CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

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.

Clinical and Laboratory Standards Institute

Setting the standard for quality in medical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advances in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

Appeal Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeal, documented in the CLSI *Standards Development Policies and Processes*, is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For additional information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

NBS09-Ed1
August 2021

Newborn Screening for X-Linked Adrenoleukodystrophy

Joseph Orsini, PhD
Ann B. Moser, BA
Adrienne Manning, BS
Heather A. Brown, MSc, PhD
Florian Eichler, MD
François Eyskens, MD, PhD
Christopher A. Haynes, PhD
Stephan Kemp, PhD
Tero Lehtonen, PhD
Olajumoke Oladipo, MD, DABCC, FACB
Peter C.J.I. Schielen, PhD
Rajendra Singh, PhD
Jennifer Taylor, PhD
Silvia Tortorelli, MD, PhD
Beth Vogel, MS, CGC

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 relevant 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.

Clinical and Laboratory Standards Institute (CLSI). *Newborn Screening for X-Linked Adrenoleukodystrophy.* 1st ed. CLSI guideline NBS09 (ISBN 978-1-68440-112-3 [Print]; ISBN 978-1-68440-113-0 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2021.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Newborn Screening for X-Linked Adrenoleukodystrophy.* 1st ed. CLSI guideline NBS09. Clinical and Laboratory Standards Institute; 2021.

NBS09-Ed1

ISBN 978-1-68440-112-3 (Print)

ISBN 978-1-68440-113-0 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 41, Number 10

Committee Membership

Consensus Council

James R. Petisce, PhD
Chairholder
BD Diagnostic Systems
USA

Tania Motschman, MS, MT(ASCP)SBB
Vice-Chairholder
USA

Deirdre Astin, MS, MT(ASCP)
USA

Anne T. Daley, MS, MT(ASCP)DLM, CMQ/OE(ASQ)CSBB
USA

Avis Danishefsky, PhD
FDA Center for Devices and Radiological Health
USA

Collette Fitzgerald, PhD
Centers for Disease Control and Prevention
USA

Michelle McLean, MS, MT(ASCP), BS
Greiner Bio-One, Inc.
USA

James H. Nichols, PhD, DABCC, FAACC
Vanderbilt University School of Medicine
USA

M. Laura Parnas, PhD, DABCC
Roche Diagnostics
USA

Victoria Petrides, MS
Abbott
USA

Matthew A. Wikler, MD, FACSA, MBA
IDTD Consulting
USA

Document Development Committee on Newborn Screening for X-Linked Adrenoleukodystrophy

Joseph Orsini, PhD
Chairholder
New York State Department of Health
USA

Ann B. Moser, BA
Vice-Chairholder
Kennedy Krieger Institute
USA

Adrienne Manning, BS
Committee Secretary
Connecticut Department of Public Health
USA

Heather A. Brown, MSc, PhD
Waters Corporation
United Kingdom

François Eyskens, MD, PhD
Antwerp University Hospital
Belgium

Christopher A. Haynes, PhD
Centers for Disease Control and Prevention
USA

Stephan Kemp, PhD
Amsterdam University Medical Center
the Netherlands

Tero Lehtonen, PhD
PerkinElmer
Finland

Peter C.J.I. Schielen, PhD
Rijksinstituut voor Volksgezondheid en Milieu
the Netherlands

Silvia Tortorelli, MD, PhD
Mayo Clinic
USA

Expert Panel on Newborn Screening

Amy Gaviglio, MS, CGC
Chairholder
Minnesota Department of Health
USA

Ronald J. Whitley, PhD, DABCC,
Vice-Chairholder
University of Kentucky Medical Center
USA

Sucheta Bhatt, MD
Illumina, Inc.
USA

Candice Brannen, PhD
Baebies, Inc.
USA

Paula V. Caposino, MA, PhD
FDA Center for Devices and Radiological Health
USA

Uttam Garg, PhD, DABCC
The Children's Mercy Hospital
USA

Dietrich Matern, MD, PhD, FACMG
Mayo Clinic
USA

Joanne Mei, PhD
Centers for Disease Control and Prevention
USA

Joseph Orsini, PhD
New York State Department of Health
USA

Scott M. Shone, PhD, HCLD(ABB)
North Carolina State Laboratory of Public Health
USA

Marci K. Sontag, PhD, MS
CI International
USA

Dianne R. Webster, Ph[illegible] FFS, RCPA, FHGSA
Auckland District Health Board
New Zealand

Staff

Clinical and Laboratory Standards Institute
USA

Lori T. Moon, MS, MT(ASCP)
Project Manager

Emily J. Gomez, MS, MLS(ASCP)^{CM}MB^{CM}
Project Manager

Megan L. Tertel, MA, ELS
Editorial Manager

Catherine E.M. Jenkins, ELS
Editor

Kristy L. Leirer, MS
Editor

Laura Martin
Editor

Acknowledgment

CLSI, the Consensus Council, and the Document Development Committee on Newborn Screening for X-Linked Adrenoleukodystrophy gratefully acknowledge the following volunteers for their important contributions to the development of this guideline:

Florian Eichler, MD
Harvard/Massachusetts General Hospital
USA

Olajumoke Oladipo, MD, DABCC, FACB
Penn State Hershey Medical Center
USA

Rajendra Singh, PhD
ARK Diagnostics, Inc.
USA

Jennifer Taylor, PhD
Maryland Department of Health
USA

Beth Vogel, MS, CGC
New York State Department of Health
USA

Contents

Contents (Continued)

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

This page is intentionally left blank.

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