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NBS09

Newborn Screening for X-Linked Adrenoleukodystrophy

Sample

This guideline discusses the detection of X-linked adrenoleukodystrophy by population-based newborn screening using dried blood spot specimens to measure C26:0-lysophosphatidylcholine.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

Newborn Screening for X-Linked Adrenoleukodystrophy

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Abstract

Clinical and Laboratory Standards Institute guideline NBS09—*Newborn Screening for X-Linked Adrenoleukodystrophy* describes the currently available laboratory tests used to measure C26:0-lysophosphatidylcholine in dried blood spot (DBS) specimens. X-linked adrenoleukodystrophy (ALD) is a peroxisomal disorder not evident at birth. ALD is caused by a variant in *ABCD1* resulting in defective ALD protein and impairment of peroxisomal oxidation of very long-chain fatty acids. Early detection is critical, because untreated male children with ALD have a 50% chance of developing adrenal insufficiency before the age of 10 and a 30% to 35% chance of developing cerebral disease, which has occurred as early as 2.75 years of age. This guideline includes a laboratory operations overview, with details about physical layout, instrumentation, protocols, automated methodologies, and potential for future expansion. Steps for implementing ALD newborn DBS screening, including validating the laboratory test, conducting pilot studies, and transitioning to routine screening, are discussed.

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Foreword

In 2006, a preliminary report found, through liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, that C26:0-lysophosphatidylcholine (LPC) was elevated in postnatal venous dried blood spot (DBS) specimens from male newborns with X-linked adrenoleukodystrophy (ALD) compared with normal controls.¹ The custom synthesis of $^2\text{H}_4$ -C26:0-LPC and the other natural very long–chain fatty acid LPCs made validation of this method possible through retrieval and testing of known positive newborn DBS specimens and comparisons with apparently normal newborn screening (NBS) specimens.² A follow-up study of 4689 newborn DBS specimens was completed, with no false-positive screen results observed, thus demonstrating that ALD NBS is feasible.³

In 2012, a negative-ion LC-MS/MS method that improved the original method by reducing key isobaric contaminants was developed.⁴ NBS for ALD was first implemented in New York State in December 2013 using a two-tiered approach. The first-tier test is a multiplexed high-throughput flow injection analysis–tandem mass spectrometry (FIA-MS/MS) method that enables screening for ALD and up to six lysosomal storage disorders (LSDs) simultaneously. Because the FIA-MS/MS method has a high false-positive rate for ALD, a second-tier test using LC-MS/MS, described in 2015,⁵ is used to reduce the false-positive rate.

This guideline provides recommendations regarding ALD newborn DBS screening. Technology selection may be complicated by regulatory considerations, reagent availability, and the other diseases (eg, LSDs) that may be combined in first-tier screening using the high-throughput FIA-MS/MS method. On a practical level, the platform choice depends on factors such as funding, internal capabilities and expertise, differences in diseases included or added to NBS programs' screening panels, and current and future test methods. Once a decision has been made, this guideline provides the user with essential information for implementing ALD newborn DBS screening.

A major challenge to development of this guideline is apparent in the analytical sections describing cutoff value determination (see Subchapters 6.2.3 and 6.2.4). A cutoff value can be difficult to determine primarily because of the long latency period of ALD, combined with the limited number of DBS specimens obtained through NBS from known clinically positive patients with ALD and the relatively recent start of screening for ALD. The long latency period, with many of the newborns detected through screening remaining asymptomatic into early childhood and even adulthood, as well as the additional challenge of detecting newborns with *ABCD1* gene variants of unknown significance, make it difficult for NBS programs to assess performance. More time is needed to fully assess the long-term effectiveness of ALD NBS.

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

KEY WORDS

Adrenoleukodystrophy

C26:0-lysophosphatidylcholine

Dried blood spots

Flow injection analysis

High-throughput tandem mass spectrometry

Negative-ion liquid chromatography–tandem mass spectrometry

Newborn dried blood spot screening

Peroxisomal disorders

Positive-ion liquid chromatography–tandem mass spectrometry

X-linked

Chapter 1

Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- Standard precautions information
- Terminology information, including:
 - Terms and definitions used in the guideline
 - Abbreviations and acronyms used in the guideline

Sample

Newborn Screening for X-Linked Adrenoleukodystrophy

1 Introduction

1.1 Scope

This guideline discusses the detection of X-linked adrenoleukodystrophy (ALD) by population-based newborn dried blood spot (DBS) screening. It focuses on high-throughput flow injection analysis–tandem mass spectrometry (FIA-MS/MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods for detecting C26:0-lysophosphatidylcholine (LPC), the primary biomarker for ALD. This guideline is intended to provide information for incorporating ALD newborn DBS screening into the routine operations of existing newborn screening (NBS) programs.

