Final Report

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Novel Technologies in Newborn Screening

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Summary

Cystic Fibrosis (CF), Congenital Hypothyroidism (CH) and Congenital Adrenal Hyperplasia (CAH): A multiplexed immunoassay using the markers T4 and TSH for the detection of CH, 17-OHP for the detection of CAH, and IRT1 and IRT2 for the detection of CF was validated with clinical samples. We are currently analyzing the data in preparation for publication.

Severe Combined Immunodeficiency (SCID): We developed and validated an immunoassay to detect T-cell immunodeficiencies in 3.2mm newborn dry blood spots using antibodies to the T-cell marker CD3. The performance of the CD3 immunoassay was compared to the TREC assay. This evaluation showed that the CD3 immunoassay could be a valuable addition to screening for SCID.

Biotinidase Deficiency Genotyping: A multiplexed biotinidase mutation analysis assay has been successfully developed. Data has been analyzed and a manuscript has been submitted to Clinical Chemistry.

Work Performed and Results Obtained

Phenotypic Multiplex Assays

- **CF, CH, and CAH Assays**

  We completed the validation of the CF, CH, and CAH multiplex assay using 1,100 newborn dry blood samples, including samples from infants diagnosed with CF, CH and CAH.

  We performed these assays by simultaneously measuring T4, TSH, 17-OHP, IRT1 and IRT2 in 3.2mm dry blood spots; we are now analyzing the data in preparation for a manuscript.

  Our assay segregates successfully samples from infants diagnosed with CF, CH and CAH from samples from infants who were negative for these conditions.

  The results from our ongoing analysis are included. Previously, we reported the development of a duplex immunoassay using IRT1 and IRT2 as markers for CF. In pathogenic conditions affecting the pancreas, the anionic trypsin, IRT2, is elevated and becomes dominant over IRT1. This assay has the advantages of detecting both forms of IRT. The results of this study were published in Clinical Chemistry 56:3 445-450 (2010).

- **SCID**

CD3 assay:
Our original immunoassay was published in Clinical Chemistry 56:9, 460-1465 (2010). We have improved on this assay by using an anti-CD3 peptide antibody raised in chicken, which we designed and had custom made by Invitrogen. We evaluated this new assay using 124 coded neonate dried blood spots obtained from the Statens Serum Institut, Copenhagen, DK. Eleven were from infants with T-cell related immunodeficiencies, all were correctly identified by our assay. We have described this study in a manuscript that was accepted on March 28, 2011 for publication in the August issue of Clinical Chemistry (copy was enclosed in our quarterly report of March 2011).

We have assessed the performance of this CD3 assay by carrying out a comparison study to the TREC assay implemented by the New York State Newborn Screening Laboratory, the results of this study are described in the National SCID Pilot Project report.

CD19 assay:
The detection of the absence of B-cells is important for identifying certain forms of SCID. Toward this purpose, we selected antibodies to B-cell markers. Using custom made anti-CD19 peptide IgY antibodies in combination with a mouse monoclonal anti-CD19 antibody, we have not yet obtained the needed sensitivity to successfully identify the absence of B-cells. In order to increase the probability of a successful immunoassay to detect B-cells, we proposed to add an additional marker, CD20 to CD19. We were unable to continue pursuing this approach to increase the sensitivity of the assay because this work was suspended in April 2011, at the request of the sponsor.

Genotypic Multiplex Assays

- **Biotinidase**

We have developed a microsphere-based array genotyping method for the simultaneous detection of six disease-causing mutations in the biotinidase gene (BTD), thereby permitting second tier molecular analysis. Genomic DNA was extracted from 3.2mm dried blood spots. BTD gene sequences, containing the mutations G98:d7i3, A171T, D444H, G451D, Q456H, and R538C were amplified by multiplexed PCR, followed by multiplexed allele-specific primer extension using universally tagged genotyping primers. The products were then hybridized to anti-tag carrying xTAG© microspheres and detected on the Luminex platform. Genotypes were verified by sequencing.

Genotyping results of 22 known biotinidase deficient samples by our multiplexed biotinidase assay was in concordance with the results obtained from DNA sequencing for all 6 mutations used in our panel.

A manuscript described this assay is currently in review at Clinical Chemistry.