

Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

TREC
Quarterly Report

Volume 5, No. 3

August 2015

INTRODUCTION

This report is the Quarterly summary of data reported within the specified data-reporting period for the Quarter 3, 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 July 13, 2015 a panel of five unknown DBS specimens was distributed to 29 domestic and 13 international laboratories 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 315R1, 315R2, 315R3, 315R4, and 315R5).

We received data from thirty-nine participants by the data reporting deadline. Tables 1 shows the certification and description of the specimens in the panel. Tables 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.

One false-negative and no false-positive assessments were reported for TREC (Table 2). False-positive assessments should be monitored and kept as low as possible.

One laboratory reported that the reference gene level for specimen 315R1 was "Within Standard Reference Range" (Table 5) using Singleplex Real Time PCR (Table 7). This specimen was formulated to mimic a SCID specimen with TREC and reference gene levels both below the standard reference range (Table 1).

The Newborn Screening Quality Assurance Program will ship next quarter's pilot PT specimens for TREC on October 5, 2015. ❖

ACKNOWLEDGMENTS

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

CDC/APHL

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

Direct inquiries to:
Centers for Disease Control and Prevention (CDC)
4770 Buford Highway, NE, MS/F43
Atlanta, GA 30341-3724

Phone: 770-488-7945
FAX: 770-488-4255
E-mail: JMei@cdc.gov

Editor: Joanne Mei
Irene Williams



NEWBORN SCREENING QUALITY ASSURANCE PROGRAM
T-CELL RECEPTOR EXCISION CIRCLE (TREC) ANALYSIS IN DRIED-BLOOD SPOTS
Quarter 3 – AUGUST 2015

TABLE 1. SPECIMEN CERTIFICATION

Specimen Number	No follow-up required Screen Negative	Follow-up Required	Specimen Description	Reference Gene Assessment
315R1		2	Blood with 'buffy-coat' removed - TREC and reference gene levels both below standard reference range.	2
315R2	1		Normal specimen; lower TREC level, reference gene level within standard reference range	1
315R3		2	SCID-like specimen; very low or no TREC, reference gene level within standard reference range	1
315R4	1		Normal specimen; higher TREC level, reference gene level within standard reference range	1
315R5	1		Normal specimen; medium TREC level , reference gene level within standard reference range	1

1 = No follow-up required (Screen Negative)
2 = Follow-up required

NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

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

Quarter 3 – AUGUST 2015

TABLE 2. FREQUENCY OF REPORTED TREC CLINICAL ASSESSMENTS

Specimen Number	No follow-up required (Screen Negative)	Follow-up required
315R1	1	38
315R2	39	0
315R3	0	39
315R4	39	0
315R5	39	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	22
70 EnLite™ Neonatal TREC kit	7
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)	10
2 EnLite™ (no DNA extraction)	7
3 DNA extracted at 99°C with washing step(s)	17
4 DNA extracted at 95°C with washing step(s)	2
5 DNA extracted at 70°C with washing step(s)	3
6 DNA extracted with no washing step	0
7 Other	0
Not provided	0

NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

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

Quarter 3– AUGUST 2015

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
315R1	1	38
315R3	39	0

TABLE 6. FREQUENCY OF REFERENCE GENES

Reference Genes	Number of Laboratories
1 RNase P coding segments	19
2 Beta-actin	18
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

METHOD	315R1		315R2		315R3		315R4		315R5	
	1*	2**	1	2	1	2	1	2	1	2
63 Real Time PCR - Singleplex	1	9	0	0	10	0	0	0	0	0
71 Real Time PCR - Multiplex	0	22	5	0	22	0	5	0	5	0
70 EnLite™ Neonatal TREC kit	0	7	0	0	7	0	0	0	0	0
Other	0	0	0	0	0	0	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**.

CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC)
ATLANTA, GA 30341

Director

Thomas R. Frieden, M.D., M.P.H.

Director

National Center for Environmental Health

Patrick Breyse, Ph.D.

Director

Division of Laboratory Sciences

James L. Pirkle, M.D., Ph.D.

Chief

Newborn Screening and Molecular Biology Branch

Carla Cuthbert, Ph.D.



Contributors:

Carter Asef
Paul Dantonio
Victor R. De Jesus, Ph.D.
Marie C. Earley, Ph.D.
Sharon Flores
Stephanie Foster
Elizabeth M. Hall
Christopher Haynes, Ph.D.
Kameron Khaksarfard
Francis Lee, Ph.D.
Lixia Li, Ph.D.
Timothy Lim, Ph.D.
Daniel Mandel, Ph.D.
Joanne Mei, Ph.D.
Gyliann Peña
Kelsey Sheard
Robert Vogt, Ph.D.
Irene Williams
Golriz Yazdanpanah
Hui Zhou, Ph.D.
Sherri Zobel

Production:

Sarah Brown
Felicia Manning
Chinh Nguyen
LoNeka Shockley

ASSOCIATION OF PUBLIC HEALTH LABORATORIES
SILVER SPRING, MD 20910



President

Judith C. Lovchik, Ph.D., D(ABMM)

Chairman, Newborn Screening and Genetics in Public Health Committee

Susan M. Tanksley, Ph.D.

Chairman, Newborn Screening Quality Assurance Quality Control Subcommittee

Patricia R. Hunt, B.A. and Joseph Orsini, Ph.D.

INQUIRIES TO:

Irene Williams, Editor • Centers for Disease Control and Prevention (CDC)
Newborn Screening Quality Assurance Program • Mailstop F-43
4770 Buford Highway, N.E. • Atlanta, GA 30341-3724
Phone (770) 488-4582 • FAX (770) 488-4255 • E-mail: IWilliams1@cdc.gov