Newborn Screening Quality Assurance Program T-Cell Receptor Circle in Dried Blood Spots Proficiency Testing Program (TRECPT)

In co-sponsorship with Association of Public Health Laboratories (APHL)
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Report Authorization

This report has been reviewed and authorized by Dr. Suzanne Cordovado, Laboratory Chief, Molecular Quality Improvement Program.

Confidentiality Statement

NSQAP participant information and evaluations are strictly confidential and shared only with individual participants, unless written authorization for release is received.

Introduction

This report summarizes all results submitted within the data-reporting period for the Quarter 1, 2020, proficiency testing (PT) program for T-cell receptor excision circle (TREC) analysis in dried blood spots (DBS) to detect severe combined immunodeficiency (SCID). The report is distributed to all participants, state laboratory directors, and program colleagues by request. The contents provide the certification profiles for the distributed specimens, screening methods, DNA extraction methods, reference genes used by participants, and the overall summary of reported clinical assessments. An evaluation of submitted data is attached to individual laboratory reports.

Certification of PT Specimens

The Quarter 1 panel consisted of five DBS specimens (specimens 2011501, 2011502, 2011503, 2011504, and 2011505) prepared from human blood, including umbilical cord blood from unaffected individuals and adult blood or modified adult blood depleted of mononuclear cells or leukocytes. Table 1 shows the certification and description of the specimens in the panel.

Table 1. Specimen Certification and Description

Specimen Number	Clinical Assessment*	Specimen Description
2011501	2	SCID-like sample with very low/undetectable TREC; reference gene within acceptable range.
2011502	3	Unsatisifactory sample - both TREC and reference gene are out-of-range.
2011503	1	Normal sample; TREC and reference gene within acceptable range.
2011504	3	Unsatisifactory sample - both TREC and reference gene are out-of-range.
2011505	1	Normal sample; TREC and reference gene within acceptable range.

* Clinical Assessment Code Key:
1 – Screen Negative (no follow-up required)
2 – Screen Positive (TREC out-of-range, reference gene in-range)
3 – Unsatisfactory sample (both TREC and reference gene out-of-range)

Distribution of PT Specimens

On January 14, 2020, NSQAP distributed a panel of five unknown DBS specimens to 75 partcipants to analyze the TREC content in peripheral blood.

Participant Results

Data was received from 68 participants by the data reporting deadline. One laboratory submitted data for the incorrect quarter and was not evaluated. Participants tested specimens by the analytical schemes they routinely use in their laboratory. Reported data includes the laboratory methods used to detect TREC levels, DNA extraction, the reference gene and the clinical assessment.

Reported Method Data

Tables 2-5 summarize the reported frequency of methods used to assess TREC levels, DNA Extraction methods used, reference genes used, clinical assessments and misclassifications. Qualitative, categorical results of Screen Negative (no follow-up required), Screen Positive (TREC out-of-range, reference gene in-range), and Unsatisfactory sample (TREC and reference gene out-of-range) were requested for each specimen.

Table 2. Reported Laboratory Methods for TREC

Method	Number of Laboratories
Real Time PCR – TREC only (reference gene run separately)	5
Real Time PCR – TREC AND Reference Gene run in a single tube	27
Real Time PCR – TREC/SMN1 AND Reference Gene run in a single tube	11
EnLite™ Neonatal TREC kit	20
Other	4

Table 3. Reported DNA Extraction Methods

Extraction Method	Number of Laboratories
In situ/on card (DNA is NOT extracted)	12
EnLite™ (DNA is NOT extracted)	20
Generations™ DNA Purification and Elution Solutions (S1/S2)	6
Generations™ Elution Solution (S2 only)	5
Extracta™ DBS with one wash	6
Other	15
No DNA Extraction Method Reported	3

Table 4. Reported Reference Genes

Reference Gene	Number of Laboratories
RNase P subunit (RPP30)	17
RNase P subunit (RPPH1)	5
Beta-actin (ACTB)	37
Other	5
No Reference Gene Reported	3

Table 5. Reported Clinical Assessments and Misclassifications

Specimen Number	1 – Screen Negative (no follow–up required)	2 - Screen Positive (TREC out-of-range reference gene in-range)	3 - Unsat Sample (TREC and reference gene out-of-range)	Clinical Assessment not Reported	Incorrect Clinical Assessments
2011501	0	66	1	0	1
2011502	0	1	66	0	1
2011503	64	2	1	0	3
2011504	0	2	65	0	2
2011505	62	0	4	1	4

Table 6. Incorrect Assessments observed by Method for each specimen number

Method	Number of Laboratories	2011501	2011502	2011503	2011504	2011505
Real Time PCR – TREC only (reference gene run separately)	5	0	0	0	0	0
Real Time PCR – TREC AND Reference Gene run in a single tube	23^	0	0	0	0	0
Real Time PCR – TREC/SMN1 AND Reference Gene run in a single tube	11	0	1	0	1	0
EnLite™ Neonatal TREC kit	20	1	0	1	1	4
Other - ImmunoIVD - SPOT-it™	6	0	0	2	0	1
Other	2	0	0	0	0	0

^ 4 labs removed from "Real Time PCR – TREC AND Reference Gene run in a single tube" method and placed into Other - ImmunoIVD SPOT-it™ method

*Methods are designated as “Other” when less than three participants report results for a given method.
“Other” methods include:
EnLite™ TREC/KREC kit
LDT (TREC/SMN1/RPP30) / EnLite™ TREC

Evaluations

Evaluations are based on the clinical assessment of the five specimens. Data was received from 68 participants and data was evaluated for 67 participants. One laboratory submitted an invalid entry and was not evaluated. Eleven incorrect clinical assessments were reported, and one clinical assessment was not reported in this quarter.

Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter’s PT specimens for TREC on June 23, 2020.

The content of this report may also be located on our website at: https://www.cdc.gov/labstandards/nsqap_reports.html

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.

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