Newborn Screening for Severe Combined Immunodeficiency: An Improved Real-time PCR Assay

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Introduction  Severe combined immunodeficiency (SCID) is a group of disorders caused by more than a dozen single gene defects. All known gene mutations cause a defect in the development of normal naïve T-cells, leading to combined cellular and humoral immunodeficiency. The Wisconsin Newborn Screening (NBS) program began statewide screening for SCID in January, 2008.1 SCID was added to the Recommended Uniform Screening Panel in May, 2010. Most NBS programs screen for SCID by assaying T-cell receptor excision circles (TRECs) using real-time quantitative PCR (RT-qPCR).2 TRECs, generated during T cell development, are abundant in healthy infants’ dried blood NBS specimens, and are very few or undetectable in the dried blood spots of patients with SCID.3 In a RT-qPCR TREC assay protocol, a reference gene amplification is used as an isolated DNA quality and quantity indicator: screened specimens are deemed as unsatisfactory specimens when low TREC results are accompanied with a poor reference gene amplification. Commonly used reference genes are β-actin and RNase P. To date, the quantitation of TRECs by RT-qPCR appears to be compatible with the state NBS programs, but challenges remain, such as to obtain sufficient DNA from NBS so there are fewer reported unsatisfactory specimens, which require repeat NBS, to obtain easily accessible and reliable DNA copy number quantitation standards to achieve accuracy and consistency of the quantitation of TRECs. A simple yet efficient DNA isolation method and reliable DNA copy number quantitation standards containing both the target and reference gene would further improve SCID newborn screening assay performance.

Objective  To develop and validate an improved method of quantitating TRECs from dried blood spots (DBS) on de-identified residual NBS cards using a novel DNA isolation method and novel gBlock constructed TREC/RPP30 DNA copy number quantitation standards.

Methods

The de-identified residual dried DBS underwent single wash with a laboratory developed DNA isolation buffer. This buffer has been patented by the Wisconsin Alumni Research Foundation and is now licensed and sold as part of Quantabio’s Extracta DBS. The DNA isolation was achieved by incubating the washed specimens with 54 μL of the same buffer at 56°C for 25 minutes. One ninth (6 μL) of the extract solution was used in a standard real-time PCR reaction with gBlock constructed TREC/RPP30 as quantitation standards. The validation process consisted of assay analytic performance (accuracy, linearity, precision, and reproducibility). The screening cutoff value was determined based on TREC value distribution in a set of 6,018 de-identified residual dried blood NBS specimens, and assay clinical performance (sensitivity, specificity, and clinical validity) was also evaluated accordingly.

Results and Conclusion

The assay showed satisfactory accuracy, linearity, precision, and reproducibility based on the results from a set of dried blood specimens with known TREC copy numbers.

We have successfully validated an improved RT-qPCR to quantitate TRECs used in NBS for SCID and other severe T cell lymphopenias.

References