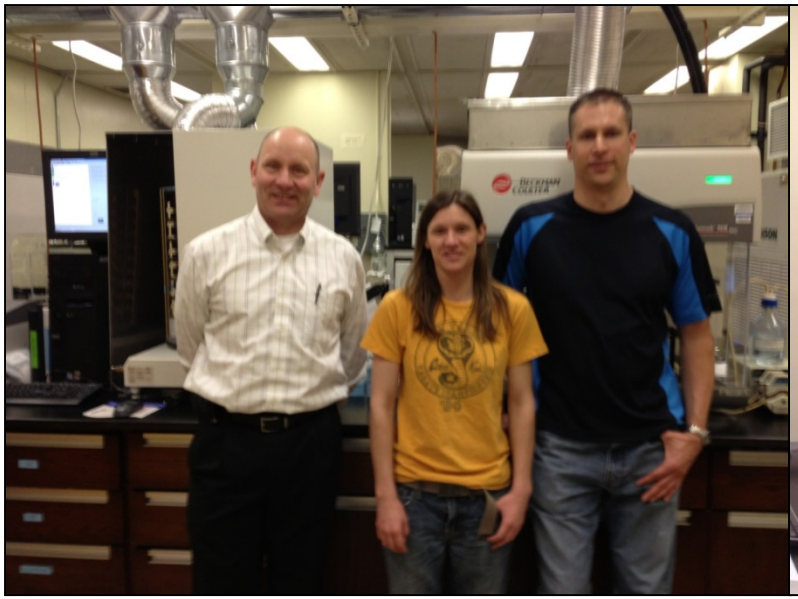
Missouri Krabbe Disease Screening

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

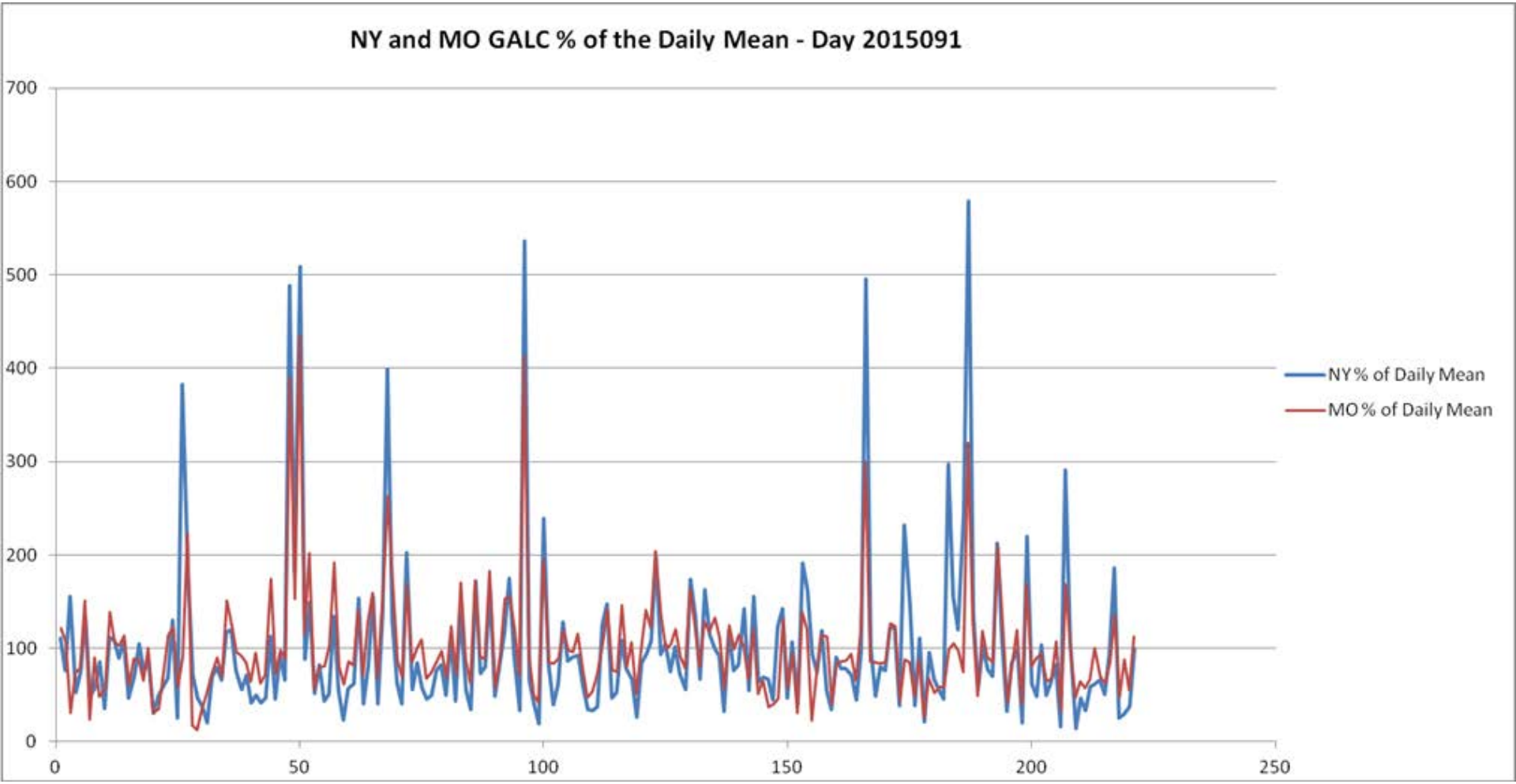
Fluorometric
Bench Method
for GALC activity

