Missouri's Experience Implementing Lysosomal Storage Disorders Screening and Follow-up for

Pompe, Gaucher, Fabry and MPS-I and Krabbe Disorders

Advisory Committee on Heritable Disorders in Newborns and Children
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Presentation Goals

- LEGISLATION
- PROCESS OF IMPLEMENTATION
- SCREENING RESULTS
- SHORT TERM FOLLOW-UP
- CONFIRMATORY RESULTS
- CHALLENGES
- LESSONS LEARNED

Missouri's Legislation

FIRST REGULAR SESSION
[TRULY AGREED TO AND FINALLY PASSED]
SENATE COMMITTEE SUBSTITUTE FOR
HOUSE BILL NO. 716
95TH GENERAL ASSEMBLY
1522S.03T 2009
AN ACT

To amend chapter 191, RSMo, by adding thereto three new sections relating to newborn screenings.

Be it enacted by the General Assembly of the state of Missouri, as follows:

Section A. Chapter 191, RSMo, is amended by adding thereto three new sections, to be known as sections 191.333, 191.1127, and 191.1130 to read as follows:

191.333. 1. This section shall be known and may be cited as the **"Brady Alan Cunningham Newborn Screening Act"**.

2. **By July 1, 2012**, the department of health and senior services **shall** expand the newborn screening requirements in section 191.331 to include the following lysosomal storage diseases: **Krabbe disease, Pompe disease, Gaucher disease, Niemann-Pick disease, and Fabry disease.** The department may by rule screen for additional lysosomal storage disorders when the following occurs:

(1) The registration of the necessary reagents with the federal Food and Drug Administration;
(2) The availability of the necessary reagents from the Centers for Disease Control and Prevention;
(3) The availability of quality assurance testing methodology for such processes; and
(4) The acquisition and installment by the department of equipment necessary to implement the expanded screening tests.

The Power of Advocacy

Jessy, Dustin (parents) and Brady Cunningham with Bob Evanosky

Process of Implementation

- Legislation passed in 2009
- Required to screen by 2012
- Developed an LSD taskforce 2012
- Developed contracts for follow-up
- Contracted with New York to screen for Krabbe August 2012
- Began full population-based pilot implementation for Pompe, Gaucher, Fabry and MPS-1 January 2013 and went live August 1, 2015
- Krabbe in-house population-based pilot implementation April 2015

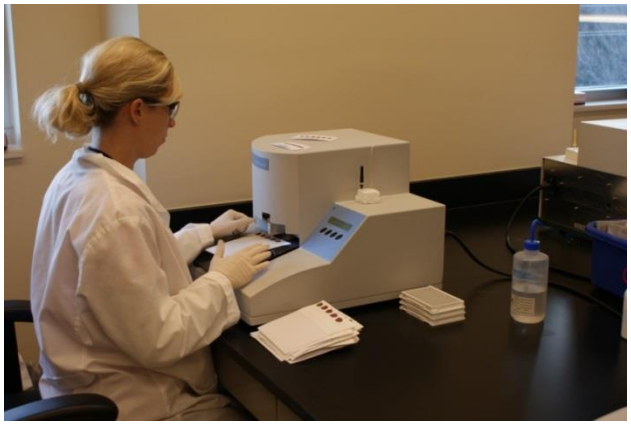
Process of Implementation (Cont'd)

- ~78,000 annual birthrate in Missouri.
- ~92,000 samples received per year. Average of 375 specimens tested per working day counting duplicate re-testing of abnormal results
- Staff of 16 scientists in lab and 3 FTE's in follow-up
- 2 lab FTE's and 1FTE in follow-up dedicated to LSD screening
- 5 lab staff are trained to conduct LSD testing
- 2 DMF workstations (8 platforms)
- 4 contracted centers for follow-up

