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)
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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 1, 2019, 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 119R1, 119R2, 119R3, 119R4, and 119R5). 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
119R1	1	1	Normal cord blood with medium TREC copy level (close to population median of term newborns)
119R2	2	2	Sample with uninterpretable results for SCID. Both TREC and reference gene out of range. Prepared from leukocyte-depleted blood.
119R3	1	1	Normal cord blood with lower TREC copy level
119R4	1	1	Normal cord blood with medium TREC copy level (close to population median of term newborns)
119R5	2	1	SCID-like sample with very low/undetectable TREC; reference gene within acceptable range. Prepared from lymphocyte-depleted blood.

*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 January 15, 2019 a panel of five unknown DBS specimens was sent to 43 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, and Table 3 shows the frequency of TREC misclassifications by each method. 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
119R1	59	0
119R2	0	59
119R3	57	2
119R4	59	0
119R5	0	59

Table 3. Laboratory Methods for TREC

Method	Number of Laboratories
Real Time PCR—Singleplex	10
EnLite™ Neonatal TREC kit	19
Real Time PCR – Multiplex	29
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)	19
DNA extracted at 99°C with washing step(s)	15
DNA extracted at 95°C with washing step(s)	7
DNA extracted at 70°C with washing step(s)	4
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
119R1	56	3
119R2	1	58
119R3	58	1
119R4	52	7
119R5	55	4

Table 6. Frequency of Reference Genes

Method	Number of Laboratories
RNase P coding segments	27
Beta-actin	32

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

Specimen 119R2

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	19
Real Time PCR – Multiplex	0	29
Other	0	1

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

Specimen 119R5

Method	Reference Gene Level Within Standard Reference Range	Reference Gene Level Outside Standard Reference Range
Real time PCR – Singleplex	9	1
EnLite™ Neonatal TREC kit	17	2
Real Time PCR – Multiplex	28	1
Other	1	0

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

Table 7c. Outside Standard Reference Range Classifications by Method for No-Followup Required Specimens

Method	119R1	119R3	119R4
Real Time PCR - Singleplex	0	0	0
EnLite™ Neonatal TREC kit	3	1	7
Real Time PCR - Multiplex	0	0	0
Other	0	0	0

Evaluations

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

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

For expected “No-followup required” specimens 119R1, 119R3 and 119R4, all reference gene assessments considered as inconsistent with expected results were attributed to the EnLite™ Neonatal TREC method.

Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter’s PT specimens for TREC on June 25, 2019.

Acknowledgements

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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

This *NEWBORN SCREENING QUALITY ASSURANCE PROGRAM* report is an internal publication distributed to program participants and selected program colleagues. The laboratory quality assurance program is a project cosponsored by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories.

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