Newborn Screening Quality Assurance Program
T-Cell Receptor Circle in Dried Blood Spots
Proficiency Testing Program (TRECPT)

In co-sponsorship with Association of Public Health Laboratories (APHL)
Provided by the Newborn Screening and Molecular Biology Branch
Centers for Disease Control and Prevention
4770 Buford Highway NE, MS/F19
Atlanta, GA 30341-3724
Email: NSQAPDMT@cdc.gov

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Report Authorization

This report has been reviewed and authorized by Dr. Joanne Mei, Laboratory Chief, Newborn Screening Quality Assurance Program.

Confidentiality Statement

NSQAP participant information and evaluations are strictly confidential and shared only with individual participants, unless written authorization for release is received.

Introduction

This report summarizes data collected within the specified period for the Quarter 4, 2018, proficiency testing (PT) program for T-cell receptor excision circle (TREC) analysis in dried blood spots (DBS) to detect severe combined immunodeficiency (SCID). The tables within this report provide the certification profiles for the specimens, summary of reported categorical results and the verification of your reported data.

Certification of PT Specimens

This panel consisted of five DBS specimens prepared from human blood, including cord blood from unaffected individuals and modified adult blood depleted of mononuclear cells or leukocytes (specimens 418R1, 418R2, 418R3, 418R4, and 418R5). Table 1 shows the certification and description of the specimens in the panel.

Table 1. Specimen Certification and Description

Specimen Number	TREC Clinical Assessment*	Reference Gene Assessment**	Specimen Description
418R1	1	1	Normal cord blood with medium TREC copy level (close to population median of term newborns)
418R2	1	1	Normal cord blood with lower TREC copy level (about 50% of population median)
418R3	2	1	SCID-like sample with very low/undetectable TREC; reference gene within acceptable range. Prepared from lymphocyte-depleted blood.
418R4	2	2	Sample with uninterpretable results for SCID. Both TREC and reference gene out of range. Prepared from leukocyte-depleted blood.
418R5	1	1	Normal cord blood with lower TREC copy level (about 75% of population median)

*1 - No Follow-up required (Screen Negative) 2 - Follow-up required

**1 - Within reference range 2 - Outside reference range

Distribution of PT Specimens

We distribute this PT report to all participants, state laboratory directors, and program colleagues by request. On September 25, 2018 a panel of five unknown DBS specimens was sent to 40 domestic, 17 international, and two manufacturer laboratories to analyze TREC content in peripheral blood.

Participant Results

TREC Level Assessment

We received data from 59 participants by the data reporting deadline. Table 2 summarizes reported frequency of clinical assessments. Table 3 provides the methods used to assess TREC levels. Table 4 shows the frequency of methods used to extract DNA from DBS. We requested only qualitative, categorical results: 'No follow-up required (Screen Negative)' or 'Follow-up required' for each specimen since quantitative results vary significantly between laboratories using different test methods and calibrators.

Table 2. Frequency of Clinical Assessments

Specimen Number	No Follow-up Required	Follow-up Required
418R1	58	1
418R2	59	0
418R3	0	59
418R4	0	59
418R5	59	0

Table 3. Laboratory Methods for TREC

Method	Number of Laboratories
Real Time PCR—Singleplex	10
EnLite™ Neonatal TREC kit	20
Real Time PCR – Multiplex	28
Other	1

Table 4. Frequency of DNA Extraction Methods

Method	Number of Laboratories
In situ/on card (no DNA extraction) with washing step(s)	13
EnLite™ (non DNA extraction)	20
DNA extracted at 99°C with washing step(s)	14
DNA extracted at 95°C with washing step(s)	7
DNA extracted at 70°C with washing step(s)	3
DNA extracted with no washing step	0
Other	1

Reference Gene Assessment

Tables 5-7 give the frequency of assessments for the reference gene, the reference genes used, and the frequency of assessments by method and specimen for detecting the reference gene, respectively.

Table 5. Reference Gene Assessment Frequency

Specimen Number	Within Standard Reference Range	Outside Standard Reference Range
418R1	57	2
418R2	59	0
418R3	54	4
418R4	1	58
418R5	59	0

Table 6. Reference Genes used by participants

Method	Number of Laboratories
RNase P coding segments	26
Beta-actin	33

Table 7a. Reference Gene Assessment Category by Method (for evaluated “Follow-up Required” Clinical Assessment Specimens)
Specimen 418R3

Method	Reference Gene Level Within Standard Reference Range	Reference Gene Level Outside Standard Reference Range
Real time PCR – Singleplex	10	0
EnLite™ Neonatal TREC kit	16	4
Real Time PCR – Multiplex	28	0
Other	1	0

Table 7b. Reference Gene Assessment Category by Method (for evaluated “Follow-up Required” Clinical Assessment Specimens)
Specimen 418R4

Method	Reference Gene Level Within Standard Reference Range	Reference Gene Level Outside Standard Reference Range
Real time PCR – Singleplex	1	9
EnLite™ Neonatal TREC kit	0	20
Real Time PCR – Multiplex	0	28
Other	0	1

Note: Reference Gene Level Within Standard Reference Range assessment is assumed when an assessment code was not provided on the data report form.

Evaluations

Evaluations are based on the source of specimen and previously established consensus categorical results from core laboratories.

No False-negatives misclassifications and one False-positive TREC misclassifications were reported.

Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter’s PT specimens for TREC on January 15, 2019.

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The content of this report may also be located on our website at:
https://www.cdc.gov/labstandards/nsqap_reports.html

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CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) ATLANTA, GA 30341

Director
Robert R. Redfield, M.D.

Director
National Center for Environmental Health
Patrick Breysse, Ph.D.

Director
Division of Laboratory Sciences
James L. Pirkle, M.D., Ph.D.

Chief
Newborn Screening and Molecular Biology Branch
Carla Cuthbert, Ph.D.

Contributors

Carter Asef
Nicolle Baird, Ph.D.
John Bernstein
Quan Bui
Paul Dantonio
Sharon Flores
Elizabeth M. Hall
Christopher Haynes, Ph.D.
Laura Centeno Hildreth
Francis Lee, Ph.D.
Lixia Li, Ph.D.
Timothy Lim, Ph.D.
Daniel Mandel, Ph.D.
Joanne Mei. Ph.D.
Kristina Mercer, Ph.D.
Gyliann Peña
Konstantinos Petritis, Ph.D.
Austin Pickens, Ph.D.
E. Shannon Torres, Ph.D.
Robert Vogt, Ph.D.
Irene Williams
Sophia Winchester
Golriz Yazdanpanah
Sherri Zobel

Production
Vinay Anamula
Kimberly Coulter
Joy Pressley
Kizzy Stewart

ASSOCIATION OF PUBLIC HEALTH LABORATORIES SILVER SPRING, MD 20910

President
Joanne Bartkus, PhD, D(ABMM)

Chairman, Newborn Screening and Genetics in Public Health Committee
Michele Caggana, Sc.D., FACMG

Chairman, Newborn Screening Quality Assurance Quality Control Subcommittee
Patricia R. Hunt, B.A. and Joseph Orsini, Ph.D.

Chairman, Newborn Screening Molecular Subcommittee
Rachel Lee, Ph.D.

INQUIRIES TO:
Irene Williams, Editor
Centers for Disease Control and Prevention (CDC), Newborn Screening Quality Assurance Program
Mailstop F-24, 4770 Buford Highway, N.E., Atlanta, GA 30341-3724
E-mail: NSQAPDMT@cdc.gov