NBS09 includes background information on the biological and clinical features of ALD, the most common peroxisomal disorder, as well as other disorders of peroxisomal fatty acid oxidation, such as the Zellweger spectrum disorders (ZSDs), that could also be identified by ALD NBS. It describes preanalytical factors that affect ALD screening, including newborn DBS collection timing and specimen storage and stability. In addition to providing details on the different tandem mass spectrometry (MS/MS) analytical methods for C26:0-LPC, this guideline discusses screening strategies, testing algorithms, cutoff value determination, case definition, and risk assessment for NBS programs to consider when implementing X-linked ALD NBS.

The intended users of this guideline are NBS laboratory, follow-up, and program personnel, public health program administrators, diagnostic medical laboratories and ALD treatment centers, health care providers (HCPs) (eg, primary care providers, neonatologists, pediatricians), regulatory agencies, public health policy makers, and manufacturers of instruments, reagents, and related products used for NBS testing.

NBS09 discusses postanalytical short-term follow-up (STFU) and long-term follow-up (LTFU) procedures, including case tracking, as well as the diagnostic tests needed to confirm an ALD diagnosis and special follow-up considerations associated with screening for a disease with a long latency period. It contains limited discussion on diagnosis and follow-up of ZSDs and other disorders of peroxisomal fatty acid oxidation that may also be identified by ALD screening. This guideline does not cover:

- DBS specimen collection for ALD NBS (see CLSI document NBS01⁶)
- Details of confirmatory diagnostic laboratory testing
 - Methods for measuring very long-chain fatty acids (VLCFA) in plasma to confirm positive ALD newborn DBS screening results
 - Methods for *ABCD1* variant analysis to confirm positive ALD newborn DBS screening results
- Guidelines for diagnosis or treatment of ALD

1.2 Background

ALD, the most common peroxisomal disorder, is caused by variants in the *ABCD1* gene,⁷ which maps to Xq28 and encodes the ALD protein, which facilitates the transport of VLCFA into the peroxisome for degradation.⁸ *ABCD1* gene variants result in a toxic accumulation of saturated VLCFA in tissues, including the brain, spinal cord, and adrenal glands. As of August 2021, more than 2900 *ABCD1* variants have been reported in the ALD Mutation Database,⁹ of which 852 are nonrecurrent.¹⁰ There is no genotype-phenotype correlation for cerebral ALD, even within the same family.⁷ However, some isolated variants are associated only with



Abbreviations: ALD, adrenoleukodystrophy; DBS, dried blood spot; LPC, lysophosphatidylcholine; LTFU, long-term follow-up; NBS, newborn screening; STFU, short-term follow-up; VLCFA, very long-chain fatty acid.

^a Three basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), and box (designates process activities).

Figure 1. Process Flow Chart for ALD NBS^a

7 Postanalytical Activities

The following subchapters discuss results reporting and follow-up for ALD screen-positive results, including descriptions of confirmatory testing and clinical evaluations needed for ALD diagnosis.

7.1 Results Reporting

The NBS program should notify HCPs of positive ALD NBS results following the program's individual policies and procedures, keeping in mind that:

- Newborns in an SCBU/NICU are more likely to have a serious peroxisomal biogenesis disorder (eg, ZSD) rather than ALD, which usually manifests later. The NBS program should not delay notification of first-tier and/or second-tier screening results while waiting for *ABCD1* gene sequencing, because reporting the biochemical findings quickly may enable a shortened diagnostic evaluation of a sick newborn.
- Some programs incorporate reporting “borderline” results when the C26:0-LPC concentration is in a borderline range set between normal (ie, in-range) and abnormal (ie, out-of-range) results and a repeat specimen is requested. If a program reports screen-positive results as likelihood of risk (eg, probable ALD), borderline results may be reported as lower risk (eg, possible ALD).
- Although ALD is not considered a “time-critical” condition by the US Department of Health and Human Services Advisory Committee on Heritable Disorders in Newborns and Children timeliness guidelines regarding notification of both screen-positive and screen-negative results,⁴² final NBS results should be reported within seven days of life to maximize health outcomes in affected newborns.
- Although ALD onset occurs after the newborn period, other male family members may be in a critical risk period for developing symptoms. Therefore:
 - In addition to the baby's HCP, the family should be notified as soon as possible (per NBS program policy).
 - NBS programs should emphasize the importance of determining the ALD status of any older male siblings. There is a 50% chance of male siblings being affected, and these family members may be old enough to already have adrenal or cerebral manifestations.

