NewSTEPs Website Model Practices for Newborn Screening
Illinois Department of Public Health
Newborn Screening for Pompe Disease

Purpose: To capture model practices from within the newborn screening community, with the purpose of providing ready access through the NewSTEPs website.

Topic, Problem, Methods, Resolution

The State of Illinois passed legislation in 2007 directing the Illinois Department of Public Health (IDPH) to establish screening for five lysosomal storage disorders (LSDs), among these being Pompe Disease. Additional legislation in 2011 expanded the number of LSDs screened to seven, and added provisions for the establishment of the following parameters:

- A method cleared either by the US Food and Drug Administration (FDA) or validated under the Clinical Laboratory Improvement Amendments (CLIA).
- Quality control and proficiency testing materials.
- Appropriate equipment for screening.
- Adequate funding.

Statewide screening is expected to begin by July 2014.

Describe How You Worked on the Topic

In November 2010, Illinois started a pilot screening program for Pompe Disease (and two other LSDs) using a newly developed microfluidic platform to assay for the enzyme GAA. During this pilot, the newborn screening (NBS) laboratory analyzed 8,012 dried blood spots (DBSs). Of these, two were reported as abnormal for GAA but were confirmed negative by second-tier tests. Due to the requirement of screening for multiple enzymes and the high volume of newborn screening in Illinois (~175,000 specimens/year), the laboratory decided to discontinue the microfluidic platform in May 2011 and began applying tandem mass spectrometry (MS/MS) technology.

In September 2011 the Illinois Department of Public Health (IDPH) initiated studies to optimize a multiplex assay that had been developed at the University of Washington for six LSD-associated enzymes, including GAA, using a single dried blood spot (DBS) punch, a single buffer, and in-line chromatographic purification. Earlier MS/MS assays had been designed for individual enzymes performed under their optimal conditions, and, following incubation, a solid-phase extraction would be performed to remove unwanted materials. The remainder of the sample would then be flow injected into the MS/MS to quantify products and internal standards.

After further improvements of the multiplex assay, the IDPH laboratory was able to show good activities for all six enzymes using a single buffer with three-hour incubation and an in-line ultra-high performance liquid chromatographic separation prior to injection in a 2.5 minute injection cycle per specimen. Using

these conditions, the laboratory can analyze more than 400 DBSs per MS/MS per day. To date, more than 10,000 de-identified DBSs have been analyzed for GAA using the six-plex assay with good reproducibility and a greater than 20-fold difference in signal between specimens with normal versus abnormal enzyme activities.

WHO WAS INVOLVED?

Development of the six-plex method at IDPH was under the supervision of Khaja Basheeruddin, Ph.D. and Rong Shao, M.D., with technical assistance from Fran Doerr, Pearlie Gardley, and Tamara Simulick. Administrative support was provided by the IDPH Office of Health Protection. Material support (substrates, internal standards, and controls) and valuable assistance in training and quality assurance were provided by the staff of the CDC Newborn Screening and Molecular Biology Branch. Technical advice was provided by Joseph Orsini, Ph.D. of the Wadsworth Center in New York and Michael Gelb, Ph.D. of the University of Washington, who also suggested method improvements and provided improved internal standards. Scientists and information technology staff of the PerkinElmer Corporation helped to provide the materials (substrates, internal standards, and controls), instrumentation, database development, and reference testing for method validation. Control dried blood spots from positive Pompe cases were provided by Dr. Barbara Burton of Lurie Children's Hospital in Chicago and Dr. Roberto Giugliani of the Hospital de Clinicas, Porto Alegre, Brazil.

WHAT ARE THE KEY THINGS YOU LEARNED FROM THIS PROCESS? WHAT WERE THE ELEMENTS THAT HELPED YOU TO SUCCEED?

Many different individuals with a wide range of skills need to work together to successfully develop a complex, high-throughput analytical assay. The process will take longer than initially anticipated and regular interaction and good communications are paramount.

WHAT WOULD YOU DO DIFFERENTLY NEXT TIME? WHAT WERE THE BARRIERS OR CHALLENGES?

Next time I would not underestimate the challenges in adapting an undeveloped test for high-throughput screening. Most challenges came from dealing with outside expectations and coordinating contracts and vendors.

WHAT ARE YOU ESPECIALLY PROUD OF IN HOW YOU MANAGED THIS PROCESS?

I am especially proud of the cooperation of all the different parties involved in seeing this project advance to the point it has.

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