Quality Assurance Program for Cystic Fibrosis Newborn Screening

Marie C. Earley, Ph.D.
Research Microbiologist

APHL Training Course
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CF Mutation Detection Proficiency Testing Program

- Began as a collaborative effort between CDC and 3 CF Centers
- Specimens drawn from adult or adolescent CF patients and are NOT enriched with IRT (No IRT testing done).
- Began quarterly shipments in February 2007
- Program has grown from 25 to 64 laboratories covering 18 countries
- Repository contains all of the ACMG recommended mutations and additional mutations
IRT PT versus CF DNA PT

- 182 Participants
- 1 Analyte
- 7 Methods
  - 2 kits used in US
  - 5 kits used internationally
  - All commercially available

- 64 Participants
- 1 to ≥71 “Analytes”
- 28 Methods
  - 3 kits (FDA-approved) + 4 LDTs used in US
  - 11 kits and 11 LDTs used internationally
  - Some assays are not commercially available
Many Different Methods*

- **Luminex xTAG CF 39/60/v2**
- **Hologic CF Inplex Assay 23 or 40+4 (Invader)**
- In-house
  - Amplification/gel electrophoresis
  - TaqMan assay
  - Luminex platform
- **Luminex xTAG CF 71 v2**
- Innogenetics (Hybridization; 19 or 36 mutations)
- Abbott Diagnostics Oligonucleotide Ligation Assay
- Elucigene (ARMS; 4, 29, 30, or 50 mutations)
- MALDI-TOF mass spectrometry
- High Resolution Melting Temperature assay
- Amplification/Heteroduplex/restriction analysis
- Sequencing

*Many international labs use 2 or more of the listed methods*
Most Common Issues

- **Laboratory space**
  - Pre- and post PCR space

- **Vocabulary**
  - Homozygote, heterozygote, compound heterozygote

- **Contamination**
  - Specific protocols must be followed

- **Complex Assays → Complex Troubleshooting**

- **Extraction**
  - Very common analytical issue
Modifications to CF DNA PT Program

- **2013**
  - Evaluations based on genotype and clinical assessment
  - Each allele counts as 5% and the clinical assessment counts for 10% of the score

- **Why?**
  - Laboratories sometimes had the correct clinical assessment but incorrect genotype – could have analytical problem
  - With treatments based on mutation, genotype is becoming more important (e.g. Kalydeco®)
Mutation-Specific Drug Therapy

- **Kalydeco™ (ivacaftor)**
  - Class III mutations cause defects that affect protein function

- **Lumacaftor**
  - Nov 2014: New drug application submitted to FDA for a combination of ivacaftor and lumacaftor for patients with F508del/F508del

- **Other pharmaceuticals**
  - Several drug companies are currently testing compounds in Phase I, Phase II, and Phase III clinical trials
Quality Control Materials
CF and Beyond
Section 10.3.9 Quality Control (2nd tier assays)

- Not practical to analyze controls for all mutations in every run
- Permissible to include
  - a common mutation (e.g., F508del)
  - a non-template control to determine contamination
  - one or more of the other mutations in the panel
- Should not report the presence of mutations for which there is no external control material
- QC material preferably in DBS matrix to evaluate entire process (DNA extraction through genotype detection)
Laboratory-Created Molecular QA Materials
CFTR Mutation Analysis

- QA materials created from transformed cell lines
- Continually working towards covering all mutations tested for in the US
- Low DNA extraction efficiency causes genotyping failures
  - Participant feedback
  - MQIP research
DNA Yields from Common NBS DNA Extraction Methods
(measured by qPCR)

- **Boil Prep**
  - ~5 fold lower than Boil Prep Generation

- **Methanol Boil Prep**
  - ~13 fold lower than Boil Prep Generation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Boil (Gen) DNA yield (ng)</th>
<th>Boil DNA yield (ng)</th>
<th>Methanol Boil DNA yield (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult PT Sample 1*</td>
<td>44.50</td>
<td>6.05</td>
<td>4.05</td>
</tr>
<tr>
<td>Adult PT Sample 2*</td>
<td>122.50</td>
<td>32.51</td>
<td>8.75</td>
</tr>
<tr>
<td>Adult PT Sample 3*</td>
<td>289.50</td>
<td>54.59</td>
<td>19.60</td>
</tr>
</tbody>
</table>

