CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

NBS04

Newborn Screening by Tandem Mass Spectrometry

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.

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

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Newborn Screening by Tandem Mass Spectrometry

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Abstract

Clinical and Laboratory Standards Institute guideline NBS04—*Newborn Screening by Tandem Mass Spectrometry* describes best practice procedures for specimen and reagent preparation, instrument and analyte calibration, method validation, QA and QC, run acceptance criteria with multianalyte platforms, external treatment effects on test results (eg, transfusions and total parenteral nutrition), results interpretation and reporting, follow-up recommendations, and the use of tandem mass spectrometry for second-tier testing.

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Foreword

Nearly 10 million newborns worldwide are screened annually for metabolic and genetic disorders. The goal of these newborn screening (NBS) tests is early diagnosis and treatment that will likely prevent or reduce the severe medical outcomes that occur in undiagnosed or untreated infants. The number of disorders screened from a newborn's dried blood spot (DBS) specimen has increased significantly in the last two decades. The essential methods for detecting fatty acylcarnitines and amino acids from DBS using tandem mass spectrometry (MS/MS) were developed in the early 1990s and put into clinical practice by private and academic laboratories in the mid-1990s. By 2000, MS/MS analysis of these metabolites had been adopted for use in three public health laboratories in the United States, and was well underway in Australia.[1] It has now expanded to almost all screening programs worldwide. The number of disorders detectable by MS/MS depends on how the analysis is performed. In its original configuration, the method could detect more than 60 biomarkers, and each biomarker could detect one or more disorders. The exact number of disorders that are reported is often debated because it relies on the number of metabolites measured, data interpretation, the way disorders are counted, and public policy. In the United States, the Recommended Uniform Screening Panel (RUSP)[2] was first accepted for NBS programs on May 21, 2010.[3] Today, the RUSP identifies 34 core conditions and 26 secondary conditions, of which 43 are detected primarily by MS/MS analysis.

MS/MS is a fundamentally different technology than systems previously used by most NBS laboratories. It is a versatile and complex system that can be easily adapted to the users' preferred testing approach. This led to numerous variations in NBS by MS/MS, and it became challenging to compare results between laboratories. There is a recognized need to develop consensus solutions to provide more consistency between MS/MS screening programs. There have been numerous workshops, training courses, and publications since the first US workgroup report,[4] with many methodological issues remaining unresolved. Variations of the original method include specimen and reagent preparation, instrument and analyte calibration, method validation, QA and QC, run acceptance criteria with multianalyte platforms, external treatment effects on test results (eg, transfusions and total parenteral nutrition), results interpretation and reporting, and follow-up recommendations. In addition to MS/MS being used as the primary screening method, the use of this technology as a second-tier test has been introduced to improve other NBS test sensitivity and specificity. A consensus guideline developed by experts for using MS/MS in NBS will ensure that babies tested using MS/MS have the opportunity to get equivalent screening services throughout the world.

Overview of Changes

This guideline replaces the previous edition of the guideline, NBS04-A, published in 2010. Several changes were made in this edition, including:

- Reorganization to follow the path of workflow
- Updating methodology and references throughout the guideline
- Removing mass spectrometer setup instructions for *m/z* peak resolution (Subchapter 4.5.4.1) and providing the reference[5]
- Updating terminology throughout the guideline

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

Acylcarnitines, amino acids, cutoffs, dried blood spot, newborn screening, second-tier testing, tandem mass spectrometry

Newborn Screening by Tandem Mass Spectrometry

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline'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 guideline
- Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline is intended to assist newborn screening (NBS) laboratory personnel in the routine use of tandem mass spectrometry (MS/MS) for the detection of metabolites that may indicate certain metabolic disorders using dried blood spot (DBS) specimens. The guideline describes:

- Preparation procedures for reagents, specimens, standards, and controls
- Calibration (both instrument and analyte)
- Standardization
- Control acceptance criteria
- Disorder profiles (interpretation of MS/MS spectra)
- External effects on results (eg, transfusion and total parenteral nutrition [TPN])
- Results reporting
- Second-tier testing
- Follow-up recommendations

This guideline:

- Is not intended to provide general information for screening on all conditions, only screening information related to MS/MS
- Does not cover confirmatory or diagnostic testing

1.2 Background

The goal of NBS is early detection of babies at increased risk for selected heritable disorders so that diagnostic testing, clinical evaluation, and, if necessary, medical treatment can be initiated promptly. The first DBS NBS began in the 1960s with the introduction of a bacterial inhibition assay for phenylketonuria (PKU).[6] Because it was reliable, simple, inexpensive, and could be scaled for large numbers of specimens,

the test was suitable for population screening. In addition, the method used DBS specimens, a simple and efficient method for collecting and transporting blood specimens.

NBS programs expanded as additional bacterial inhibition assays and other technologies (eg, immunochemistry and electrophoresis) were developed, laboratories screened for more disorders, and the number of babies screened increased. A significant addition was the introduction of MS/MS to the NBS laboratory. Because MS/MS is a multiplex method that measures several analytes on the same sample simultaneously, it is possible to detect multiple disorders on the same testing platform.

The current list of common metabolic disorders detectable by routine screening on MS/MS is provided in Appendix A. They are organic acid disorders (Table A1), fatty acid oxidation (FAO) disorders (Table A2), and aminoacidopathies (Table A3). The current list of metabolites that may be measured to detect common metabolic disorders is provided in Appendix B for amino acids and Appendix C for acylcarnitines.

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.[7] 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.[8]

1.4 Terminology

1.4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in different countries and regions, and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. CLSI recognizes its important role in these efforts, and its consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines.

NOTE: Mandates are generally reserved for CLSI standards, but are occasionally allowed in CLSI guidelines. In CLSI guidelines, use of the term "must" is either 1) based on a requirement or 2) indicative of a necessary step to ensure patient safety or proper fulfillment of a procedure. The document development committee evaluated use of the term "must" and deemed it appropriate.

CLSI uses the globally applicable terms *preexamination, examination,* and *postexamination* in its documents. However, in the NBS laboratory, DBS specimens are *examined* to ensure they are satisfactory before they are "analyzed." Hence, for the purposes of CLSI NBS documents, the terms *preanalytical, analytical,* and *postanalytical* are used in place of *preexamination, examination,* and *postexamination*. Additionally, the term *analysis* is used in place of *examination*. Although contradictions among these terms may exist between new CLSI NBS documents and already published NBS documents, these contradictions will be reconciled as documents go through the routine revision process.