Establishing Quality Assurance Protocols for Low Frequency Events in a High Throughput SCID NBS Laboratory

2011 Newborn Screening and Genetics Testing Symposium
San Diego, CA

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Associate Professor, Pediatrics
202,298 infants screened for SCID

4 SCID

~1 : 50,000

Surveillance: No known false negative

Through guthrie date 10/25/2011
Implementing SCID NBS with Multiplexed Assays in an Integrated Program Approach
CDC National Center for Environmental Health
Grant # IV01-EH000362-03

New England Newborn Screening Program Staff
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GRANT COMPONENTS

• ASSAY DEVELOPMENT
• SCREENING IMPLEMENTATION
• ALGORITHM REFINEMENT
• TECHNOLOGY TRANSFER
• PUBLICATIONS
# US PILOT STUDIES
## INTERIM FINDINGS
Combined data from CDC and NICHD-funded pilots (through April 2011)

<table>
<thead>
<tr>
<th>State</th>
<th>WI</th>
<th>MA</th>
<th>NY</th>
<th>CA</th>
<th>PR</th>
<th>LA</th>
<th>TOT</th>
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<tbody>
<tr>
<td>screened</td>
<td>243,707</td>
<td>161,707</td>
<td>136,635</td>
<td>358,000</td>
<td>29,000</td>
<td>31,464</td>
<td>960,513</td>
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<tr>
<td>SCID</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>rate (1/x)</td>
<td>61,000</td>
<td>161,707</td>
<td>34,000</td>
<td>72,000</td>
<td></td>
<td></td>
<td>69,000</td>
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</tbody>
</table>
Are we generating false negative results?

If so, what could be the cause?

Review data:
What is the potential for

Contamination?

Imprecision of the assay?
96-Well DNA Sample Plates on Biomek Deck

Individual wells remain hermetically sealed until punctured with the pipette tip for transfer to the 384-well reagent-containing plate.

Convex appearance of perfectly sealed wells

Pierced wells
Laboratory Algorithm

Components that might fail a run if copies detected

• No template controls, DNA from “no TREC” samples
• “blank spots” that are prepped with clinical specimens
Laboratory Algorithm

Components that might fail a run if copies detected

- No template controls, DNA from “no TREC” samples
- “blank spots” that are prepped with clinical specimens

Other “blank spots”
SCID NBS Algorithm

(All TREC & RNaseP Values are copies/ul)
Dried Blood Spot Specimen

Multiplex assay for TREC and RNaseP

TREC ≥ 503

and

RNaseP ≥ 4032

→ SCREEN NEGATIVE
(automated report)

RNaseP < 4032

Prompts retest in duplicate of same specimen

TREC < 503

Prompts retest in duplicate of same specimen
RNaseP Values for 80 BSD-punched Guthrie spots and Companion Blanks
RNaseP Values for 80 BSD or Hand-punched Guthrie spots and Companion Blanks
TREC Values for 80 BSD-or Hand-punched Guthrie Spots and Companion Blanks
SCID NBS Algorithm

(All TREC & RNaseP Values are copies/ul)

Dried Blood Spot Specimen

Multiplex assay for TREC and RNaseP

TREC ≥ 503

and

RNaseP ≥ 4032

SCREEN NEGATIVE

(automated report)

TREC < 503

Prompts retest in duplicate of same specimen

Two or Three tests with RNaseP < 4032

SCID SCREEN UNSATISFACTORY

Phone call to PCP office to Request Repeat NBS specimen

Two or Three tests with RNaseP ≥ 4032

And

TREC ≥ 252

SCREEN NEGATIVE

(automated report)

Two or Three tests with RNaseP ≥ 4032

And

TREC < 252

SCREEN POSITIVE

PHONE CONSULT with PCP and recommendation for repeat NBS and/or Flow Cytometry followed by fax of Screen Positive report packet
Precision of the Assay

Considerations

• intrinsic to the assay
  • within eluate
  • Between instruments, calibration curves
• intrinsic to the sample
  • Across a spot; DNA preparation
20 cards

anonymized

Per NBS card

16 punches
16 eluates

Per eluate

10 reactions

Instruments 1 and 2

160 data points per NBS card
### Precision of the Assay – eluates

(sample from 320 eluates)

<table>
<thead>
<tr>
<th>Cq values</th>
<th>Cq values</th>
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<tbody>
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<td>31.51</td>
<td>31.27</td>
</tr>
<tr>
<td>31.14</td>
<td>31.11</td>
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<tr>
<td>31.13</td>
<td>31.24</td>
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<td>31.07</td>
<td>30.82</td>
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</table>

- **471 copies/ul**
- **463 copies/ul**

Mean SD all eluates 0.21
Precision of the Assay – across a spot
TREC Cq Values from Sampling across 20 Guthrie Spots
Min, Max, Mean for 16 Punches
RNase P Cq Values from Sampling across 20 Guthrie Spots
Min, Max, Mean for 16 Punches
Thoughts

Confirms High Quality Robust Assay

It’s always a good thing to check…for things that might be reasonably preventable.

Punching considerations
Use of replicate data within algorithm
SCID NBS Diagnoses

MA SCID NBS Working group conference