7.2 Follow-up for Adrenoleukodystrophy, Zellweger Spectrum Disorders, and Other Peroxisomal and Nonperoxisomal Disorders

STFU and ITFU for individuals diagnosed with ALD, ZSDs, and other peroxisomal and nonperoxisomal disorders include genetic counseling; referral to a pediatric endocrinologist, pediatric metabolic specialist, pediatric neurologist, pediatric gastroenterologist, metabolic physician, and other specialists depending on symptoms and health care system; and referral to a family support group⁵⁶ (see Additional Resources).

7.3 Short-Term Follow-up on Screen-Positive Results for Adrenoleukodystrophy

STFU begins as soon as a repeat specimen is requested (eg, because of a borderline result) or the newborn is referred for a diagnostic evaluation. STFU continues until a repeat specimen is received or a final biochemical and genetic diagnosis is determined. Diagnostic evaluation depends on whether the NBS program performs *ABCD1* sequencing before referral and on the newborn's sex and health status.²⁶ An individual is considered biochemically and genetically diagnosed if he or she has elevated VLCFA and an *ABCD1* variant that is known to be pathogenic (see Subchapter 6.2 for information on case definitions, cutoffs, and VUS). For females, VLCFA are not always elevated in plasma, so females with a known *ABCD1* pathogenic gene variant are considered to have a genetic diagnosis of ALD despite inconsistent VLCFA elevations. Per clinical guidelines, a biochemically

Related CLSI Reference Materials^a

- C24** **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th ed., 2016.** This guideline provides definitions, principles, and approaches to laboratory quality control design, implementation, and assessment.
- C62** **Liquid Chromatography-Mass Spectrometry Methods. 1st ed., 2014.** This document provides guidance to the clinical laboratorian for the reduction of interlaboratory variance and the evaluation of interferences, assay performance, and other pertinent characteristics of clinical assays. This guideline emphasizes particular areas related to assay development and presents a standardized approach for method verification that is specific to mass spectrometry technology.
- EP05** **Evaluation of Precision of Quantitative Measurement Procedures. 3rd ed., 2014.** This document provides guidance for evaluating the precision performance of quantitative measurement procedures. It is intended for manufacturers of quantitative measurement procedures and for laboratories that develop or modify such procedures.
- EP06** **Evaluation of Linearity of Quantitative Measurement Procedures. 2nd ed., 2020.** This guideline provides information for characterizing the linearity interval of a measurement procedure, validating a linearity interval claim (to be performed by the manufacturer), and verifying an established linearity interval claim (to be performed by the end user).
- EP09** **Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed., 2018.** This guideline covers the design of measurement procedure comparison experiments using patient samples and subsequent data analysis techniques used to determine the bias between two *in vitro* diagnostic measurement procedures.
- EP10** **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures. 3rd ed., 2014.** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.
- EP14** **Evaluation of Commutability of Processed Samples. 3rd ed., 2014.** This document provides guidance for evaluating the commutability of processed samples by determining if they behave differently than unprocessed patient samples when two quantitative measurement procedures are compared.
- EP15** **User Verification of Precision and Estimation of Bias. 3rd ed., 2014.** This document describes the estimation of imprecision and of bias for clinical laboratory quantitative measurement procedures using a protocol that can be completed within as few as five days.

^a CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

Related CLSI Reference Materials (Continued)

- 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.
- EP19** **A Framework for Using CLSI Documents to Evaluate Clinical Laboratory Measurement Procedures. 2nd ed., 2015.** This report uses the "measurement procedure lifecycle" framework to aid users of CLSI evaluation protocols documents during establishment and implementation of measurement procedures developed by both commercial manufacturers and clinical laboratories, ie, for laboratory-developed tests.
- EP21** **Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures. 2nd ed., 2016.** This guideline provides manufacturers and end users with an understanding of concepts related to total analytical error (TAE) for quantitative measurement procedures. An experimental protocol and data analysis method are provided to estimate TAE based upon a comparison of methods experiment with patient specimens, and to assess it relative to a pre-established goal for clinical acceptability.
- 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** **Dried Blood Spot Specimen Collection for Newborn Screening. 7th ed., 2021.** This standard highlights specimen collection methods, discusses acceptable techniques for applying blood drops or aliquots to the filter paper section of the specimen collection device, and provides instructions on proper specimen drying, 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. 2nd ed., 2019.** This guideline describes the recommended protocols for screening preterm, low birth weight, and sick newborns for hearing loss, critical congenital heart defects, and diseases detectable through newborn dried blood spot screening.
- NBS04** **Newborn Screening by Tandem Mass Spectrometry. 2nd ed., 2017.** This guideline serves as a reference for the multiple activities related to operating a tandem mass spectrometry laboratory as part of public and private newborn screening programs.
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Related CLSI Reference Materials (Continued)

- QMS01** **A Quality Management System Model for Laboratory Services. 5th ed., 2019.** This guideline provides a model for medical laboratories to organize the implementation and maintenance of an effective quality management system.
- 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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