CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

CLSI NBS13™

Newborn Screening for Spinal Muscular Atrophy

CLSI NBS13 describes a newborn screening system for detecting spinal muscular atrophy (SMA). It discusses both *SMN1* exon 7 deletion testing and *SMN2* copy number analysis performed on newborn dried blood spot specimens, as well as screening strategies for identifying newborns at increased risk for SMA.

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

CLSI NBS13-Ed1
March 2025

Newborn Screening for Spinal Muscular Atrophy

Jennifer Kwon, MD, MPH
Veronica Wiley, PhD, FHGSA, FFSc(RCPA)
Jennifer Taylor, PhD
Abul Kalam Azad, MD, MSc, PhD
Mei Baker, MD
Mary Barrioz, BS
Francois Boemer, PharmD, PhD
Iulia Coliban, MS
Kendra Croak, MS
Francis Lee, MSc, PhD
Blanca Martinez, MLS(ASCP)CM
Devin Oglesbee, PhD
Rebecca Shaulis, MS
Sonu Shrestha, PhD
Colleen Stevens, PhD
Els Voorhoeve, MSc
Raquel Yahyaoui Macias, MD, PhD

Abstract

Clinical and Laboratory Standards Institute NBS13—*Newborn Screening for Spinal Muscular Atrophy* describes newborn screening (NBS) laboratory tests and screening strategies used to identify newborns at increased risk of developing spinal muscular atrophy (SMA). SMA is a serious condition that causes weakness, motor decline, and death in otherwise cognitively normal children. Early detection and intervention are critical to improving the strength and survival of newborns with SMA, allowing them to live healthier and more productive lives. SMA is caused by loss of function of the survival of motor neuron (SMN) gene, with about 95% of SMA cases characterized by a homozygous deletion in *SMN1* exon 7, leading to deficiency of the SMN protein and degeneration of motor neurons followed by progressive muscle weakness. The severity of the disease is typically inversely correlated to the number of *SMN2* copies. CLSI NBS13 describes the various methods used for *SMN1* exon 7 deletion testing and *SMN2* copy number analysis, validation of assays, integrating SMA NBS into NBS laboratory operations, interpreting and reporting screening results, as well as short-term and long-term follow-up.

Clinical and Laboratory Standards Institute (CLSI). *Newborn Screening for Spinal Muscular Atrophy.* 1st ed. CLSI guideline NBS13 (ISBN 978-1-68440-274-8 [Print]; ISBN 978-1-68440-275-5 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2025.

Suggested Citation

CLSI. *Newborn Screening for Spinal Muscular Atrophy.* 1st ed. CLSI guideline NBS13. Clinical and Laboratory Standards Institute; 2025.

CLSI NBS13-Ed1

ISBN 978-1-68440-274-8 (Print)

ISBN 978-1-68440-275-5 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 45, Number 5

Committee Membership

Consensus Council

The Consensus Council sets priorities for CLSI standards development and votes on Final Draft documents to confirm that process requirements have been met. Consensus Council members are listed on the CLSI website: https://clsi.org/standards-development/consensus-council/

Document Development Committee on Newborn Screening for Spinal Muscular Atrophy

Jennifer Kwon, MD, MPH
Chairholder
University of Wisconsin—Madison Medical School
USA

Veronica Wiley, PhD, FHGSA, FFSc(RCPA)
Vice-Chairholder
The Children's Hospital at Westmead
Australia

Jennifer Taylor, PhD
Committee Secretary
American College of Medical Genetics
USA

Mei Baker, MD
Wisconsin State Laboratory of Hygiene
USA

Francois Boemer, PharmD, PhD
CHU de Liège
Belgium

Kendra Croak, MS
Colorado Department of Public Health and Environment
USA

Devin Oglesbee, PhD
Mayo Clinic
USA

Sonu Shrestha, PhD
Thermo Fisher Scientific
USA

Colleen Steve[illegible] Pr[illegible]
New York State Department of Health
USA

Rac[illegible]el Yahyaoui Macias, MD, PhD
M[illegible]laga Regional University Hospital
[illegible]pain

Expert Panel on Newborn Screening

Expert panel volunteers support the develop[illegible]nt of CLSI documents by providing technical expertise in specialty areas. Expert panel members are listed by area of exp[illegible]tise on the CLSI website: https://clsi.org/standards-development/expert-panels/

Acknowledgment

CLSI, the Consensus Council, and the Document Development Committee on Newborn Screening for Spinal Muscular Atrophy gratefully acknowledge the following volunteers for their important contributions to the development of CLSI NBS13:

