Missouri Krabbe Disease Screening

APHL NBSGTS; St. Louis, MO; February 29, 2016

Patrick Hopkins, Chief of Missouri NBS Laboratory
Missouri Goes Alone August 1st, 2015

• Sincere thanks to New York for 3 years of conducting Missouri’s Krabbe screening.

• After 4 months of parallel testing, Missouri stopped sending samples to New York on 7/31/15 and continued our Krabbe full population pilot going solo.

Thank You, New York!
Missouri Krabbe Screening Method

Fluorometric Bench Method for GALC activity

HMU-β-D Galactopyranoside + DBS (GALC) → HMU + Galactose
Workflow for Fluorometric Bench Assay

1. Extract DBS (100 ul of extraction solution – 30 minutes)
2. Add 10 ul of GALC substrate to each well of a new plate
3. Transfer 10 ul of sample extract to the new plate with GALC substrate
4. Seal plates and incubate at 37°C (17 hours)
5. Add calibrants (70 ul in wells A1 – H1)
6. Add stop buffer (50 ul /well)
7. Read plates in fluorometer (BioTek Synergy HTX)
First Day to be Parallel Tested
GALC Data Comparison

NY’s method and MO’s method

• Correlation between the two methods is tight: $R^2 = 0.92$

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Median</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO Activity</td>
<td>1,137</td>
<td>2.12</td>
<td>1.54</td>
<td>0.26</td>
<td>1.81</td>
<td>21.86</td>
</tr>
<tr>
<td>NY Activity</td>
<td>1,727</td>
<td>4.82</td>
<td>3.76</td>
<td>0.46</td>
<td>3.92</td>
<td>50.06</td>
</tr>
</tbody>
</table>

Correlation

<table>
<thead>
<tr>
<th></th>
<th>NY Activity</th>
<th>MO Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY Activity</td>
<td>NY Activity</td>
<td></td>
</tr>
<tr>
<td>MO Activity</td>
<td>0.92</td>
<td></td>
</tr>
</tbody>
</table>
Implementation Process

• Validation and verification of bench fluorometric method for Krabbe.
• Four month parallel testing with NY (April, May, June, and July 2015).
• MSPHL Molecular Unit conducts 2\textsuperscript{nd} tier DNA testing for 30 Kb del.
• Discontinued sending samples to New York as the full population Krabbe pilot continued on in Missouri (July 31\textsuperscript{st}, 2015).
Validation Results

• Tested 34 of previous Missouri positive Krabbe referrals (4 with two mutations, 30 with one mutation):
  o All flagged as abnormal except one carrier of the Y303C mutation (was slightly above our proposed cutoff).
• Tested 29 of previous Missouri’s Poly’s Only and all flagged as abnormal.
• Tested blind positive samples provided by NY and results flagged very well.
• Tested 12 Proficiency Test sample sets and all flagged correctly.
• Accuracy, Precision, and Linearity all are very good.
• All abnormal results found by NY during the parallel testing were flagged abnormal by MO also.
30 Kb deletion test

Control Ladders

30 Kb deletion not present

30 Kb del is present
Reporting Algorithm

• Fixed cutoff for DNA prompt ≤ 0.50 umol/L/hr.
• Any baby with a 30 Kb deletion is referred.
• “Failsafe” cutoff level to refer low GALC screens with no mutation found ≤ 0.20 umol/L/hr.
• We have scenarios that only require a repeat screen (no DNA testing on first screen):
  o **Inconclusive** – have other LSDs flagging with GALC
  o **No Result** – some premature infants and early collects
  o **Borderline** – GALC in the borderline range but not at DNA prompt level.
Missouri Algorithm for Full Population Krabbe Pilot Screening

Screening Test → Result Below Provisional Cut-off?
  - NO → No Further Action
  - YES → Retest Testing in Duplicate

Retest Testing in Duplicate → Average of 3 Runs Below Action Cut-off?
  - NO → Assess Risk
  - YES → Average of 3 Runs Below Action Cut-off?

Obtain Repeat/Second Screen → Borderline or Inconclusive

Borderline or Inconclusive → 2nd Tier DNA Test for 30Kb
  - 30Kb Deletion Present → NO
  - 30Kb Deletion Not Present → YES

2nd Tier DNA Test for 30Kb → GALC Level Below Failsafe Low Cut-off?
  - NO → Borderline or Inconclusive
  - YES → Assess Risk

Assess Risk → Notification to Referral Center

Notification to Referral Center → Referral Center Notifies Parents
  - YES

Referral Center Notifies Parents → Referral Center Coordinates Diagnostics Laboratory Tests
  - NO

Referral Center Coordinates Diagnostics Laboratory Tests → Referral Center Informs NBS Follow-up and Lab of Results and Diagnosis
  - NO
Current GALC Cutoffs

• Provisional (Instrument) Cutoff ≤ 0.60 umol/L/hr

• Borderline Range Cutoff = 0.51 – 0.55 umol/L/hr

• DNA Prompt Cutoff ≤ 0.50 umol/L/hr

• Failsafe Referral Cutoff ≤ 0.20 umol/L/hr
Missouri Newborn Screening Disorders Tested