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)

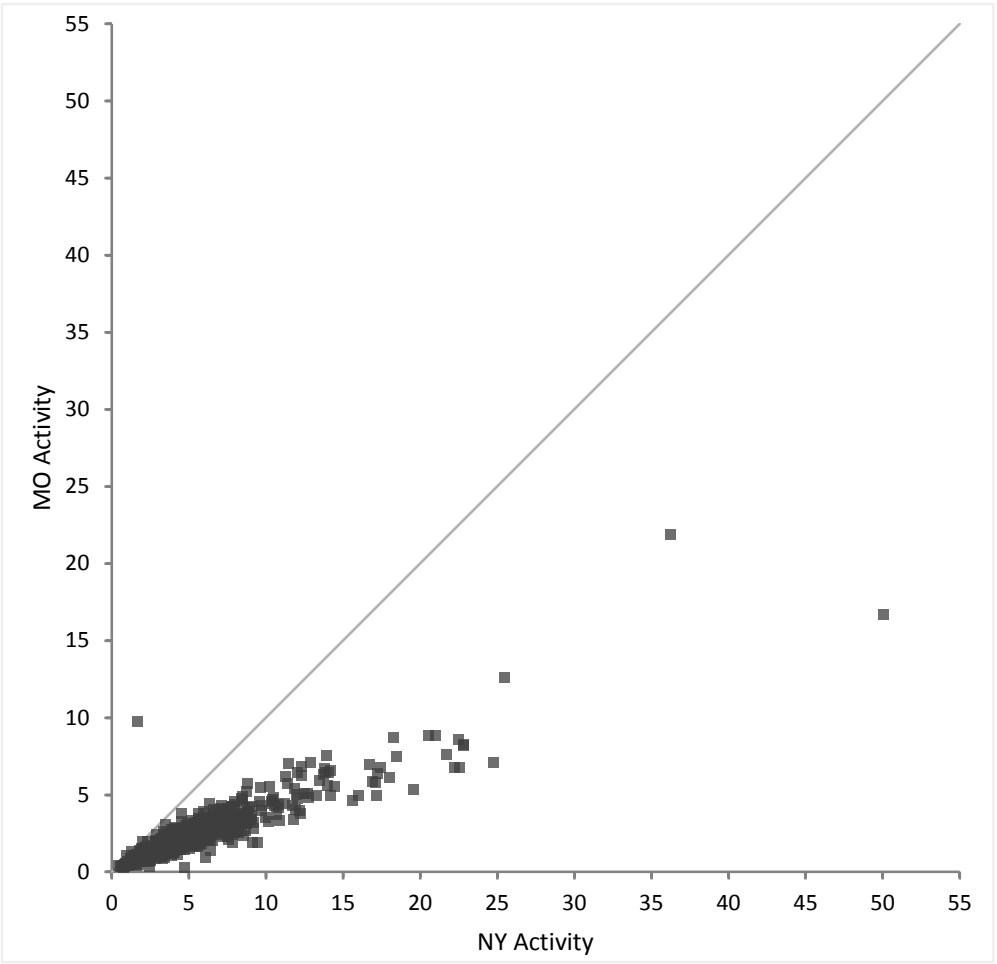
First Day to be Parallel Tested



GALC Data Comparison
NY's method and MO's method

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

Variable	N	Mean	SD	Min	Median	Max
MO Activity	1,137	2.12	1.54	0.26	1.81	21.86
NY Activity	1,727	4.82	3.76	0.46	3.92	50.06
Correlation						
	NY Activity					
MO Activity	0.92					



