Evaluation of Dried Blood Spot Quality Control Materials for Cystic Fibrosis Molecular Tests

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Objectives of Study

- Molecular testing in newborn screening (NBS) laboratories has become increasingly common.
- Quality control QC materials in the DBS matrix for *CFTR* testing are not readily available.
- NSQAP developed a method for constructing DBS QC materials for CF molecular testing.
- U.S. NBS laboratories participating in NSQAP’s CF Mutation Detection PT program have evaluated pilot materials.
Flowchart of General Procedure

Grow cell lines (Coriell Cell Repositories)

- Mix lymphoblasts, red blood cells, and serum
- Adjust hematocrit and spot
- Confirm genotypes of DBS
- Send to CF Mutation Detection PT participants
- Evaluate performance based on reported genotype
Classification of Methods and Mutations Used by Proficiency Testing Participants

<table>
<thead>
<tr>
<th></th>
<th>U.S.</th>
<th>International</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Participating Laboratories</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td># of Methods Used*</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>kits</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>in-house</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Total # of Mutations Covered</td>
<td>44</td>
<td>119†</td>
</tr>
<tr>
<td>Total # of Variants Covered</td>
<td>6</td>
<td>6</td>
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</tbody>
</table>

* Does not count multiple versions of same kit
† Minimum number as some methods can find many rare mutations
CF Mutation Detection Proficiency Testing

Program Growth

<table>
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<tr>
<th>Year</th>
<th>Domestic Participants</th>
<th>International Participants</th>
<th>Total Participants</th>
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<tbody>
<tr>
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<td>10</td>
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</tr>
<tr>
<td>2009</td>
<td>20</td>
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<td>2010</td>
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<td>80</td>
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<tr>
<td>2012</td>
<td>50</td>
<td>50</td>
<td>100</td>
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Concentration of Cells vs Genotyping Results

Based on results, all future materials were made using 3.5 X 10^6 cells/mL total volume
Laboratories that could not genotype the specimen were the same for each genotype.
Commonly Reported Issues

- “Low Signal”, “Equivocal” or “Sample Failure” were reported
- Did not always interfere with data interpretation
- 7 laboratories could not provide a genotype in Rnd 1
- 3 laboratories could not genotype in Rnd 2
- Of the remaining 4 laboratories,
  - 1 laboratory stopped participating
  - 1 laboratory did not specify any changes to the procedure
  - 2 laboratories extracted DNA from more punches or changed the DNA extraction protocol
Conclusions

- Appropriate QC materials are important to the quality management system.
- DBS controls are needed to monitor the testing process from beginning to end.
- NSQAP’s pilot materials were correctly genotyped in the majority of laboratories.
- An increase in cell concentration did not make a substantial difference in performance.
- Difficulties in genotyping were resolved by increasing the amount of DNA extracted or the efficiency of the extraction method.
Future Activities

- Continue to monitor the performance of the materials
- Collaborate with NBS laboratories and CF Centers to add other mutations needed
- Prepare pilot materials to cover the recommended panel of 23 mutation and others
- Prepare pilot materials for other NBS disorders that use a DNA-based confirmatory test
Acknowledgements

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