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NBS09

Newborn Screening for X-Linked Adrenoleukodystrophy

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.

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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 $^{2}H_{4}$-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

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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

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 NBS01[6])
- 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