Amino Acid Disorders
- Argininosuccinic aciduria
- Arginase deficiency
- Argininosuccinate lyase deficiency
- Argininosuccinate lyase deficiency (ASL, Agmargamourea)
- Citrullinemia type 1 (CIT-1, argininosuccinate synthetase deficiency)
- Citrullinemia type 2 (CIT-2, argininosuccinate synthetase deficiency)
- Defects of dibutylcarnitine biosynthesis (DEFDBT-BS)
- Defects of dibutylcarnitine biosynthesis (DEFDBT-1)
- Homocystinuria (HCY, cystathionine beta-synthase)
- Hyperphenylalaninemia (HPA, PHE)
- Hypophosphatasia (HPT)
- Maple syrup urine disease (MSUD, branched-chain ketoacid dehydrogenase)
- Phenylketonuria (PKU, phenylalanine hydroxylase)
- Tyrosinemia type 1 (TYR-1, fumarylacetoacetate hydrolase deficiency)
- Tyrosinemia type 2 (TYR-2, tyrosine amino transferase)
- Tyrosinemia type 3 (TYR-3, hydroxyphenylpyruvate dioxygenase)

Fatty Acid Disorders
- Carnitine acetyltransferase deficiency (CACT)
- Carnitine uptake defect (CUD, carnitine transport defect)
- Carnitine palmitoyltransferase deficiency (CPT-1, CPT-2)
- Carnitine palmitoyltransferase deficiency (CPT-1A, CPT-1B)
- Deficiency of acyl-CoA dehydrogenase deficiency (LCHAD, LCHAD)
- Deficiency of acyl-CoA dehydrogenase deficiency (MCAD, MCAD)
- Medium-Chain Acyl-CoA dehydrogenase deficiency (MCD, MCD)
- Medium-Chain Acyl-CoA dehydrogenase deficiency (MCAD, MCAD)
- Short-Chain Acyl-CoA dehydrogenase deficiency (SCAD, SCAD)
- Trifunctional protein deficiency (TPP)

Organic Acid Disorders
- 2-Methyl-3-hydroxybutyric aciduria (2MBH)
- 3-Methylbutyl-4-Ca-A deficiency (MBA)
- 3-Hydroxy-3-methylglutaryl-Co-A synthetase (HMG, 3-Hydroxy-3-methylglutaryl-Co-A synthase)
- 2-Methylcrotonyl-CoA carboxylase deficiency (MC-CoA)
- 2-Methylcrotonyl-CoA carboxylase deficiency (MC-CoA)
- 3-Hydroxy-3-methylglutaryl-Co-A synthetase (HMG, 3-Hydroxy-3-methylglutaryl-Co-A synthase)
- Beta-ketoaciduria (BKT, mthithreonine, acetone, ketoaciduria, short-chain ketone aciduria)
- Glyceric aciduria type 1 (GA-1, glyceraldehyde-3-phosphate dehydrogenase)
- Isocitrate-CoA dehydrogenase deficiency (ICD)

Organic Acid Disorders (continued)
- Isocitrate dehydrogenase (I.A, isocitrate-CoA dehydrogenase)
- Methylmalonic aciduria (CRL, A, B, vitamin B12 disorder)
- Methylmalonic aciduria (CRL, C, D)
- Methylmalonic aciduria (MMA, methylmalonic-CoA mutase)
- Multiple carboxylase deficiency (MCAD, holocarboxylase synthetase)
- Propionic acidemia (PROP, propionyl-CoA carboxylase)

Hemoglobinopathies
- Sickle cell disease (Hb S)
- Sickle hemoglobin C disease (Hb SC)
- Sickle beta zero thalassemia disease
- Sickle beta plus thalassemia disease
- Sickle hemoglobin D disease
- Sickle hemoglobin E disease
- Sickle hemoglobin G-6-A disease
- Sickle hemoglobin Lepore Boston disease
- Sickle HIEF disorder
- Sickle Unidentified
- Hemoglobin C beta zero thalassemia disease
- Hemoglobin C beta plus thalassemia disease
- Hemoglobin E beta zero thalassemia disease
- Hemoglobin E beta plus thalassemia disease
- Hemoglobin G-6-A disease
- Hemoglobin G-6-A disease
- Homozygous beta zero thalassemia disease
- Homozygous beta plus thalassemia disease
- Homozygous E disease

Lysosomal Storage Disorders
- Fabry (GLA)
- Gaucher (GBA)
- Refsum MP1 (IDUA)
- Krabbe (GALC)
- Pompe (GAA)

Others
- Critical Congenital Heart Defects (CCCHD)
- Kinking

* There is a lower probability of detection of this disorder during the immediate newborn period.
** Currently conducting statewide pilot implementation testing

The Missouri Newborn Screening Laboratory's goal is to identify infants at risk and in need of diagnostic testing for the above disorders. A normal screening result does not rule out the possibility of an underlying metabolic genetic disease.