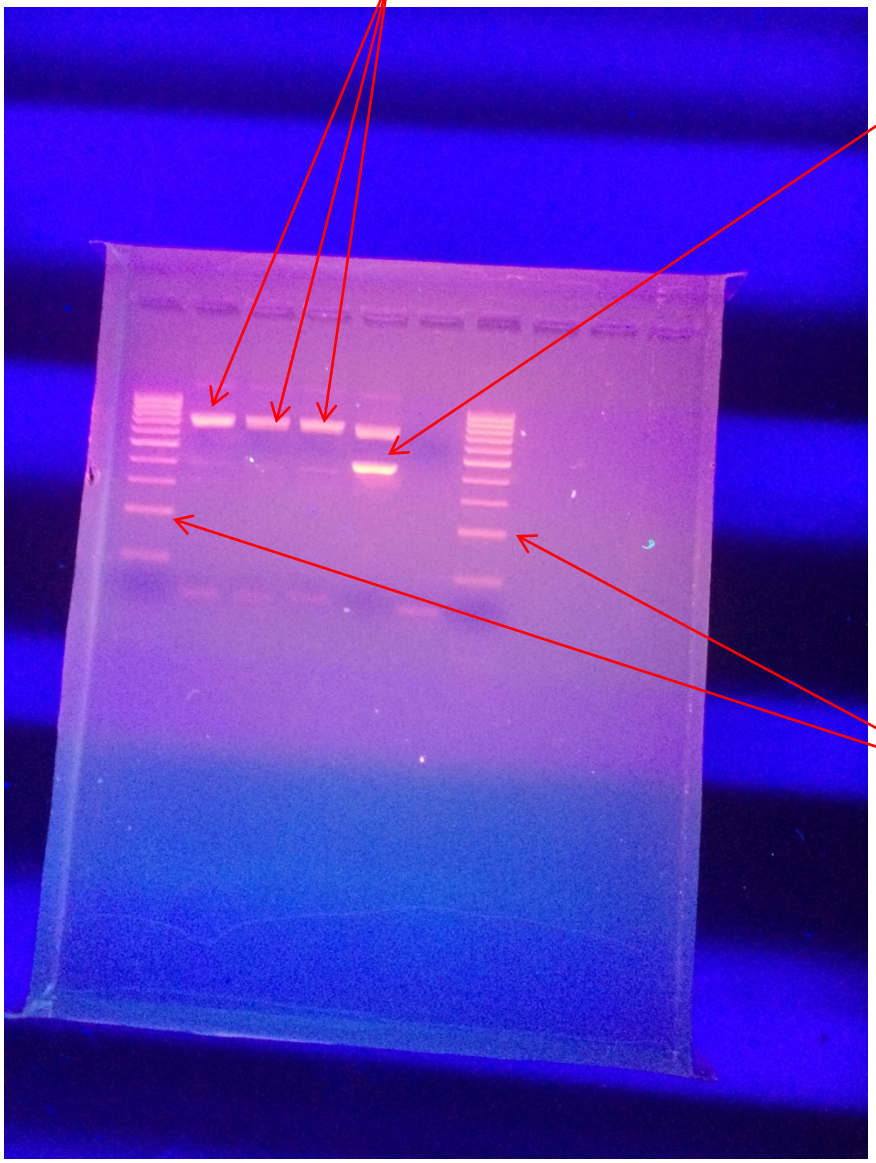
Implementation Process

- 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 2nd tier DNA testing for 30 Kb del.
- Discontinued sending samples to New York as the full population Krabbe pilot continued on in Missouri (July 31st, 2015).

Validation Results

- Tested 34 of previous Missouri positive Krabbe referrals (4 with two mutations, 30 with one mutation):
  - 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.