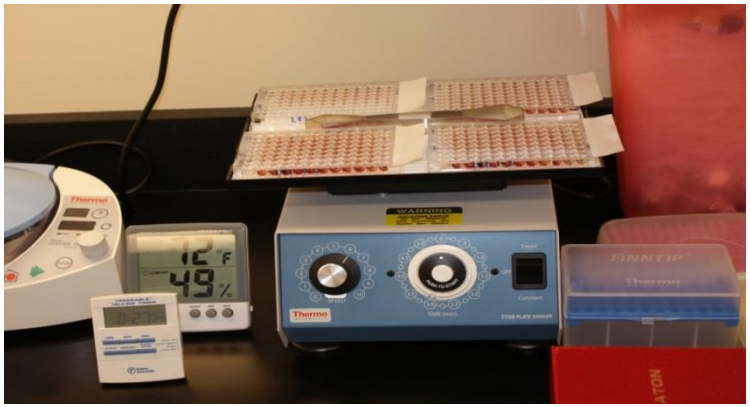
Equipment for Pompe, Gaucher, Fabry and MPS-I

Courtesy of Missouri State Public Health Lab

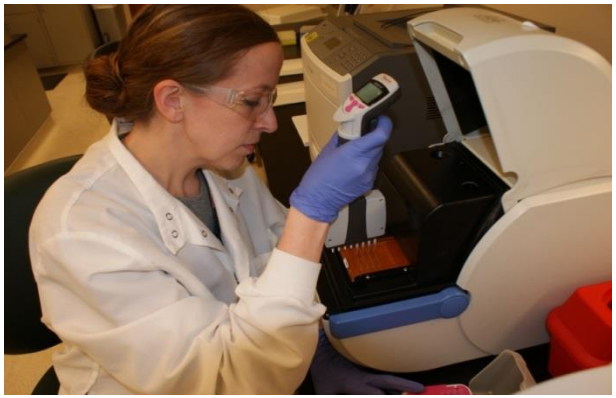
Workflow for LSD Testing



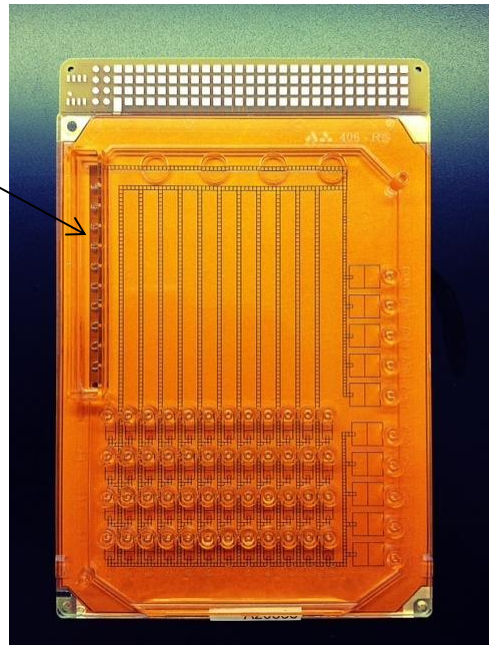
Punch samples (15 min per 96-well plate)



Extract samples (30 min at room temp)