Reviewed: 3/3/15
Normal Report

Newborn Screening Laboratory
Phone: 573-751-2652 Fax: 573-751-8155
http://health.mo.gov/lab/newborn/
Bill Whiteman Laboratory Director

LABORATORY REPORT
Submitter: MISSOURI HOSPITAL
Address: MO

Mother: NEWBORN, MOMMY
ABC LANE
JEFFERSON CITY, MO 65101

Physician: LR JOHN SAVEBABY
Address:

Baby’s Name: NEWBORN, BABY
Date of Birth: 07/06/2015 @ 00:01
Sex: M
Race: NP
Med Rod#: NP
Birth Weight: 3600 gms
Gestation Age: NP
Feeding Type: Breast

Specimen Type: Initial
Age @ Collection: 1 day(s)
Date Collected: 07/02/2015 @ 00:00:02
Date Received: 07/12/2015
Date Reported: 07/21/2015
Copy Printed: 07/21/2015

*NP = Not Provided

DISORDER
Primary Congenital Hypothyroidism Normal
Congenital Adrenal Hyperplasia Normal
Hemoglobinopathy Normal
Biotinidase Deficiency Normal
Galactosemia Normal
Fatty Acid Disorders Normal
Organic Acid Disorders Normal
Amino Acid Disorders Normal
Cystic Fibrosis Normal
Lysoasomal Storage Disorders Normal

SCREENING RESULT

The above screening results are not intended to directly indicate or confirm the need of diagnostic testing. A normal screening result does NOT rule out the possibility of an undiagnosed metabolic disease.

REMEMBER: Do you know your patient’s newborn hearing screening results?
**LABORATORY REPORT** (Duplicate)

<table>
<thead>
<tr>
<th>Item</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby's Name</td>
<td>NEWBORN, BABY</td>
</tr>
<tr>
<td>Date of Birth</td>
<td>07/01/2015@00:01</td>
</tr>
<tr>
<td>Sex M</td>
<td>Male</td>
</tr>
<tr>
<td>Med Rec#</td>
<td>NP</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>3500 gms</td>
</tr>
<tr>
<td>Gestation Age</td>
<td>38 weeks</td>
</tr>
<tr>
<td>Feeding Type</td>
<td>Breast</td>
</tr>
<tr>
<td>Specimen Type</td>
<td>Initial</td>
</tr>
<tr>
<td>Age @ Collection</td>
<td>1 day(s)</td>
</tr>
<tr>
<td>Date Collected</td>
<td>07/02/2015@00:00</td>
</tr>
<tr>
<td>Date Received</td>
<td>07/17/2015</td>
</tr>
<tr>
<td>Mother</td>
<td>NEWBORN, MOMMY</td>
</tr>
<tr>
<td>Physician</td>
<td>DR. JOHN SAVEDANLY</td>
</tr>
<tr>
<td>Address</td>
<td>, MO</td>
</tr>
<tr>
<td>Lab ID Number</td>
<td>2015BJ0020</td>
</tr>
<tr>
<td>Form ID Number</td>
<td>B1224557</td>
</tr>
</tbody>
</table>

**DISORDER** | **SCREENING RESULT** | **EXPECTED RANGE**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Congenital Hypothyroidism</td>
<td>Normal</td>
</tr>
<tr>
<td>Congenital Anorectal Hypeplasia</td>
<td>Normal</td>
</tr>
<tr>
<td>Hemoglobinopathy</td>
<td>Normal</td>
</tr>
<tr>
<td>Redimide Deficiency</td>
<td>Normal</td>
</tr>
<tr>
<td>Galectosena</td>
<td>Normal</td>
</tr>
<tr>
<td>Fatty Acid Disorders</td>
<td>Normal</td>
</tr>
<tr>
<td>Organic Acid Disorders</td>
<td>Normal</td>
</tr>
<tr>
<td>Amino Acid Disorders</td>
<td>Normal</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>Normal</td>
</tr>
<tr>
<td>Lysosomal Storage Disorders</td>
<td>NO RESULT</td>
</tr>
</tbody>
</table>

**Comments**

NO RESULT: Multiple lysosomal activity levels are decreased, therefore results are inconclusive. A repeat newborn screening test is necessary.

*NP = Not Provided

---

The above screening results are meant to identify infants at risk and are used for diagnostic testing. A normal screening result does not rule out the possibility of an underlying metabolic/genetic disease.

**REMINDER:** Do you know your patient's newborn hearing screening results?
Positive Krabbe Result (provided only to genetic referral center during pilot phase)
Missouri Stats Since Going Solo
(August 1, 2015 – February 29, 2016)

• Approximately 45,500 births screened.
• 260 Reflexed to 2nd Tier DNA
• 7 Referrals with heterozygous 30 Kb del.
  – Six of those confirmed as 30 Kb del carriers only (one still pending).
• 5 Referred from GALC level alone.
  – One confirmed as a carrier of a Krabbe mutation other than 30 Kb del.
Acknowledgements

- Tracy Klug, Lacey Vermette and the Missouri NBS LSD laboratory team
- Dr. Sharmini Rogers, Julie Raburn, Jami Kiesling and the Missouri NBS follow-up team
- The Missouri LSD Task Force
- Dr. Joe Orsini and the NY LSD laboratory team
- The Baebies Inc. team
Thank You