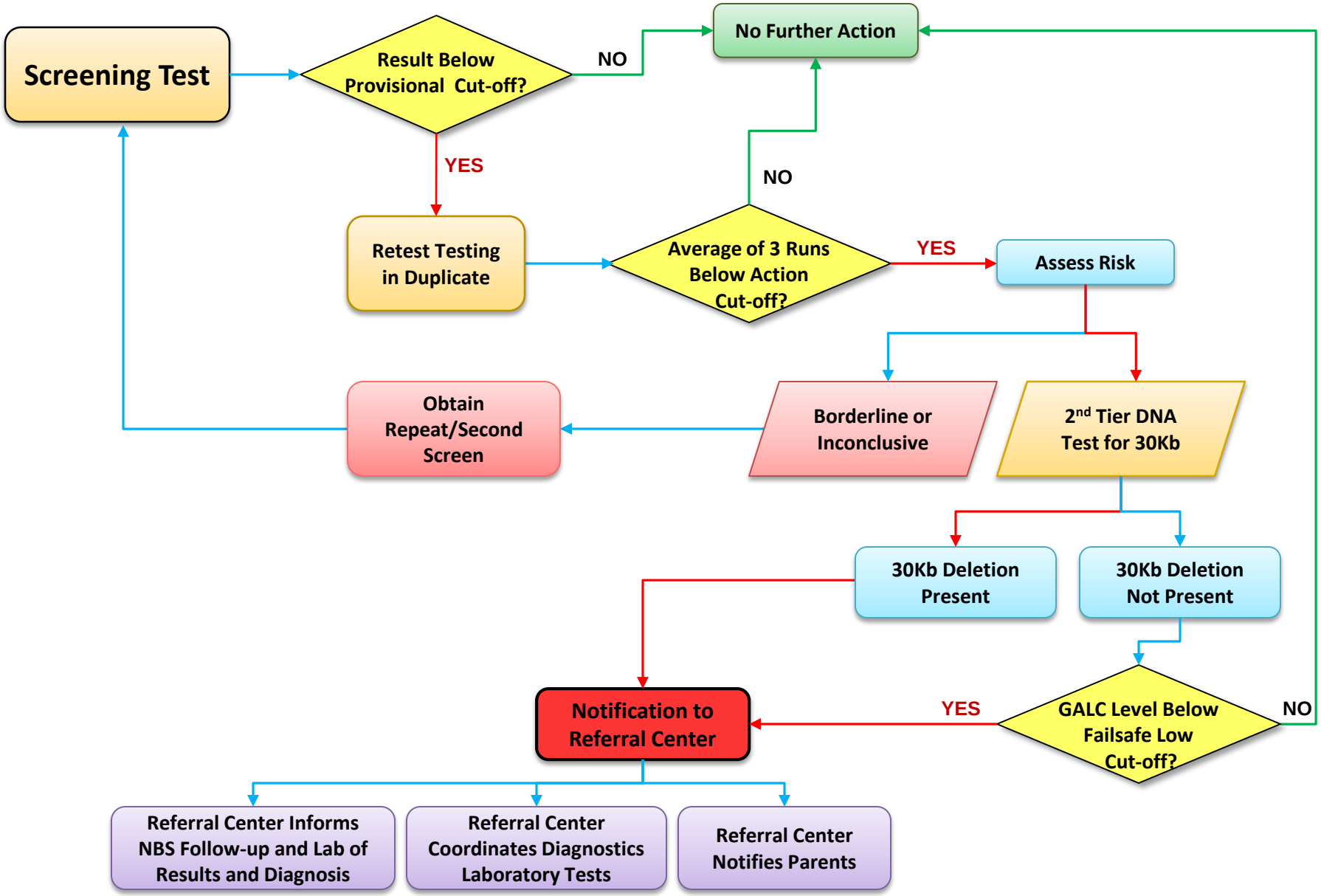
30 Kb deletion test



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):
  - **Inconclusive** – have other LSDs flagging with GALC
  - **No Result** – some premature infants and early collects
  - **Borderline** – GALC in the borderline range but not at DNA prompt level.

Missouri Algorithm for Full Population Krabbe Pilot Screening



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

Biotinidase deficiency (BIOT)
Classical galactosemia (GALT)
Congenital adrenal hyperplasia (CAH)
Congenital primary hypothyroidism (CH)
Cystic fibrosis (CF)

Amino Acid Disorders
- Argininemia (ARG, arginase deficiency)
- Argininosuccinate acidemia (ASA, argininosuccinase)
- Citrullinemia type I (CIT-I, argininosuccinate synthetase)
- Citrullinemia type II (CIT-II, citrin deficiency)
- Defects of biopterin cofactor biosynthesis (BIOPT-BS)
- Defects of biopterin cofactor regeneration (BIOPT-RG)
- Homocystinuria (HCY, cystathionine beta synthase)
- Hyperphenylalaninemia (H-PHE)
- Hypermethioninemia (MET)
- Maple syrup urine disease (MSUD, branched-chain ketoacid dehydrogenase)
- Phenylketonuria (PKU, phenylalanine hydroxylase)
- Tyrosinemia type I (TYR-1, fumarylacetoacetate hydrolase) *
- Tyrosinemia type II (TYR-II, tyrosine aminotransferase)
- Tyrosinemia type III (TYR-III, hydroxyphenylpyruvate dioxygenase)