* Extracted from NSQAP’s Adult Cystic Fibrosis PT specimens with known high, medium and low concentrations
### Newborn Screening Needs Compared to the Coriell Cell Repositories

<table>
<thead>
<tr>
<th>Cell Line Information</th>
<th>Needed</th>
<th>Coriell</th>
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</thead>
<tbody>
<tr>
<td>Number of ACMG recommended mutations</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Number of additional mutations found in commercial assays used in U.S. newborn screening laboratories</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
<td>Number of California-specific mutations</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Total number of unique mutations</td>
<td>72</td>
<td>44</td>
</tr>
</tbody>
</table>
Laboratory prepared DBS for molecular assays

Current Efforts

Laboratory efforts
- Test DBS created with cell lines for CF and Galactosemia
- Transform cells to immortalize
- Collect blood with rare mutations

Based on pilot testing
- Determine criteria for use as PT specimens
- Determine certification criteria for use as QC specimens
### Additions to NSMBB CF Repository (DBS & cryopreserved cells)

<table>
<thead>
<tr>
<th>New Additions to NSMBB CF Repository</th>
<th>Received</th>
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<tbody>
<tr>
<td>Total number of mutations received* (February 2015)</td>
<td>71</td>
</tr>
<tr>
<td>Number of ACMG recommended mutations replenished</td>
<td>19</td>
</tr>
<tr>
<td>Number of mutations in commercial assays used by U.S. newborn screening laboratories (non-ACMG)</td>
<td>21</td>
</tr>
<tr>
<td>Number of California-specific mutations</td>
<td>7</td>
</tr>
</tbody>
</table>

*Mutations requested and found through sequencing
<table>
<thead>
<tr>
<th>Country</th>
<th>Number of Donors</th>
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<tbody>
<tr>
<td>United States</td>
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<tr>
<td>Mexico</td>
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<tr>
<td>Guatemala</td>
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<tr>
<td>El Salvador</td>
<td>3</td>
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<td>Iran</td>
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<tr>
<td>India</td>
<td>2</td>
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<tr>
<td>Canada</td>
<td>2</td>
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<tr>
<td>Iceland</td>
<td>1</td>
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<tr>
<td>Germany</td>
<td>1</td>
</tr>
<tr>
<td>Japan</td>
<td>1</td>
</tr>
<tr>
<td>Not provided</td>
<td>6</td>
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</tbody>
</table>
Moving Forward

- Pilot DBS for galactosemia mutations created by MQIP
  - Good news: labs testing for 9 mutations or less
  - Bad news: all laboratory developed assays

- DBS derived from immortalized cell lines

- Molecular PT program expansion
  - Disorders on RUSP that use a molecular assay (galactosemia)
  - Exploring development of a CAH 2nd tier molecular test
  - Disorders recommended by APHL Molecular Subcommittee
NSMBB CF DBS Repository

- Proficiency testing
- Validation/Verification of methods
- Troubleshooting
- Working toward covering mutation panels for all of the methods used in the US
Take-Home Messages

- **Typical challenges in NBS labs doing molecular assays**
  - Lab space – Unidirectional work flow
  - Contamination – previous amplicons contaminate new runs
  - Vocabulary/nomenclature – may not be familiar with terms
  - Complex assays to troubleshoot – many steps or many mutations
  - DNA extraction - efficiency and purity may affect assay

- **CF PT program evaluates genotype & clinical assessments**

- **Mutation specific drug therapy now available**
  - Kalydeco
  - Another being evaluated by FDA
  - More in Phase II and Phase III trials

- **NSMBB has repository of DBS for PT, validation, etc.**

- **NSMBB is developing QC materials for CF molecular assays**
If you need CFTR materials, please contact:

Marie Earley 770-488-7828 mearley@cdc.gov
NSQAP email nsqapdmt@cdc.gov

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

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