Newborn Screening Quality Assurance Program

Quarterly Report
Volume 8, No.1

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

Report Issued: February 26, 2018

Introduction

This is a summary of data reported within the specified data-reporting period for the Quarter 1, 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 118R1, 118R2, 118R3, 118R4, and 118R5). 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
118R1	1		Normal specimen; Lower TREC level , reference gene level within standard reference range	1
118R2		2	SCID-like specimen; very low or no TREC, reference gene level within standard reference range	1
118R3	1		Normal specimen; Medium TREC level, reference gene level within standard reference range	1
118R4	1		Normal specimen; Medium TREC level, reference gene level within standard reference range	1
118R5		2	Blood with 'buffy-coat' removed - TREC and reference gene levels both below standard reference range	2

Distribution of PT Specimens

We distribute this PT report to all participants, state laboratory directors, and program colleagues by request. On January 9, 2018 a panel of five unknown DBS specimens was distributed to 39 domestic, 20 international, and two manufacturer laboratories to analyze TREC content in peripheral blood.

Participant Results

TREC Level Assessment

We received data from 54 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
118R1	52	2
118R2	0	54
118R3	54	0
118R4	39	15
118R5	0	54

Table 3a. Laboratory Methods For TREC

Method	Number of Laboratories
Real Time PCR—Singleplex	9
EnLite™ Neonatal TREC kit	17
Real Time PCR - Multiplex	27
Other	1

Table 3b. Frequency of TREC Assessments by Method for Specimen 118R4

Method	False-positive results
Real Time PCR—Singleplex	0
EnLite™ Neonatal TREC kit	15
Real Time PCR - Multiplex	0
Other	0

Table 4. Frequency of DNA Preparation Methods

Method	Number of Laboratories
In situ/on card (no DNA extraction) with washing step(s)	14
EnLite™ (no DNA extraction)	17
DNA extracted at 99°C with washing step(s)	15
DNA extracted at 95°C with washing step(s)	4
DNA extracted at 70°C with washing step(s)	3
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
118R1	50	4
118R2	53	1
118R3	53	1
118R4	40	14
118R5	0	54

Table 6. Frequency of Reference Genes

Method	Number of Laboratories
RNase P coding segments	24
Beta-actin	29
Other	1

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

	118R2		118R5	
	1	2	1	2
63 Real Time PCR - Singleplex	9	0	0	9
70 EnLite™ Neonatal TREC kit	16	1	0	17
71 Real Time PCR - Multiplex	27	0	0	27
Other	1	0	0	1

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

Note: Normal assessment 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 and 17 False-positive TREC assessments were reported. Fifteen of the False-positive results were attributed to Specimen 118R4 for laboratories reporting the EnLite™ Neonatal TREC method. Committee consensus determined the evaluation of this specimen should be based on the reported method.

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

118R1—Four laboratories using the EnLite™ Neonatal TREC kit reported a reference gene assessment as “Outside Reference Range”. This specimen represented a lower TREC level, and reference gene level within standard reference range.

118R2—One laboratory 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, and reference gene level within standard reference range.

118R3—One laboratory using the EnLite™ Neonatal TREC kit reported a reference gene assessment as “Outside Reference Range”. This is a normal specimen with medium TREC level and reference gene level within standard reference range.

118R4—Fourteen laboratories using the EnLite™ Neonatal TREC kit reported a reference gene assessment as “Outside Reference Range”. This is a normal specimen with medium TREC level and reference gene level within standard reference range. The inability of the EnLite™ assay to amplify both TREC and the beta-actin gene in this sample may suggest PCR inhibition.

118R5 - All laboratories reported the expected TREC clinical assessment as "Follow-up Required" and reference gene assessment as “Outside Reference Range”. This specimen was prepared from blood with 'buffy-coat' removed to give both TREC and reference gene levels as below standard reference ranges.

Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter’s PT specimens for TREC on July 10, 2018.

REPORT AUTHORIZATION

This report has been reviewed and authorized by Dr. Joanne Mei, Lab 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.

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NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

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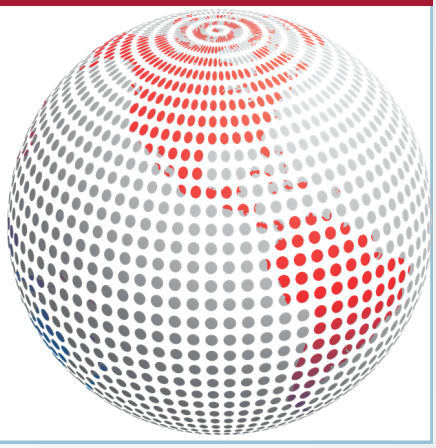

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