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NBS06-A

Newborn Blood Spot Screening for Severe Combined Immunodeficiency by Measurement of T-cell Receptor Excision Circles; Approved Guideline

This document addresses the detection of severe combined immunodeficiency (SCID) by population-based newborn screening using dried blood spot specimens to measure T-cell receptor excision circles. SCID is a lethal disorder of infancy that is not evident at birth, and effective treatment requires presymptomatic detection.

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

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Abstract

Severe combined immunodeficiency (SCID) is a congenital clinical disorder that is not evident at birth. Without treatment, most babies with SCID will die in infancy from virulent infection. This guideline addresses the detection of SCID by population-based newborn screening (NBS) using dried blood spot (DBS) specimens to measure T-cell receptor excision circles (TREC). Responding to recent US recommendations, the document is intended to facilitate the incorporation of SCID NBS into the routine operation of NBS programs worldwide. Based on extensive input from NBS laboratories, it describes the laboratory tests currently used to measure TREC in DBS by real-time quantitative PCR. The document also describes biological and clinical features of SCID and of other conditions potentially identified by SCID NBS. It provides an overview of laboratory operations including physical layout, instrumentation, TREC assay protocols, automated methodologies, and alternative platforms. The document includes a summary of diagnostic tests used for follow-up of abnormal TREC results as well as other short-term and long-term follow-up activities, including case tracking. It describes variants of SCID that may not be detected by TREC assays in newborn DBS. The guideline delineates the steps for implementing SCID NBS: validating the laboratory test, conducting pilot studies, and transitioning to routine screening. It is directed toward NBS laboratory personnel, public health program personnel, producers of laboratory products related to NBS, and those involved with oversight of NBS testing.

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Foreword

The use of newborn screening (NBS) to detect severe combined immunodeficiency (SCID) and other T-cell defects represents a new frontier in public health and epidemiological endeavors. From its inception almost 50 years ago as a public health program to detect phenylketonuria,[1-3] NBS has grown into a global pursuit capable of detecting a wide spectrum of congenital metabolic and other disorders using a variety of laboratory analyses and dried blood spots (DBS) as the primary NBS specimen. The first NBS test, the bacterial inhibition assay, led to the creation of a new public health practice. The use of radioimmunoassay to detect congenital hypothyroidism[4] coalesced public health laboratories into an international consortium and fueled the continuing expansion of NBS into new populations with more disease conditions studied. By the late 1990s, the process for selecting diseases to be included in population-based NBS had grown much more intricate[5] due to technical advances in laboratory science and to greater public awareness, legislative action, and parental education. During this time, SCID became widely recognized as a promising candidate for NBS, provided that a suitable screening test could be developed.

The general public awareness of SCID arose largely from the life of David Vetter, often referred to as the "boy in the bubble."[6] His case served as the forerunner for the medical interventions used today to treat children with SCID, most notably temporary protective isolation and restoration of immune function by hematopoietic cell transplantation (HCT). Although David died from complications related to his transplant, his case prompted the refinement of HCT into a highly effective treatment for SCID when it is performed in early infancy. The first few weeks of life, before the loss of maternal antibodies leads to severe infections, provide a critical window of opportunity. Thereafter, morbidity from infections makes HCT much less effective.[7] Early identification in the asymptomatic infant is therefore essential to successful treatment.

As the benefit of HCT in early infancy became increasingly apparent, some pediatric immunologists began to call for neonatal testing for SCID. However, a reliable high-throughput assay that uses newborn DBS was required to exploit the existing infrastructure for population-based NBS. The importance of a SCID NBS test was highlighted as a top priority at a 2001 conference at the US Centers for Disease Control and Prevention (CDC).[8] These discussions helped to bring attention to the development and validation of a SCID NBS test at the US National Institutes of Health (NIH).[9] This test used absolute quantitative real-time PCR to measure T-cell receptor excision circles (TREC), extra-chromosomal DNA fragments uniquely created during T-cell formation.[10-13] The initial results from 23 infants newly diagnosed with SCID and 239 residual newborn DBS suggested that the TREC assay could be a sensitive and specific method for SCID NBS.[9] NBS programs would have to develop a high-throughput capacity and demonstrate the feasibility of implementing a sufficiently controlled DNA-based technology in their population-based services.

In 2008, a partnership among the Children's Hospital of Wisconsin (CHW), the Wisconsin State Laboratory of Hygiene (WSLH), and the Jeffrey Modell Foundation for primary immunodeficiency led to the first population-based application of the TREC assay in an NBS public health program.[14,15] The TREC assay used in the program was established at WSLH and CHW and was shown to be suitable for high-throughput routine NBS. By 2009, Massachusetts had developed an internally controlled multiplex TREC assay and initiated a second population-based SCID NBS program,[16,17] while the University of California, San Francisco began SCID NBS in certain high-risk Native American populations. During this initial phase, the CDC developed and worked with the laboratories in Wisconsin and Massachusetts to validate the DBS reference materials needed to support the expanding number of public health laboratories preparing for SCID NBS. The Wisconsin and Massachusetts experiences documented the feasibility of including SCID in routine NBS and, together with the CDC, paved the way for wider implementation. In 2010, an advisory committee to the US Department of Health and Human Services recommended the addition of SCID as a core condition in its Recommended Uniform Screening Panel, as well as the addition of related T-cell lymphocyte deficiencies to the list of secondary targets.[18] The US National