Load filler fluid into cartridge while samples are on shaker, and thaw reagents



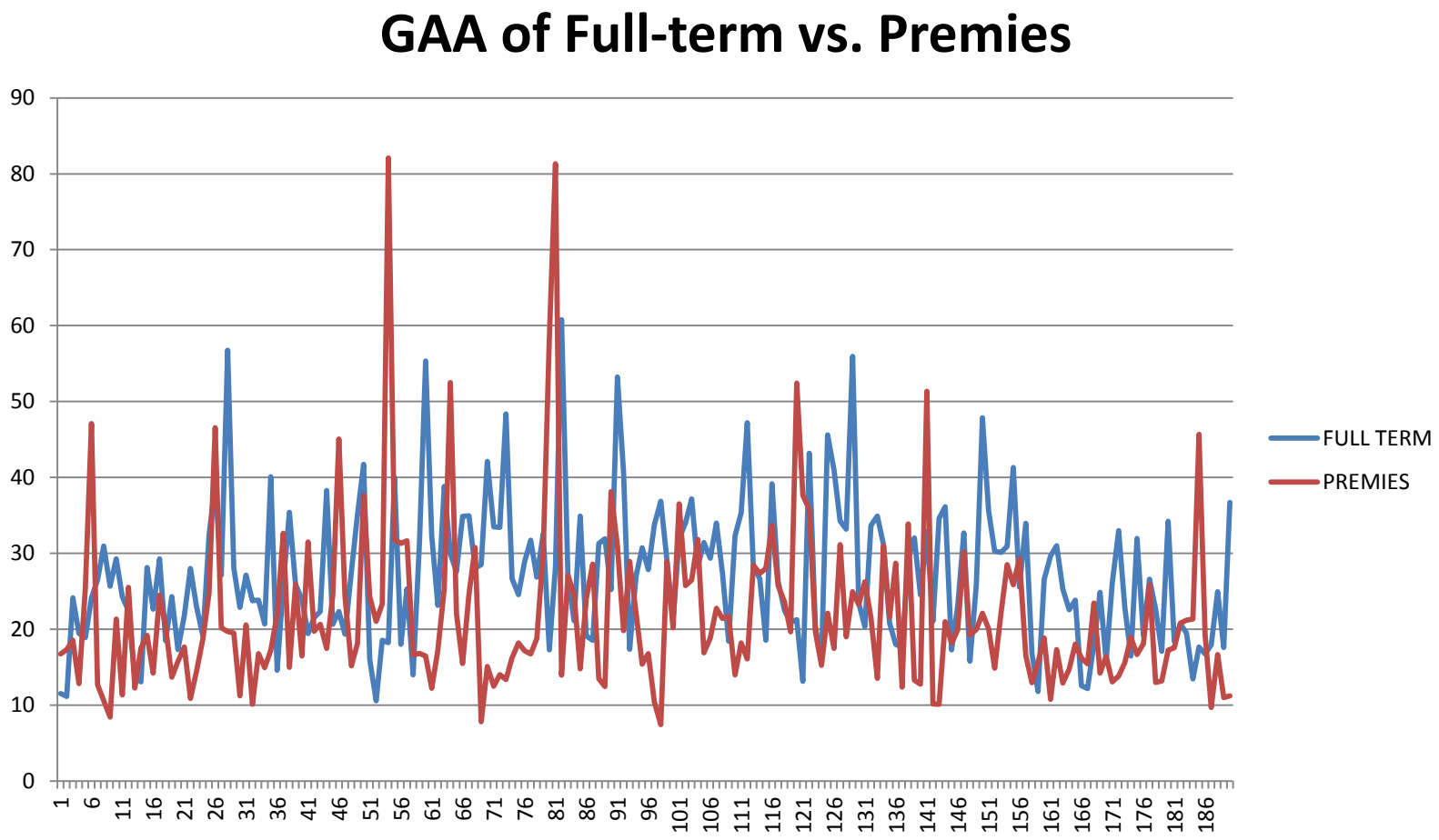
Load samples (3.5 ul) and reagents (12ul) into cartridge; Instrument run time ~ 2 hrs and 45 min

