“An improved method for DNA extraction from Dried Blood Spots for T receptor excision (TREC) analysis and other Newborn Screening assays”

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Overall Analysis Scheme

3 mm DBS is punched into a 96 well plate

Partially automated DNA extraction using an epMotion 5075

Automated set up of real time qPCR in a 384 well format using the epMotion 5075

Duplex qPCR amplification and analysis (TREC and beta actin) on a 7900HT.
epMotion 5075

Prevent Disease – Promote Wellness – Improve Quality of Life
Solution 1

WASH
Shake 10 min
room temperature 500 rpm

WASH
Shake 10 min
room temperature 500 rpm

WASH
Shake 10 min
room temperature 500 rpm

ELUTE into 50 μl

Incubate 95 degrees for 15 min

Solution 2

Prevent Disease – Promote Wellness – Improve Quality of Life
Reasons to change the TREC method in MI

- High repeat rate in the lab
- High “abnormal” rate reported (≈0.4%).
- Too many tips used
- Extraction method was time consuming.
- CDC SCID grant aims included:
  » A new extraction method that is faster, less expensive, and more efficient.
  » Utilizing the DNA extract for other molecular methods in the MI NBS lab (cystic fibrosis).
Temperature is the key variable
Summary for extraction conditions tested

B actin copy per microliter of blood with varying extraction conditions

Previous method:
- Previous extraction: RT, 500 rpm; 15 min at 95°C; 2 x sol 1; 10 min shake

Current method:
- 60°C TMX; 1000 rpm; 30 min at 95°C
- 1 x sol 1; 10 min shake
- 1 x sol 2; 5 min shake
- 2 x sol 1; no sol 2; 10 min shake
- No sol 1; 1 x sol 2; 10 min shake
- No sol 1; 1 x sol 1; 5 min shake

Extraction conditions:
60°C TMX, 1000 rpm, 30 min 95°C
Comparison of the two DNA extraction methods

**Previous method**

1. **WASH**
   - Shake 10 min room temperature 500 rpm

2. **WASH**
   - Shake 10 min room temperature 500 rpm

3. **WASH**
   - Shake 10 min room temperature 500 rpm

4. **ELUTE into 50 µl**
5. **Incubate 95 degrees for 15 min**

**Current method**

1. **WASH**
   - Shake 10 min room temperature 500 rpm

2. **WASH**
   - Shake 10 min room temperature 500 rpm

3. **WASH**
   - Shake 10 min 60 degrees C 100 rpm

4. **ELUTE into 50 µl**
5. **Incubate 95 degrees for 30 min**

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Prevent Disease – Promote Wellness – Improve Quality of Life
### Comparison of the two DNA extraction methods

<table>
<thead>
<tr>
<th></th>
<th>Previous Method</th>
<th>Current Method</th>
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<tbody>
<tr>
<td>Time on epMotion</td>
<td>75 minutes</td>
<td>20 minutes</td>
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<tr>
<td>Tips used per 96 well plate</td>
<td>404</td>
<td>130</td>
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<tr>
<td>Average repeat rate in lab</td>
<td>10%</td>
<td>&lt;3%</td>
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</table>
Summary of cost savings

• Reduction in consumables $\approx$ $0.24$ per sample.
• Less storage space needed for tips, less autoclaving $\approx$ $5,000$ a year.
• No more solution 1 used $\approx$ $23,000$ a year.
• Less time spent doing the bench work. Able to reduce down to one FTE technician. $\approx$ $80,000$ a year (benefits and salary).
Summary of cost savings

- Lower repeat rate in the lab from nearly 10% to down to less than 3%. Equates to over $15,000 dollars in savings (reagents and consumables).
- Estimated cost savings by utilizing DNA extract for Cystic Fibrosis testing ≈ $50,000 (reagents, consumables, and technician time).
- Additional measures saves nearly $30,000 a year in lab savings.
New Method Validation completed in 2012; go live date September 1, 2012

• Compared a new and improved DNA extraction method to the former DNA extraction method used for TREC analysis and for Cystic fibrosis.

• Compared Gene Expression master mix to Environmental master mix.
Comparison of master mixes from ABI

<table>
<thead>
<tr>
<th>Heparin in Units/mL of blood</th>
<th>B actin quantity per µl of blood</th>
<th>Gene Expression Master Mix</th>
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<tr>
<td>10</td>
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<td>Environmental Master Mix</td>
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*Each data point is an average of 6 DBS replicates with various heparin levels with 2 master mixes.

Environmental master mix much better!
This DNA extraction works with the following molecular assays:

- **Methods run by MI**
  - qPCR (duplex of TREC and β-actin)
  - Rnase P
  - Cystic Fibrosis InPlex® - (full validation)
  - Melting curve analysis of spinal muscular atrophy (SMA)- (several thousand samples)
  - Biotinidase sequencing (run by Henry Ford Hospital)
This DNA extraction works with the following molecular assays:

- Methods run by CDC’s NSMBB
  - RNAse P qPCR
  - Microsatellite short tandem repeat (STR) analysis
  - Sequencing
    - CF exon 11 (501 bp) and 12 (477 bp)
    - HBA2 (1350 bp)
    - HBA1 (1622 bp)
    - HBB (1932 bp)
    - GALT (3974 bp)
    - CAH (5624 bp) *Note: A faint 5624 bp amplicon was present, however not enough to perform sequencing assay.
Where do we go from here?

- Implement “cherry” picking using epMotion 5075 from extracted DNA to use for CF mutation analysis.
- Host other NBS labs for training and assist with SCID startup
- Investigate other primary and secondary targets.
Acknowledgements

- MI NBS laboratory
- This work was partially funded by a research cooperative agreement from CDC (Grant # 01EH000936) and does not represent the official view of CDC.
- MAP team members
- NY & WI laboratories
- CDC/NCEH/NSMBB
- Other NBS labs