

Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

TREC
Quarterly Report

Volume 6, No. 1

February 2016

INTRODUCTION

This report is the Quarterly summary of data reported within the specified data-reporting period for the Quarter 1, 2016, 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 January 11, 2016 a panel of five unknown DBS specimens was distributed to 34 domestic, 16 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 116R1, 116R2, 116R3, 116R4, and 116R5).

We received data from 48 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 or false-positive assessments were reported this quarter. False-positive assessments should be monitored and kept as low as possible.

Three laboratories reported that the reference gene level for specimen 116R1 was "Outside Standard Reference Range" (Table 5) using the EnLite™ Neonatal TREC kit (Table 7). This specimen represented a SCID-like specimen with very low or no TREC and reference gene level within the standard reference range (Table 1). Specimen 116R4 was prepared using blood with "buffy-coat" removed. The expected TREC and reference gene levels were designed to assay below the standard reference range (Table 1). One laboratory using the EnLite Neonatal TREC kit and one laboratory using Multiplex Real Time PCR reported the reference gene level as "Within Standard Reference Range" (Tables 5 and 7).

The Newborn Screening Quality Assurance Program will ship next quarter's pilot PT specimens for TREC on April 4, 2016. ❖

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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NEWBORN SCREENING QUALITY ASSURANCE PROGRAM
T-CELL RECEPTOR EXCISION CIRCLE (TREC) ANALYSIS IN DRIED-BLOOD SPOTS
Quarter 1 – FEBRUARY 2016

TABLE 1. SPECIMEN CERTIFICATION

Specimen Number	No follow-up required Screen Negative	Follow-up Required	Specimen Description	Reference Gene Assessment
116R1		2	SCID-like specimen; very low or no TREC, reference gene level within standard reference range	1
116R2	1		Normal specimen; lower TREC level, reference gene level within standard reference range	1
116R3	1		Normal specimen; medium TREC level , reference gene level within standard reference range	1
116R4		2	Blood with 'buffy-coat' removed - TREC and reference gene levels both below standard reference range.	2
116R5	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
116R1	0	48
116R2	48	0
116R3	48	0
116R4	0	48
116R5	48	0

TABLE 3. LABORATORY METHODS FOR TREC AND REFERENCE GENE

Method	Number of Laboratories
63 Real Time PCR - Singleplex	10
70 EnLite™ Neonatal TREC kit	15
71 Real Time PCR - Multiplex	23
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)	14
3 DNA extracted at 99°C with washing step(s)	15
4 DNA extracted at 95°C with washing step(s)	5
5 DNA extracted at 70°C with washing step(s)	2
6 DNA extracted with no washing step	0
7 Other	2
Not provided	1

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Quarter 1 – FEBRUARY 2016

TABLE 5. FREQUENCY OF REFERENCE GENE ASSESSMENT CATEGORY
(for expected Follow-up Required specimens)

Specimen Number	1 - Within Standard Reference Range	2- Outside Standard Reference Range
116R1	43	3
116R4	2	44

TABLE 6. FREQUENCY OF REFERENCE GENES

Reference Genes	Number of Laboratories
1 RNase P coding segments	20
2 Beta-actin	27
3 Serum albumin	0
4 TERT - Telomerase Reverse	0
5 Other	0
Not provided	1

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

METHOD	116R1		116R4	
	1	2	1	2
63 Real Time PCR - Singleplex	10	0	0	10
70 EnLite™ Neonatal TREC kit	10	3	1	12
71 Real Time PCR - Multiplex	23	0	1	22
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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