Fatty Acid Disorders
- Carnitine acylcarnitine translocase deficiency (CACT)
- Carnitine uptake defect (CUD, carnitine transport defect) *
- Carnitine palmitoyl transferase deficiency I (CPT-1a)
- Carnitine palmitoyl transferase deficiency II (CPT-II)
- Dienoyl-CoA reductase deficiency (DE-RED)
- Glutaric acidemia type II (GA-II, multiple acyl-CoA dehydrogenase deficiency)
- Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)
- Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)
- Medium-chain ketoacyl-CoA thiolase deficiency (MCKAT)
- Medium/Short chain L-3-hydroxy acyl-CoA dehydrogenase deficiency (M/SCHAD)
- Short-chain acyl-CoA dehydrogenase deficiency (SCAD)
- Trifunctional protein deficiency (TFP)
- Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)

Organic Acid Disorders
- 2-Methyl-3-hydroxybutyric aciduria (2M3HBA)
- 2-Methylbutyryl-CoA dehydrogenase deficiency (2MBG)
- 3-Hydroxy 3-methylglutaric aciduria (HMG, 3-Hydrox 3-methylglutaryl-CoA lyase)
- 3-Methylcrotonyl-CoA carboxylase deficiency (3-MCC)
- 3-Methylglutaconic aciduria (3MGA, Type I hydratase deficiency)
- Beta ketothiolase (BKT, mitochondrial acetoacetyl-CoA thiolase, short-chain ketoacyl thiolase)
- Glutaric acidemia type I (GA-1, glutaryl-CoA dehydrogenase)
- Isobutyryl-CoA dehydrogenase deficiency (IBG)

Organic Acid Disorders (continued)
- Isovaleric acidemia (IVA, Isovaleryl-CoA dehydrogenase)
- Malonic acidemia (MAL, malonyl-CoA decarboxylase)
- Methylmalonic acidemia (CBL A,B; vitamin B12 disorders)
- Methylmalonic acidemia (CBL C,D)
- Methylmalonic acidemia (MUT, methylmalonyl-CoA mutase)
- Multiple carboxylase deficiency (MCD, holocarboxylase synthetase)
- Propionic acidemia (PROP, propionyl-CoA carboxylase)

Hemoglobinopathies
- Sickle cell disease (Hb S/S)
- Sickle hemoglobin-C disease (Hb S/C)
- Sickle beta zero thalassemia disease
- Sickle beta plus thalassemia disease
- Sickle hemoglobin-D disease
- Sickle hemoglobin-E disease
- Sickle hemoglobin-O-Arab disease
- Sickle hemoglobin Lepore Boston disease
- Sickle HPFH 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-H disease
- Homozygous beta zero thalassemia disease
- Homozygous-C disease
- Homozygous-E disorder
- Double heterozygous beta thalassemia disease

Lysosomal Storage Disorders
- Fabry (GLA)
- Gaucher (GBA)
- Hurler/MPS-I (IDUA)
- Krabbe (GALC) **
- Pompe (GAA)