Abul Kalam Azad, MD, MSc, PhD
Montefiore Medical Center
USA

Mary Barrioz, BS
Missouri State Public Health Laboratory
USA

Iulia Coliban, MS
Institute of Mother and Child
Republic of Moldova

Francis Lee, MSc, PhD
Centers for Disease Control and Prevention
USA

Blanca Martinez, MLS(ASCP)^{CM}
Tennessee Department of Health
USA

Rebecca Shaulis, MS
Michigan Department of Health and Human Services
USA

Els Voorhoeve, MSc
National Institute for Public Health and the Environment (RIVM)
Netherlands

Contents

Contents (Continued)

Foreword

Spinal muscular atrophy (SMA) is a disorder of progressive weakness associated with loss of lower motor neurons (LMNs), which are the nerve cells that originate in the anterior horn of the spinal cord. Although rare, SMA is one of the more common inherited causes of childhood death. SMA is caused by deficiency of the survival of motor neuron (SMN) protein, which is usually due to loss of function of *SMN1* on chromosome 5q. SMN protein is not absent, however, because of the presence of a nearly identical pseudogene, *SMN2*, that can produce small amounts of SMN protein. Historically, SMA has been categorized into a variety of types, largely based on how much residual SMN protein is produced by *SMN2*. Children with SMA have weakness that progresses as their LMNs continue to die. It is estimated that in SMA type 1, the severe infantile form of SMA, up to 90% of LMNs die by age 6 months, leaving the infant unable to sit and unable to breathe without chronic ventilatory support. Since 2016, three effective disease-modifying treatments (DMTs) for SMA have been approved, all of which effectively increase the amount of SMN protein and, if given early enough, prevent LMN degeneration. With these treatments, children with SMA who are treated before any LMN loss can make ongoing and normal motor gains, rather than motor declines.

The commercial availability of the first DMT, nusinersen, at the end of 2016 meant that if treated early enough, children with SMA type 1 could make typical motor gains in infancy without needing respiratory support. The remarkable changes in outcomes seen in the clinical trials of nusinersen helped SMA receive approval for inclusion on the United States' Federal Recommended Uniform Screening Panel in 2018. Since then, two additional DMTs for SMA have been approved by the US Food and Drug Administration and SMA newborn screening (NBS) has been introduced in nearly every US state, as well as internationally. The notable speed of implementation of SMA NBS in the United States was helped by the earlier introduction of severe combined immunodeficiency NBS, which also uses DNA-based technology rather than determination of an analyte concentration that is more typical of NBS for other diseases.

NOTE: The content of CLSI NBS13 is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

dried blood spots

lower motor neurons

newborn screening

real-time quantitative polymerase chain reaction

***SMN1* homozygous exon 7**

***SMN2* copy number**

spinal muscular atrophy

Chapter 1

Introduction

Newborn Screening for Spinal Muscular Atrophy

1 Introduction

1.1 Scope

CLSI NBS13 discusses the detection of 5q spinal muscular atrophy (SMA) by population-based newborn dried blood spot (DBS) screening and preanalytical, analytical, and postanalytical aspects of newborn screening (NBS) for SMA. It focuses on analytical methods to detect the homozygous absence of *SMN1* exon 7, which accounts for approximately 95% of SMA cases, as well as methods for *SMN2* copy number analysis. CLSI NBS13 describes:

- Background information on the biological and clinical features of SMA, and the value of the *SMN2* copy number in predicting disease severity
- Preanalytical considerations, including population education and issues of consent before screening
- Analytical methodologies for detecting the homozygous absence of *SMN1* exon 7, including:
  - Real-time quantitative PCR (qPCR)
  - High-resolution melting analysis (HRMA)
  - Multiplex ligation-dependent probe amplification (MLPA)
  - Digital PCR (dPCR)/droplet digital PCR (ddPCR)
  - Capillary electrophoresis
  - Mass spectrometry
- Analytical methodologies for *SMN2* copy number analysis, including real-time qPCR, dPCR, and capillary electrophoresis
- Screening strategies and laboratory screening algorithms, cutoff value determinations, case definition, and risk assessment for NBS programs to consider when implementing SMA NBS
- Postanalytical short-term follow-up (STFU) and long-term follow-up (LTFU) procedures, including case tracking, diagnostic testing needed to confirm an SMA diagnosis, and recent treatments available for SMA

The intended users of CLSI NBS13 include NBS laboratories, follow-up and program personnel, public health program administrators, diagnostic medical laboratories, SMA treatment centers, health care providers (HCPs; eg, primary care providers, neonatologists, pediatricians, neurologists), regulatory agencies, public health policy makers, and manufacturers of instruments, reagents, and related products used for NBS.

CLSI NBS13 does not include:

- Details of carrier screening
- Details of confirmatory diagnostic laboratory testing
- Details of clinical treatments and monitoring
- Comparative cost information