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SCID Pilot Study funded by the NIH subsequently provided support to introduce SCID NBS in California, New York, Louisiana (through testing at the Wisconsin laboratory), and Puerto Rico (through testing at the Massachusetts laboratory).[19] Taiwan has also initiated SCID NBS. Currently, NBS programs are testing over 2 million newborns each year for SCID, with that number expected to increase rapidly worldwide.

Through close collaboration among NBS programs, government agencies, academic centers, and private foundations, the early growth of SCID NBS has proceeded rapidly and effectively. The purpose of this document is to assimilate this early experience into consensus guidelines for laboratory practice and program implementation. There is some sense of urgency to this task. Presently, the programs that have implemented SCID NBS are among the most experienced, and capturing their SCID NBS knowledge is critical to methodical and effective expansion of this program. SCID NBS is still technologically evolving with respect to both laboratory practices and outcome measures. The document development committee is fully aware that this guideline must establish the groundwork for refinements in the near future. Therefore, it will be subject to modification and updates in a more rapid fashion than normal CLSI policy. Feedback based on the experience from NBS programs will be critical to this enhancement.

Key Words

Dried blood spots, newborn screening, polymerase chain reaction, severe combined immunodeficiency, T-cell receptor excision circles

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Newborn Blood Spot Screening for Severe Combined Immunodeficiency by Measurement of T-cell Receptor Excision Circles; Approved Guideline

1 Scope

This guideline addresses the detection of severe combined immunodeficiency (SCID) by population-based newborn screening (NBS) using dried blood spot (DBS) specimens. The guideline is intended to facilitate the incorporation of SCID NBS into the routine operation of existing NBS programs. Methodologically, it focuses on measuring T-cell receptor excision circles (TREC) in DBS by real-time quantitative PCR[a] (qPCR), the method in use by all NBS laboratories at the time of guideline publication. It also describes other qPCR methods for measuring TREC in DBS that may come into future use.

This guideline includes detailed information for laboratory practice including calibration, QC, and proficiency testing (PT). It also addresses program issues such as short-term follow-up (notification and tracking to establish or rule out a diagnosis). The guideline includes clinical and immunological background on SCID and other immunodeficiency disorders that may present with low or no TREC content in newborns. It draws heavily on the experience of the NBS programs that have already operationalized the TREC assay for population-based NBS. The document includes several appendixes that provide additional information important to the guideline. In particular, Appendix C includes the operational algorithms in use by four of the NBS programs conducting SCID NBS at the time this document development committee was convened. The guideline is primarily intended for use by NBS laboratory personnel, producers of laboratory products related to NBS, and those involved with oversight of NBS programs.

The guideline is limited to NBS applications. It discusses, but does not detail, the methods used in diagnostic laboratory tests for immune deficiencies on whole blood, including immunophenotyping by flow cytometry (addressed in CLSI document H42)[20] and lymphocyte function assays (some of which are addressed in CLSI document I/LA26).[21] It does not discuss blood spot collection for NBS, which is the subject of a separate guideline (see CLSI document LA04).[22] While the document includes general guidelines for short- and long-term follow-up, the knowledge base for assessing sensitivity, specificity, and predictive value is not yet sufficient to express accurate quantitative values for these parameters. These parameters will be revisited in future editions of this guideline.

2 Introduction

SCID is a lethal disorder of infancy that is often not clinically apparent until several weeks after birth. SCID can be effectively treated by hematopoietic cell transplantation (HCT), and there is strong evidence that early intervention during the asymptomatic period results in better outcomes and increased survival.[7] Historically, the only babies tested at birth were those with a family history of SCID. A family-based survey revealed that the survival rate was 85% for those newborns tested at birth compared to 58% for newborns not tested at birth.[23] In 2001, the cumulative evidence from clinical experience led to SCID being identified as a target for public health NBS.[8] In 2005, Chan and Puck published a method to detect SCID from newborn blood spot specimens by measuring the content of TREC using real-time qPCR.[9] In 2008, Wisconsin began the first prospective population-based pilot study of SCID NBS using a laboratory-developed real-time qPCR assay to measure TREC.[15,24] In 2009, Massachusetts initiated its SCID NBS pilot program using an internally controlled multiplex real-time qPCR TREC assay.[17,25] Since then, several additional US states and other global NBS programs have incorporated SCID into their

[a] *Absolute quantitative PCR* refers to qPCR assays that incorporate calibrator reference materials having values assigned in absolute copy numbers, as contrasted to calibration in relative (proportionate) values. Because the term *absolute* may be misinterpreted to mean independent of any calibrator reference material, it will not be used in the remaining sections of this guideline.

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