Course Objectives and Outline

NBS Molecular Training Workshop

March 9 - 13, 2015

Rachel Lee, PhD
Texas Department of State Health Services

Suzanne Cordovado, PhD
Molecular Quality Improvement Program, CDC
Course Objectives

After completion of the NBS Molecular Workshop, participants will be able to:

- Identify scenarios when a molecular test is appropriate in the newborn screening laboratory.
- Describe the benefits of using a molecular test for different newborn screening disorders.
- Name at least two applications of molecular testing in newborn screening.
- Describe the common protocols for DNA extraction and DNA quantitation from dried blood spots.
Course Objectives – cont.

- Describe a PCR-based assay to detect a single or small number of mutations.
- Conduct DNA extraction, DNA quantitation, PCR amplification, gel electrophoresis, and mutation detection methods.
- Understand quality control procedures and requirements specific for molecular testing.
- Understand data reporting and interpretation as it relates to molecular testing.
Day 1 Lectures:

- Overview of NBS Molecular Assays - When to use a molecular test and why
- Overview of Hemoglobinopathies and Galactosemia
- DNA Extraction and Quantitation
- ARMS PCR Overview
Day 2 Lectures:

- Overview of SCID and the TREC assay
- Case Study of Molecular Assay Validation: Primary Molecular Screening
- Lab Math - Molarity
Day 3 Lectures:

- Molecular Methods Used in NBS Genotyping
- Overview of Cystic Fibrosis
- Case Study of Molecular Assay Validation: 2nd tier Molecular Screening
- Lab Math – Primer Preparation and Dilutions
- Post-analytic Issues - Data Reporting and Clinical Interpretation
Day 4 Lectures:

- Molecular Lab Design, QA/QC Considerations
- Whole Genome Sequencing and Molecular Medicine
- NSMBB Molecular Programs:
  - CF PT (NSQAP)
  - SCID PT/MPES (NSTRI)
- Q&A: Lab Issues
Day 5 Lectures and Test:

- Test
- Molecular Assessment Program
- NBS Molecular Website
- Tour of NSQAP and BMSL laboratories
- Course Evaluation
- Final Remarks
Day 1 Lab Activity:

- DNA Extraction: Boil Prep Method
- DNA Extraction: Boil Prep Generation Method
- Single Mutation Detection: ARMS-PCR Set Up
Day 2 Lab Activity:

- qPCR: TREC and RNAse P Set Up
Day 3 Lab Activity:

- Completion of ARMS-PCR: Gel Electrophoresis and Analysis
- Analysis of qPCR: RNAse P
- Analysis of qPCR: TREC
- DBS in-situ TREC Assay
- Automated Liquid Handling Demonstration
Day 4 Lab Activity:

- Allelic Discrimination (TaqMan) Set Up
- Analysis of Allelic Discrimination (TaqMan)
- DNA Sequencing Platform Discussion
Other Laboratory Activities:
- Analysis of all Laboratory Assays
- Liquid Handling Robotics Demonstration
- DBS in-situ TREC assay set up
- DNA Sequencing Platforms Discussion
<table>
<thead>
<tr>
<th>Day 1</th>
<th>DNA Extraction</th>
<th>Mutation Detection</th>
<th>qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen Lysis DNA Extraction</td>
<td>4 samples for GALT mutation detection</td>
<td>GALT ARMS PCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 samples for TREC/RNase P</td>
<td>Using the 4 samples Qiagen Lysis</td>
<td></td>
</tr>
<tr>
<td>Boil Prep DNA Extraction</td>
<td>7 samples for TREC/RNase P</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 2</th>
<th></th>
<th>TREC/RNase P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Using 7 samples Qiagen Lysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Using 7 samples Boil Prep</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 3</th>
<th></th>
<th>TREC Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel Electrophoresis of ARMS PCR</td>
<td>RNase P Analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TREC/RNase P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Using DBS in-situ method</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 4</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GALT TaqMan Allelic Discrimination</td>
<td></td>
</tr>
</tbody>
</table>