

Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

TREC
Quarterly Report

Volume 5, No. 4

November 2015

INTRODUCTION

This report is the Quarterly summary of data reported within the specified data-reporting period for the Quarter 4, 2015, proficiency testing (PT) program for T-cell receptor excision circle (TREC) analysis in dried blood spots (DBS) to detect severe combined immunodeficiency (SCID). The attached tables provide the certification profiles for the specimens, summary of reported categorical results and the verification of your reported data. We distribute this PT report to all participants, state laboratory directors, and program colleagues by request.

On October 5, 2015 a panel of five unknown DBS specimens was distributed to 33 domestic, 15 international, and one manufacturer laboratory to analyze TREC content in peripheral blood.

PARTICIPANT RESULTS

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 415R1, 415R2, 415R3, 415R4, and 415R5).

We received data from 46 participants by the data reporting deadline. Table 1 shows the certification and description of the specimens in the panel. Table 2 summarizes reported frequency of clinical assessments. Table 3 gives the methods used to assess TREC levels, and Table 4 shows the frequency of methods used to prepare DNA from dried blood spots. 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.

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.

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

No false-negative assessments were reported this quarter. One false-positive assessment was reported for specimen 415R2 (Table 2). False-positive assessments should be monitored and kept as low as possible.

One laboratory reported that the reference gene level for specimen 415R3 was "Within Standard Reference Range" (Table 5) using Multiplex Real Time PCR (Table 7). This specimen (buffy-coat removed) was formulated to mimic an abnormal specimen with TREC and reference gene levels both below the standard reference range (Table 1). Specimen 415R4 represented a SCID-like specimen with very low or no TREC and the reference gene level within standard reference range. Two laboratories using the EnLite Neonatal TREC kit, one laboratory using Multiplex Real Time PCR, and one laboratory using Singleplex reported the reference gene level as "Outside Reference Range" (Table 7).

CDC/APHL

This program is cosponsored by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL).

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The Newborn Screening Quality Assurance Program will ship next quarter's pilot PT specimens for TREC on January 11, 2016. ❖

ACKNOWLEDGMENTS

We would like to thank Barbara Waters-Pick (Duke University Medical Center) for the supply of umbilical cord blood.

NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

T-CELL RECEPTOR EXCISION CIRCLE (TREC) ANALYSIS IN DRIED-BLOOD SPOTS

Quarter 4 – NOVEMBER 2015

TABLE 1. SPECIMEN CERTIFICATION

Specimen Number	No follow-up required Screen Negative	Follow-up Required	Specimen Description	Reference Gene Assessment
415R1	1		Normal specimen; lower TREC level, reference gene level within standard reference range	1
415R2	1		Normal specimen; medium TREC level , reference gene level within standard reference range	1
415R3		2	Blood with 'buffy-coat' removed - TREC and reference gene levels both below standard reference range.	2
415R4		2	SCID-like specimen; very low or no TREC, reference gene level within standard reference range	1
415R5	1		Normal specimen; lower TREC level, reference gene level within standard reference range	1

1 = No follow-up required (Screen Negative)

2 = Follow-up required

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TABLE 2. FREQUENCY OF REPORTED TREC CLINICAL ASSESSMENTS

Specimen Number	No follow-up required (Screen Negative)	Follow-up required
415R1	46	0
415R2	45	1
415R3	0	46
415R4	0	46
415R5	46	0

TABLE 3. LABORATORY METHODS FOR TREC AND REFERENCE GENE

Method	Number of Laboratories
63 Real Time PCR - Singleplex	10
71 Real Time PCR - Multiplex	23
70 EnLite™ Neonatal TREC kit	13
Other	0

TABLE 4. FREQUENCY OF DNA PREPARATION METHODS

DNA Preparation Method	Number of Laboratories
1 In situ/on card (no DNA extraction) with washing step(s)	9
2 EnLite™ (no DNA extraction)	13
3 DNA extracted at 99°C with washing step(s)	16
4 DNA extracted at 95°C with washing step(s)	3
5 DNA extracted at 70°C with washing step(s)	3
6 DNA extracted with no washing step	0
7 Other	1
Not provided	1

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TABLE 5. FREQUENCY OF REFERENCE GENE ASSESSMENT CATEGORY
(for Specimens with the Follow-up Required Clinical Assessment)

Specimen Number	1- Within normal range	2- Outside normal range
415R3	1	44
415R4	41	4

TABLE 6. FREQUENCY OF REFERENCE GENES

Reference Genes	Number of Laboratories
1 RNase P coding segments	19
2 Beta-actin	25
3 Serum albumin	0
4 TERT - Telomerase Reverse	0
5 Other	1
Not provided	1

TABLE 7. REFERENCE GENE ASSESSMENT CATEGORY
RESULTS BY LABORATORY METHOD
(for Specimens with the "Follow-up Required" Clinical Assessment)

METHOD	415R3		415R4	
	1	2	1	2
63 Real Time PCR - Singleplex	0	10	9	1
70 EnLite™ Neonatal TREC kit	0	12	10	2
71 Real Time PCR - Multiplex	1	22	22	1
Other	0	0	0	0

1 = Reference Gene Level Within Standard Reference Range
2 = Reference Gene Level Outside Standard Reference Range

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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