Newborn Screening Quality Assurance Program

Quarterly Report
Volume 7, No.3

T-Cell Receptor Circle in Dried Blood Spots Proficiency Testing Program (TRECPT)

Report Issued: November 4, 2017

Introduction

This is a summary of data reported within the specified data-reporting period for the Quarter 4, 2017, 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 417R1, 417R2, 417R3, 417R4, and 417R5). Table 1 shows the certification and description of the specimens in the panel.

Table 1. Specimen Certification and Description

Specimen Number	No Follow-up Required	Follow-up Required	Specimen Description	Reference Gene Assessment
417R1		2	SCID-like specimen; very low or no TREC, reference gene level within standard reference range	1
417R2	1		Normal specimen; Medium TREC level, reference gene level within standard reference range	1
417R3		2	Blood with 'buffy-coat' removed - TREC and reference gene levels both below standard reference range	2
417R4		2	SCID-like specimen; very low or no TREC, reference gene level within standard reference range	1
417R5	1		Normal specimen; Medium TREC level, reference gene level within standard reference range	1

Distribution of PT Specimens

We distribute this PT report to all participants, state laboratory directors, and program colleagues by request. On October 2, 2017 a panel of five unknown DBS specimens was distributed to 37 domestic, 20 international, and two manufacturer laboratories to analyze TREC content in peripheral blood.

Participant Results

TREC Level Assessment

We received data from 52 participants by the data reporting deadline. For this quarter, Table 2 summarizes reported frequency of clinical assessments. Table 3a provides the methods used to assess TREC levels, and table 3b shows the frequency of False-positive results by each method. Table 4 shows the frequency of methods used to prepare 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
417R1	0	52
417R2*	40	12
417R3	0	52
417R4	0	52
417R5	52	0

*Specimen 417R2 was not evaluated due to lack of 80% consensus.

Table 3a. Laboratory Methods For TREC

Method	Number of Laboratories
63 Real Time PCR—Singleplex	8
70 EnLite™ Neonatal TREC kit	15
71 Real Time PCR - Multiplex	28
Other	1

Table 3b. Frequency of TREC Assessments by Method for Specimen 417R2*

Method	False-positive results
63 Real Time PCR—Singleplex	0
70 EnLite™ Neonatal TREC kit	10
71 Real Time PCR - Multiplex	1
19 Other	1

*Specimen 417R2 was not evaluated

Table 4. Frequency of DNA Preparation Methods

Method	Number of Laboratories
In situ/on card (no DNA extraction) with washing step(s)	13
EnLite™ (no DNA extraction)	14
DNA extracted at 99°C with washing step(s)	15
DNA extracted at 95°C with washing step(s)	5
DNA extracted at 70°C with washing step(s)	2
DNA extracted with no washing step	1
Other	1
Not provided	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
417R1	51	1
417R2	Not evaluated	Not evaluated
417R3*	2	50
417R4	51	1
417R5	52	0

*Reference Gene Assessment expected to be “Outside of Standard Reference Range”.

Table 6. Frequency of Reference Genes

Method	Number of Laboratories
RNase P coding segments	24
Beta-actin	27
Serum albumin	0
TERT - Telomerase Reverse	0
Other	0
Not provided	1

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

	417R1		417R4	
	1	2	1	2
63 Real Time PCR - Singleplex	8	0	8	0
70 EnLite™ Neonatal TREC kit	14	1	14	1
71 Real Time PCR - Multiplex	28	0	28	0
Other	1	0	0	1

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

Note: A normal assessment was assumed when an assessment code when 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 and 2 False-positive assessments were reported for evaluated specimens. The TREC Assessment for Specimen 417T2 was reported as "Within normal limits" by 40 labs and "Outside normal limits" by 12 labs and was considered "not evaluated" due to lack of 80% consensus. Ten of the 12 labs reported using the EnLite™ Neonatal TREC kit . Because 80% consensus was not reached, these misclassifications were not considered to be False-positive. False-positive assessments should be monitored and kept as low as possible.

The following is additional information describing the reference gene assessment frequency (Table 5):

417R1—One lab of 15 using the EnLite™ Neonatal TREC kit reported a reference gene assessment as "Outside Reference Range". This was a SCID-like specimen with very low or no TREC, however the reference gene level was within standard reference range.

417R3—Two labs of 15 using the EnLite™ Neonatal TREC kit reported a reference gene assessment as "Within normal limits". This specimen was prepared by removing the 'buffy-coat' to give an expected result of below the standard reference range for both the TREC and reference gene.

417R4—One lab of 15 using the EnLite™ Neonatal TREC kit reported a reference gene assessment as "Outside Reference Range". This was a SCID-like specimen with very low or no TREC, however the reference gene level was within the standard reference range.

Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter's PT specimens for TREC in January 2018.

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NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

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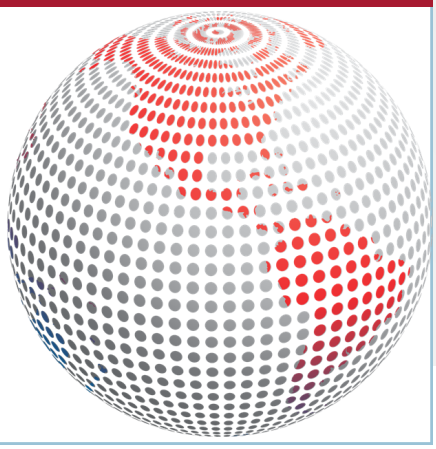

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