Others
- Critical Congenital Heart Defects (CCHD)
- Hearing

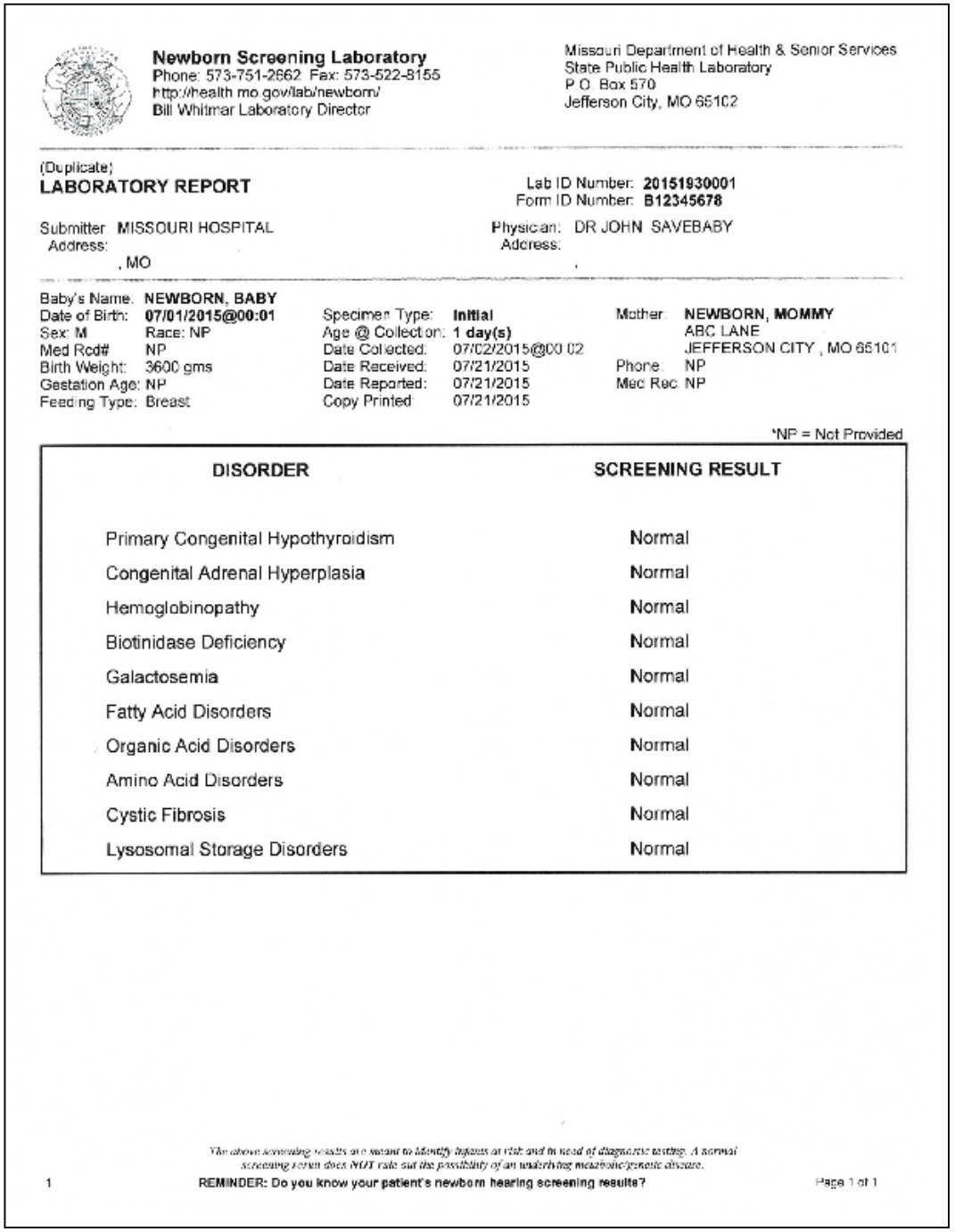
* There is a lower probability of detection of this disorder during the immediate newborn period.

** 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: 8/3/15

Normal Report



Newborn Screening Laboratory
Phone: 573-751-2662 Fax: 573-522-8155
http://health.mo.gov/lab/newborn/
Bill Whitmar Laboratory Director

Missouri Department of Health & Senior Services
State Public Health Laboratory
P O Box 570
Jefferson City, MO 65102

(Duplicate)
LABORATORY REPORT

Lab ID Number: **20151930001**
Form ID Number: **B12345678**

Submitter: MISSOURI HOSPITAL
Address:
, MO

Physician: DR JOHN SAVEBABY
Address:
,

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

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

Mother: **NEWBORN, MOMMY**
ABC LANE
JEFFERSON CITY , MO 65101
Phone: NP
Med Rec: NP

*NP = Not Provided

DISORDER	SCREENING RESULT
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
Lysosomal Storage Disorders	Normal

The above screening results are meant to identify infants at risk and in need of diagnostic testing. A normal screening screen does NOT rule out the possibility of an underlying metabolic/genetic disease.

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REMINDER: Do you know your patient's newborn hearing screening results?

