Second tier tests and newborn screening

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Outline

- Utah Newborn Screening Program
- ARUP Newborn screening laboratory
- Second tier tests algorithms/workflow
The efficiency and effectiveness of a newborn screening program is dependent upon the smooth integration of sample collection, laboratory testing, follow-up, diagnosis, timely treatment, and tracking of outcomes.
Biochemical Genetics and Newborn Screening Laboratories at ARUP

- Technical oversight
- Scientific oversight

Technical Supervisor

- BCG 17 FTEs
- NBS 4 FTEs
- 13 LC-MS/MS
Second Tier Tests

- Tests run on the SAME sample used for the primary screen
- Different target analytes
- Often a different methodology is used
What is the purpose of second tier tests?

- Identify infants at risk of having a metabolic condition, while

- Reducing false positives (proportion of non-affected individuals who test positive), and

- Reducing false negatives (proportion of true affected individuals who test negative)
Impact of false positive results

- Anxiety
- Increased costs to parents
- Increased costs to society
- Decreased credibility for NBS program
- “Cry wolf” effect
- Potential for missing appropriate follow-up of a real patient
Impact of false negative results

• Missing a diagnosis of a potentially treatable metabolic condition, resulting in

• Morbidity and mortality associated with the condition
Second tier tests and newborn screening

• Biochemical/small molecule analysis based tests
  – LC-MS/MS

• Molecular testing
  – Cystic Fibrosis
  – Lysosomal storage diseases
  – DNA testing for metabolic disorders
Strategy for 2\textsuperscript{nd} tier tests

• Presence of compounds that produce ions with the same mass/charge ratio: chromatographic separation
  – Antibiotics
  – Isomers/Isobars (allo-isoleucine, hydroxyproline, C10-OH-carnitine)
2nd tier test for elevated C5-carnitine

- Normal
- Pivaloylcarnitine
- 2-Methylbutyrylcarnitine
- Isovalerylcarnitine
- Isovaleric Acidemia
2\textsuperscript{nd} tier test for elevated C5DC-carnitine

**Glutaryl carnitine**
- Normal: 0.02 umol/L
- MCAD deficiency: 0.04 umol/L
- Glutaric acidemia type I: 0.18 umol/L

**C10OH-carnitines**

**Methylsuccinyl carnitine**
- Kidney disease: 0.19 umol/L

**SRM 388.3 > 85**

**Ketosis**

**Normal**
Strategy for 2nd tier tests

• Specific markers for metabolic conditions:
  – Elevated C3-carnitine
    • Methylmalonic, methylcitric, 3-hydroxypropionic acids
    • Total homocysteine
  – Elevated methionine
    • Total homocysteine
  – Low methionine
    • Methylmalonic acid, total homocysteine
Specific markers for metabolic conditions:
- Elevated C3-carnitine
- Methylmalonic/methylcitric acid

2nd tier test for elevated C3-carnitine

- Methylcitric acid (Propionic acidemia)
- Succinic acid
- Methylymalonic acid (Methylymalonic acidemia)
2nd tier tests available

- Steroid profile for CAH
- Total homocysteine (elevated/low methionine)
- Glutaryl carnitine (elevated C5DC-carnitine)
- Methylmalonic/methylcitric acid (elevated C3)
- Allo-isoleucine (elevated Xle)
- Ethylmalonic acid (elevated C4-carnitine)
- Guanidinoacetate for GAMT deficiency
2nd tier tests

- Steroid profile for CAH (~150/month)
- Methylmalonic/methylcitric acid (~100/month)
- Glutaryl carnitine (~50/month)
- Total homocysteine (~30/month)
- Allo-isoleucine (~1/month)
- Ethylmalonic acid (~5/month)
- Guanidinoacetate for GAMT deficiency (?)
Choice of second-tier tests

- Identify the biggest “offender”
  - Positive predictive value for a specific marker
    (probability of being affected when the test is positive)

<table>
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<th>Year</th>
<th>Marker(s)</th>
<th>Condition</th>
<th># of positives</th>
<th># of diagnosis</th>
<th>PPV %</th>
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## Effectiveness of second-tier tests

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<td>PA, MMA</td>
<td>11</td>
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<td>100</td>
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</tbody>
</table>
Algorithm for CAH

Elevated 17-OHP (after correction for Birth Weight)

2nd tier test
By LC-MS/MS

17-OHP > 12.5 ng/mL serum
(17-OHP+A)/C > 1

Confirmatory tests
Referral to Pediatric Endocrinologist

17-OHP < 12.5 ng/mL serum
(17-OHP+A)/C > 4

Has infant received corticosteroid prior to sample collection?

