

Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

**TREC
Quarterly Report**

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May 2015

INTRODUCTION

This report is the Quarterly summary of data reported within the specified data-reporting period for the Quarter 2, 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 April 6, 2015 a panel of five unknown DBS specimens was distributed to 26 domestic and 14 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 215R1, 215R2, 215R3, 215R4, and 215R5).

Tables 1 shows the certification and description of the specimens in the panel. Tables 2 summarizes the 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

We processed data from 38 participants. No 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.

Two laboratories reported that the reference gene level for specimen 215R2 was "Outside Standard Reference Range" (Table 5) using the EnLite™ Neonatal TREC kit (Table 7). This specimen was formulated to mimic a SCID specimen with low or no TREC and a reference gene level within the standard reference range (Table 1). This result indicates the method may be returning inconsistent results for the reference gene.

The Newborn Screening Quality Assurance Program will ship next quarter's pilot PT specimens for TREC on July 13, 2015. ❖

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CDC/APHL

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

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

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
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TABLE 1. SPECIMEN CERTIFICATION

Specimen Number	No follow-up required Screen Negative	Follow-up Required	Specimen Description	Reference Gene Assessment
215R1	1		Normal specimen; medium TREC level, reference gene level within standard reference range	1
215R2		2	SCID-like specimen; low or no TREC, reference gene level within standard reference range	1
215R3	1		Normal specimen; medium TREC level, reference gene level within standard reference range	1
215R4	1		Normal specimen; below average TREC level, reference gene level within standard reference range	1
215R5		2	Blood with 'buffy-coat' removed - TREC and reference gene levels both below standard reference range.	2

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
215R1	38	0
215R2	0	38
215R3	38	0
215R4	38	0
215R5	0	38

TABLE 3. LABORATORY METHODS FOR TREC AND REFERENCE GENE

Method	Number of Laboratories
63 Real Time PCR - Singleplex	11
71 Real Time PCR - Multiplex	20
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)	8
2 EnLite™ (no DNA extraction)	6
3 DNA extracted at 99°C with washing step(s)	16
4 DNA extracted at 95°C with washing step(s)	3
5 DNA extracted at 70°C with washing step(s)	2
6 DNA extracted with no washing step	0
7 Other	1
Not provided	2

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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
215R2	36	2
215R5	0	38

TABLE 6. FREQUENCY OF REFERENCE GENES

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

METHOD	215R1		215R2		215R3		215R4		215R5	
	1*	2**	1	2	1	2	1	2	1	2
63 Real Time PCR - Singleplex	11	0	11	0	11	0	11	0	0	11
71 Real Time PCR - Multiplex	20	0	20	0	20	0	20	0	0	20
70 EnLite™ Neonatal TREC kit	7	0	5	2	7	0	7	0	0	7
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

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Director

Division of Laboratory Sciences

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

Chief

Newborn Screening and Molecular Biology Branch

Carla Cuthbert, Ph.D.



Contributors: Barbara W. Adam
Paul Dantonio
Victor R. De Jesus, Ph.D.
Marie C. Earley, Ph.D.
Sharon Flores
David Foreman
Stephanie Foster
Elizabeth M. Hall
Christopher Haynes, Ph.D.
Sarah Klass
Francis Lee, Ph.D.
Lixia Li, Ph.D.
Timothy Lim, Ph.D.
Daniel Mandel, Ph.D.
Joanne Mei, Ph.D.
Patrick Pickens
Kelsey Sheard
Jennifer Taylor, Ph.D.
Robert Vogt, Ph.D.
Irene Williams
Golriz Yazdanpanah
Hui Zhou, Ph.D.
Sherri Zobel

Production: Sarah Brown
Iris Landers
Felicia Manning
LoNeka Shockley

ASSOCIATION OF PUBLIC HEALTH LABORATORIES
SILVER SPRING, MD 20910

President

Dan Rice, DrPH, MS.

Chairman, Newborn Screening and Genetics in Public Health Committee

Susan M. Tanksley, Ph.D.

Chairman, Newborn Screening Quality Assurance Quality Control Subcommittee

Patrick Hopkins, B.S.



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