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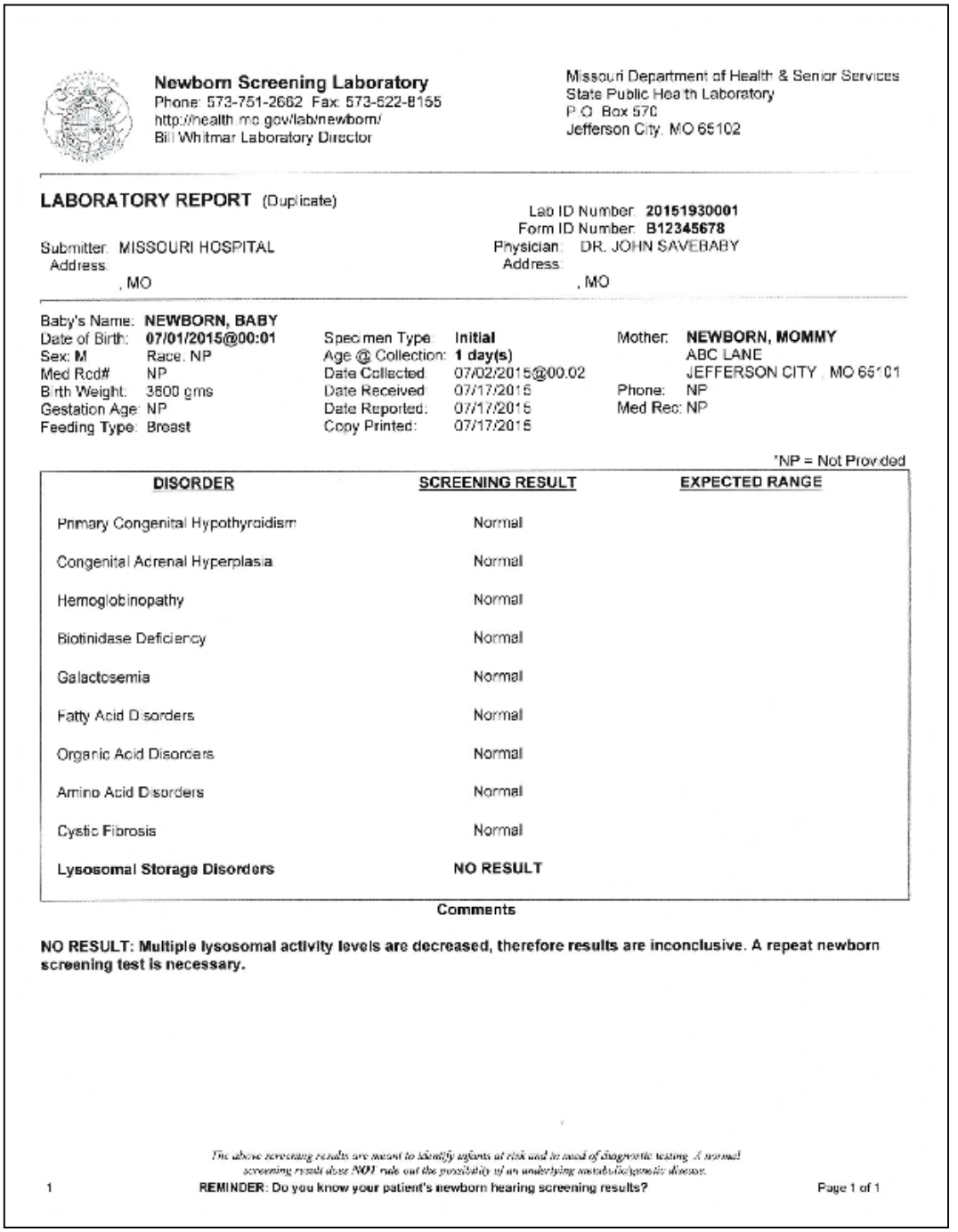
Multiple LSD Activities Decreased



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Jefferson City, MO 65102

LABORATORY REPORT (Duplicate)

Lab ID Number: **20151930001**
Form ID Number: **B12345678**

Submitter: MISSOURI HOSPITAL
Address:
, MO

Physician: DR. JOHN SAVEBABY
Address:
, MO

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

Specimen Type: **Initial**
Age @ Collection: **1 day(s)**
Date Collected: 07/02/2015@00:02
Date Received: 07/17/2015
Date Reported: 07/17/2015
Copy Printed: 07/17/2015

Mother: **NEWBORN, MOMMY**
ABC LANE
JEFFERSON CITY, MO 65101
Phone: NP
Med Rec: NP

*NP = Not Provided

DISORDER	SCREENING RESULT	EXPECTED RANGE
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	
Lysosomal Storage Disorders	**NO RESULT**	

