Newborn Screening Quality Assurance Program

Quarterly Report
Volume 7, No.2

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

2017 Quarter 3 August

Introduction

This is a summary of data reported within the specified data-reporting period for the Quarter 3, 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 317R1, 317R2, 317R3, 317R4, and 317R5). 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
317R1	1		Normal specimen; Lower TREC level , reference gene level within standard reference range	1
317R2		2	SCID-like specimen; very low or no TREC, reference gene level within standard reference range	1
317R3		2	Blood with 'buffy-coat' removed - TREC and reference gene levels both below standard reference range	2
317R4	1		Normal specimen; Lower TREC level , reference gene level within standard reference range	1
317R5	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 July 10, 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 50 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
317R1	45	4
317R2	0	50
317R3	0	50
317R4	50	0
317R5	50	0

Table 3a. Laboratory Methods For TREC

Method	Number of Laboratories
63 Real Time PCR—Singleplex	9
70 EnLite™ Neonatal TREC kit	14
71 Real Time PCR - Multiplex	26
Other	1

Table 3b. Frequency of False-positive TREC Assessments by Method

Method	False-positive results
63 Real Time PCR—Singleplex	1
70 EnLite™ Neonatal TREC kit	2
71 Real Time PCR - Multiplex	1

Table 4. Frequency of DNA Preparation Methods

Method	Number of Laboratories
1 In situ/on card (no DNA extraction) with washing step(s)	12
2 EnLite™ (no DNA extraction)	14
3 DNA extracted at 99°C with washing step(s)	14
4 DNA extracted at 95°C with washing step(s)	6
5 DNA extracted at 70°C with washing step(s)	3
6 DNA extracted with no washing step	0
7 Other	1
Not provided	0

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. Frequency of Reference Gene Assessment for Expected Follow-up Required Specimens

Specimen Number	Within Std Reference Range	Outside Std Reference Range
317R2	50	0
317R3	0	50

Table 6. Frequency of Reference Genes

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

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

	317R2		317R3	
	1	2	1	2
63 Real Time PCR - Singleplex	9	0	0	9
70 EnLite™ Neonatal TREC kit	14	0	0	14
71 Real Time PCR - Multiplex	26	0	0	26
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 four False-positive assessments were reported this quarter. False-positive assessments should be monitored and kept as low as possible.

One laboratory reported the reference gene level of specimen 317R1 as “Outside Reference Range”, using using Real Time PCR Singleplex. This specimen represented a normal specimen with lower TREC level and reference gene level within standard reference range. Another laboratory reported the reference gene level of specimen 317R4 as “Outside Reference Range” using the EnLite™ Neonatal TREC kit. This specimen also represented a normal specimen with lower TREC level and reference gene level within standard reference range.

Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter’s PT specimens for TREC on October 2, 2017.

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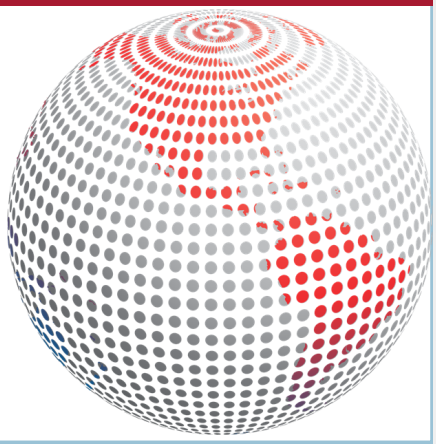
NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

Direct inquiries to:

Centers for Disease Control and Prevention
4770 Buford Highway NE, MS/F19
Atlanta, GA 30341-3724
Phone: 404-488-7945 Email: jvm0@cdc.gov

Editors
Joanne Mei
Irene Williams



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INQUIRIES TO:

Irene Williams, Editor • Centers for Disease Control and Prevention (CDC) • Newborn Screening Quality Assurance Program
Mailstop F-24 • 4770 Buford Highway, N.E. • Atlanta, GA 30341-3724
Phone (770) 488-4582 • NSQAPDMT@cdc.gov
E-mail: IWilliams1@cdc.gov