Yes:
Confirmatory tests
Referral to Pediatric Endocrinologist

No:
Normal

17-OHP < 12.5 ng/mL serum
(17-OHP+A)/C < 4

Normal
Algorithm for C3-carnitine

C3 > 4 umol/L
and
C3/C2 > 0.19 or C3/C16 > 1.9
C3 > 5 umol/L

2nd tier test for
MMA
MCA

MMA > 5 umol/L
MCA = Normal
Methylmalonic acidemia
Confirmatory tests
Referral to metabolic center

MMA = Normal
MCA > 5 umol/L
Propionic acidemia
Confirmatory tests
Referral to metabolic center

MMA = Normal
MCA = Normal
Normal
Algorithm for Methionine

**Met>45 µmol/L or Met<8 µmol/L**

2nd tier test for Total Homocysteine

- **60 µmol/L < Met < 100 µmol/L** and **tHcys < 8 µmol/L**
  - **Normal**

- **Met = normal or elevated** and **tHcys > 8 µmol/L**
  - **Homocystinuria** (Cystathionine ß-synthase def)
  - Confirmatory tests
  - Referral to metabolic center

- **Met < 10 µmol/L** and **tHcys > 8 µmol/L**
  - **Homocystinuria** (Remethylation defect)
  - Confirmatory tests
  - Referral to metabolic center

- **Met > 100 µmol/L**
  - **tHcys = normal or mildly elevated**
  - **MAT deficiency and others**
  - Confirmatory tests
  - Referral to metabolic center
Algorithm for X-Leu

X-Leu > 200 µmol/L and X-Leu/Ala > 1.75

2nd tier test for Allo-isoleucine

- Allo-isoleucine < 2 µmol/L: Normal
- Allo-isoleucine > 2 µmol/L: MSUD Confirmatory tests Referral to metabolic center
- Allo-isoleucine < 2 µmol/L Hydroxyproline > 100 µmol/L: Hydroxyprolinemia Confirmatory tests Referral to metabolic center
Newborn screening workflow

• Primary screen run and reported daily

• 2nd tier tests:
  – CAH run daily
  – MMA/MCA run 3+/week
  – Hcys run weekly
  – Allo-isoleucine run as needed
  – C5DC run as needed
Barriers to implementation of 2nd tier tests

• Availability of resources
  – Instruments
  – Personnel

• Cost effectiveness
  – Economy of scale
Regional approach to 2nd tier testing

- Evaluation and implementation of second tier testing for disorders identified by MS/MS in newborn blood spots in the Mountain States Region (CDC- grant 5U01EH000453-02)
  - Coordinate with the Mountain States (Region 6) the submission of samples (blood spots) to be analyzed with a second tier method.
  - Compare the number of positive results after the 2nd tier tests with the number of positive results obtained with the primary screen.
  - Evaluate the feasibility of a regional center for second tier tests.
Region 6: 2\textsuperscript{nd} tier tests

- Steroid profile for CAH
- Methylmalonic/methylcitric acids for elevated C3-carnitine
- Total homocysteine for high methionine
- Allo-isoleucine for MSUD
- Succinylacetone for Tyrosinemia type I
Utah: 2nd tier tests

• Steroid profile for CAH
• Methylmalonic/methylcitric acids for elevated C3-carnitine
• Total homocysteine for high methionine
• Allo-isoleucine for MSUD
• C5DC for Glutaric acidemia type I
• Ethylmalonic acid for SCAD
Region 6 study: results

- Results: 9650 second tier tests
Region 6 study: results

• Proportion of tests run on babies weighing <2,000 g at birth:
  – Allo-isoleucine (Xle) 38%
  – Total homocysteine (Met) 37%
  – Succinylacetone (Tyr) 33%
  – CAH 17%
  – Methylmalonic/methylcitric 14%
  – Ethylmalonic 2%
Region 6 study: results

- Abnormal 2nd tier results by test
  - HCY 1 (0.1%)
  - MMA/MCA 8 (1%)
  - CAH 91 (3.1%)
  - SUAC 0
  - Allo-isoleucine 0
  - EMA 0
2nd tier for methylmalonic/methylcitric acid

N=888

2nd tier
- 8 confirmatory tests
  - 3 True Positives
  - PPV = 37.5%

traditional
- 130 confirmatory tests
  - 3 True Positives
  - PPV = 2.3%
2\textsuperscript{nd} tier test for CAH (Utah)

- 4 years data
  - Number of infants screened = 271,784
  - Number of abnormal 2\textsuperscript{nd} tier tests requiring confirmatory tests = 58
  - Number of true positives = 23
  - False positive rate = 0.013%
  - Positive Predictive Value = 39.7%
Summary

• Second tier tests are effective in reducing false positives.

• Implementation of second tier tests can also reduce the stress to the NBS program, families, medical homes caused by false positives.

• They can be integrated in the laboratory workflow provided adequate instrumentation and personnel resources are available.
Acknowledgments

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