Comments

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

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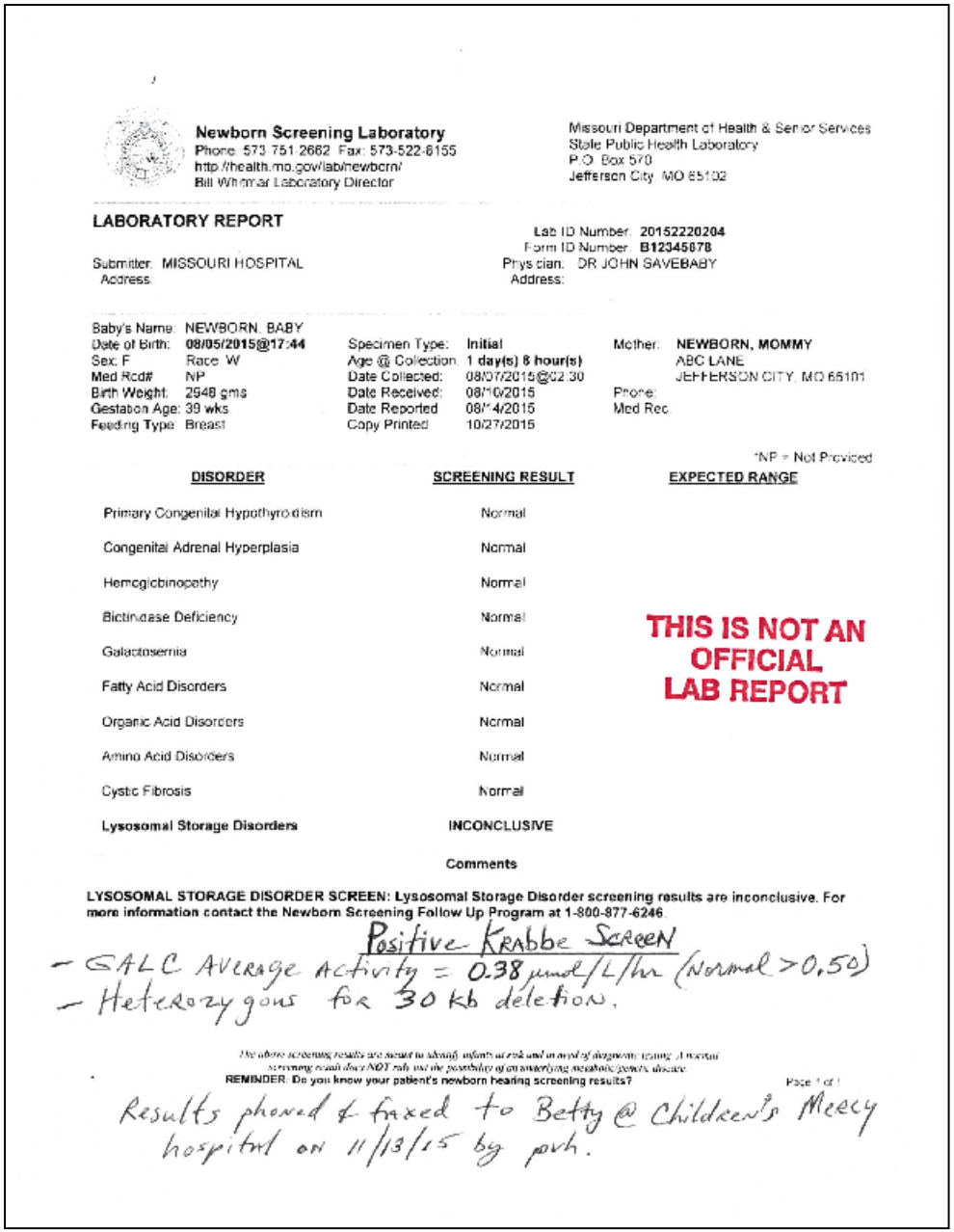
1 **REMINDER**: Do you know your patient's newborn hearing screening results? Page 1 of 1

Positive Krabbe Result
(provided only to genetic referral center during pilot phase)



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State Public Health Laboratory
P.O. Box 570
Jefferson City, MO 65102

LABORATORY REPORT

Lab ID Number: **20152220204**
Form ID Number: **B12345678**

Submitter: MISSOURI HOSPITAL
Address:

Physician: DR JOHN SAVEBABY
Address:

Baby's Name: NEWBORN, BABY
Date of Birth: **08/05/2015@17:44**
Sex: F Race: W
Med Rcd#: NP
Birth Weight: 2948 gms
Gestation Age: 39 wks
Feeding Type: Breast

Specimen Type: **Initial**
Age @ Collection: **1 day(s) 8 hour(s)**
Date Collected: 08/07/2015@02:30
Date Received: 08/10/2015
Date Reported: 08/14/2015
Copy Printed: 10/27/2015

Mother: **NEWBORN, MOMMY**
ABC LANE
JEFFERSON CITY, MO 65101
Phone:
Med Rec:

*NP = Not Provided

DISORDER	SCREENING RESULT	EXPECTED RANGE
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	
Lysosomal Storage Disorders	**INCONCLUSIVE**	

THIS IS NOT AN OFFICIAL LAB REPORT

Comments

LYSOSOMAL STORAGE DISORDER SCREEN: Lysosomal Storage Disorder screening results are inconclusive. For more information contact the Newborn Screening Follow Up Program at 1-800-877-6246.

Positive Krabbe Screen
- GALC Average Activity = 0.38 µmol/L/hr (Normal >0.50)
- Heterozygous for 30 kb deletion.

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REMINDER: Do you know your patient's newborn hearing screening results?

Page 1 of 1

Results phoned & faxed to Betty @ Children's Mercy hospital on 11/13/15 by pvh.

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