# 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 3, 2019, 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

This Quarter 3 panel consisted of five DBS specimens prepared from human blood, including cord blood from unaffected individuals and adult blood or modified adult blood depleted of mononuclear cells or leukocytes (specimens 319R1, 319R2, 319R3, 319R4, and 319R5). 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 |
|---|---|---|
| 319R1 | 2 | SCID-like sample with very low/undetectable TREC; reference gene within acceptable range. |
| 319R2 | 1 | Normal sample; TREC and reference gene within acceptable range. |
| 319R3 | 3 | Unsatisifactory sample - both TREC and reference gene are out of range. |
| 319R4 | 1 | Normal sample; TREC and reference gene within acceptable range. |
| 319R5 | 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 June 25, 2019 NSQAP distributed a panel of five unknown DBS specimens to 67 partcipants to analyze the TREC content in peripheral blood.

## Participant Results

Data was received from 61 participants by the data reporting deadline. One laboratory submitted data on an incorrect form and was not evaluated. Participants tested specmens by the analytical schemes they routinely use in their laboratory. Reported data includes the laboratory method used to detect TREC levels, DNA extraction method, 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—Singleplex | 9 |
| Real Time PCR – TREC/Reference Gene Multiplex | 20 |
| Real Time PCR – TREC/SMN1/Reference Gene Multiplex | 8 |
| EnLite™ Neonatal TREC kit | 19 |
| Other | 4 |

Table 3. Reported DNA Extraction Methods

| Extraction Method | Number of Laboratories |
|---|---|
| In situ/on card (no DNA extraction) | 12 |
| EnLite™ (non DNA extraction) | 20 |
| Generations DNA Purification and Elution Solutions (S1/S2) | 6 |
| Generations Elution Solution (S2 only) | 7 |
| Extracta DBS with one wash | 4 |
| Other | 11 |

Table 4. Reported Reference Genes

| Reference Gene | Number of Laboratories |
|---|---|
| RNase P subunit (RPP30) | 17 |
| RNase P subunit (RPPH1) | 6 |
| Beta-actin (ACTB) | 31 |
| Other | 5 |

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) | Incorrect Clinical Assessments |
|---|---|---|---|---|
| 319R1 | 1 | 59 | - | 1 |
| 319R2 | 59 | 1 | - | 1 |
| 319R3 | - | 2 | 58 | 2 |
| 319R4 | 59 | 1 | - | 1 |
| 319R5 | 60 | - | - | - |

## Evaluations

Evaluations are based on the clinical assessment of the five specimens where each counts for 20% of the evaluation. Five incorrect clinical assessments were reported in this quarter.

## Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter's PT specimens for TREC on September 24, 2019.

## Acknowledgements

We would like to thank Ann Kaestner, MT(ASCP) (Carolinas Cord Blood Bank) for the supply of umbilical cord blood.

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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