Courtesy of Missouri State Public Health Lab

Current Referral Cutoffs

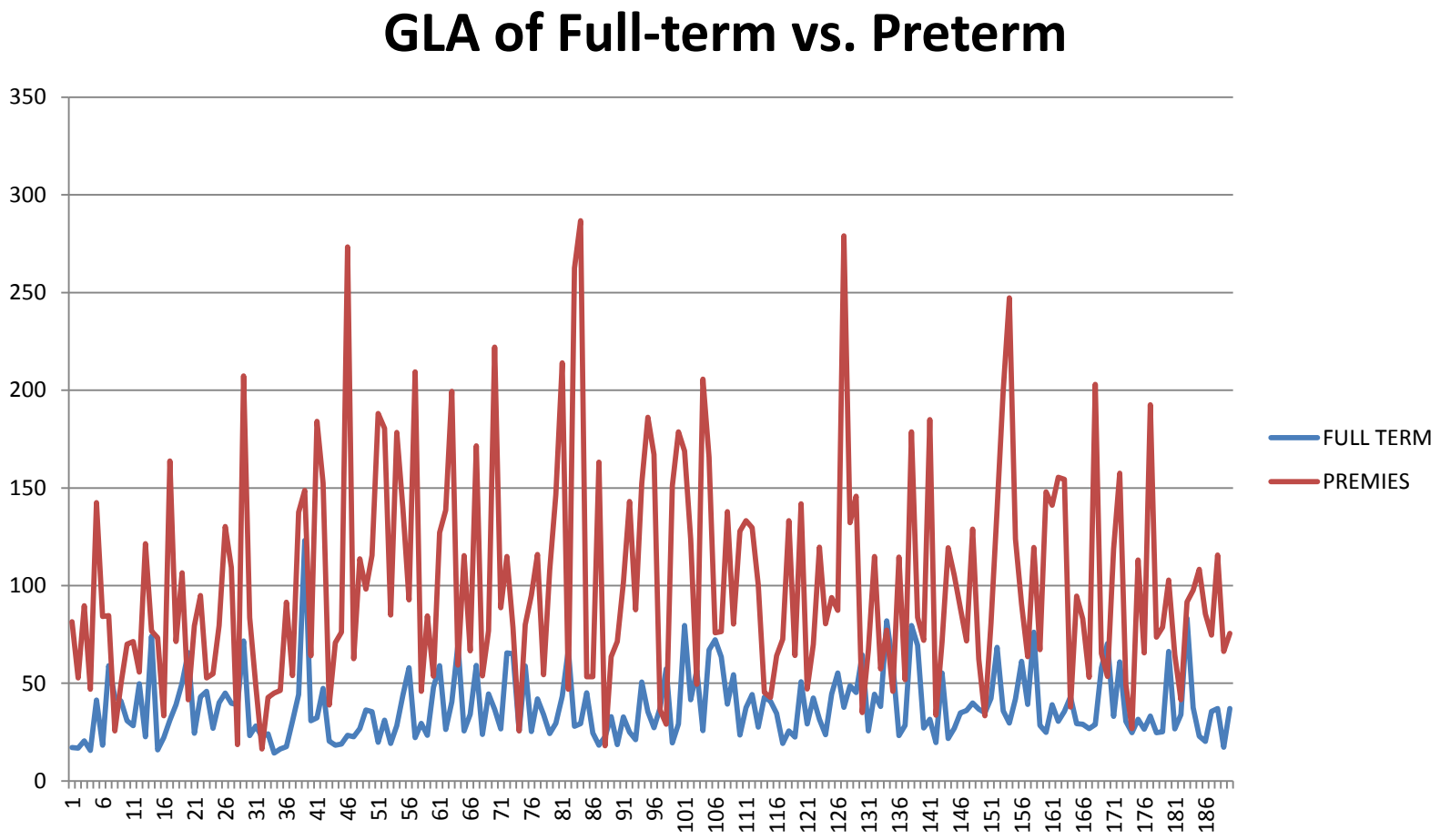
- GAA (Pompe) cutoff = ≤ 7.8 umol/L/hr (0.25% percentile)
- GBA (Gaucher) cutoff = ≤ 6.7 umol/L/hr (0.15% percentile)
- GLA (Fabry) cutoff = ≤ 7.4 umol/L/hr (0.52% percentile)
- IDUA (MPS1) cutoff = ≤ 1.4 umol/L/hr (0.07% percentile)

