MPS I [Hurler syndrome]) was recently added to the RUSP.

1.3   Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[12] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[13]

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) approach in the development of standards and guidelines, which facilitates project management; defines a document structure using a template; and provides a process to identify needed documents. The QMS approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

NBS07 covers the QSE indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29				X EP12 EP17 H26 NBS01 NBS02 NBS03 NBS04					H26
POCT04		POCT04	POCT04	POCT04	NBS04 POCT04	POCT04 POCT08 QMS24	QMS02			QMS24	QMS24

Related CLSI Reference Materials*

EP12   **User Protocol for Evaluation of Qualitative Test Performance. 2nd ed., 2008.** This document provides a consistent approach for protocol design and data analysis when evaluating qualitative diagnostic tests. Guidance is provided for both precision and method-comparison studies.

EP17   **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. 2nd ed., 2012.** This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates.

H26   **Validation, Verification, and Quality Assurance of Automated Hematology Analyzers. 2nd ed., 2010.** This document provides guidance for the validation, verification, calibration, quality assurance (QA), and quality control (QC) of automated multichannel hematology analyzers for manufacturers, end-user clinical laboratories, accrediting organizations, and regulatory bodies. In addition, end-user clinical laboratories will find guidance for establishment of clinically reportable intervals and for QA for preexamination and examination aspects of their systems.

M29   **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

NBS01   **Blood Collection on Filter Paper for Newborn Screening Programs. 6th ed., 2013.** This document highlights specimen collection methods, discusses acceptable techniques for applying blood drops or aliquots to the filter paper segment of the specimen collection device, and provides instructions on proper specimen handling and transport to ensure quality specimens are consistently obtained for newborn screening analysis.

NBS02   **Newborn Screening Follow-up. 2nd ed., 2013.** This guideline describes the basic principles, scope, and range of follow-up activities within the newborn screening system.

NBS03   **Newborn Screening for Preterm, Low Birth Weight, and Sick Newborns. 1st ed., 2009.** This guideline outlines the recommended protocols for screening preterm, sick, or low birth weight infants for hearing loss and disorders detectable through dried blood spot testing.

NBS04   **Newborn Screening by Tandem Mass Spectrometry. 1st ed., 2010.** This guideline serves as a reference source for the numerous activities related to operating a tandem mass spectrometry laboratory as part of public and private newborn screening programs with the goal of creating greater test accuracy, performance, and consistency among laboratories, thereby ensuring data quality that will ultimately benefit all newborns worldwide.

POCT04   **Essential Tools for Implementation and Management of a Point-of-Care Testing Program. 3rd ed. 2016.** This guideline provides direction to users of *in vitro* diagnostic devices outside the medical laboratory on how to ensure reliable results that are comparable to those obtained from medical laboratory instruments.

POCT08   **Quality Practices in Noninstrumented Point-of-Care Testing: An Instructional Manual and Resources for Health Care Workers. 1st ed., 2010.** This instructional guideline delivers laboratory science concepts and activities with the goal of increasing knowledge and quality of laboratory testing for testing personnel with no laboratory background.

QMS02   **Quality Management System: Development and Management of Laboratory Documents. 6th ed., 2013.** This document provides guidance on the processes needed for document management, including creating, controlling, changing, and retiring a laboratory's policy, process, procedure, and form documents in both paper and electronic environments.

QMS24   **Using Proficiency Testing and Alternative Assessment to Improve Medical Laboratory Quality. 3rd ed., 2016.** This guideline describes an approach for a complete proficiency testing (PT) process and provides assistance to laboratories in using PT as a quality improvement tool.

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