Health Status Effect



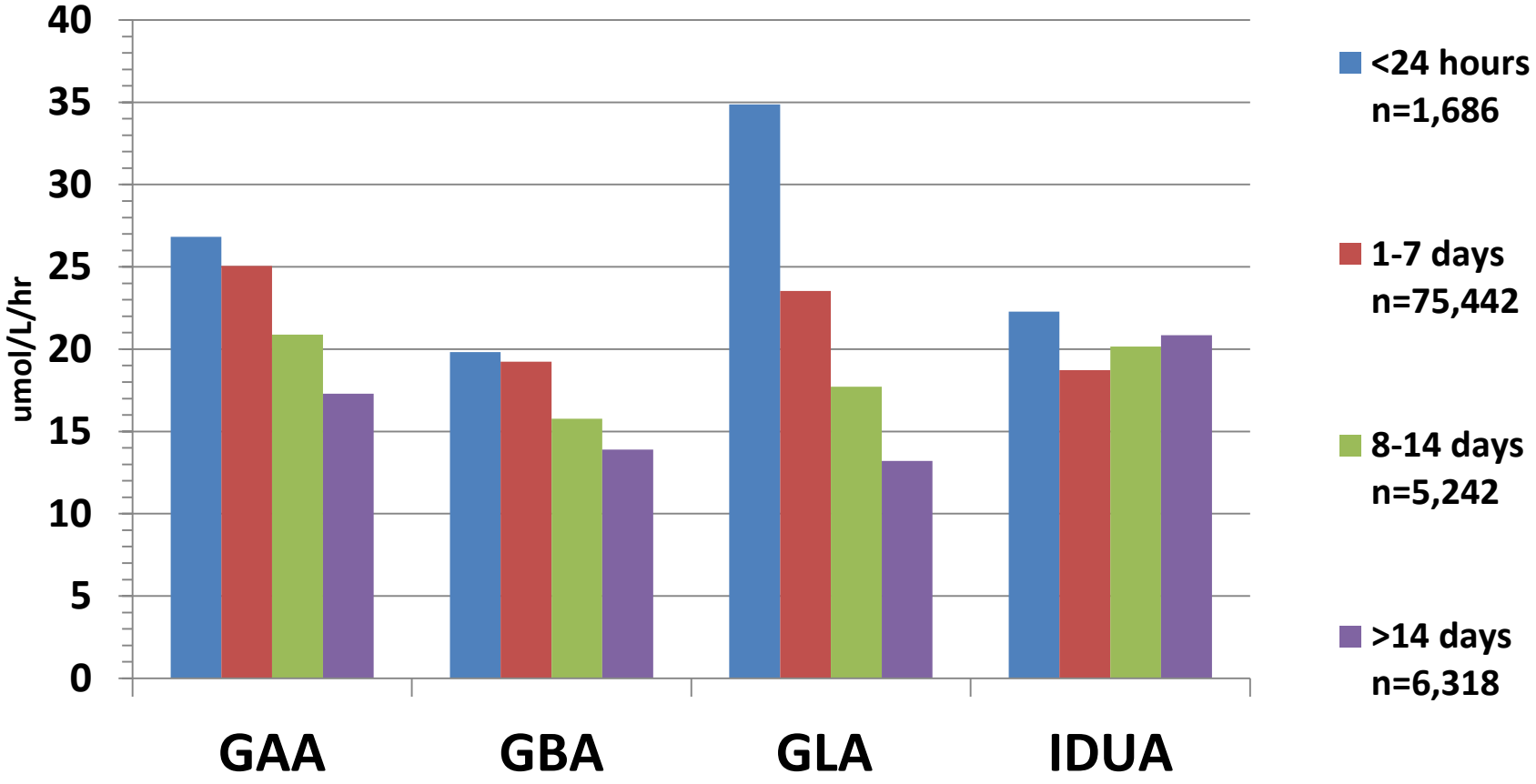
Courtesy of Missouri State Public Health Lab

Health Status Effect



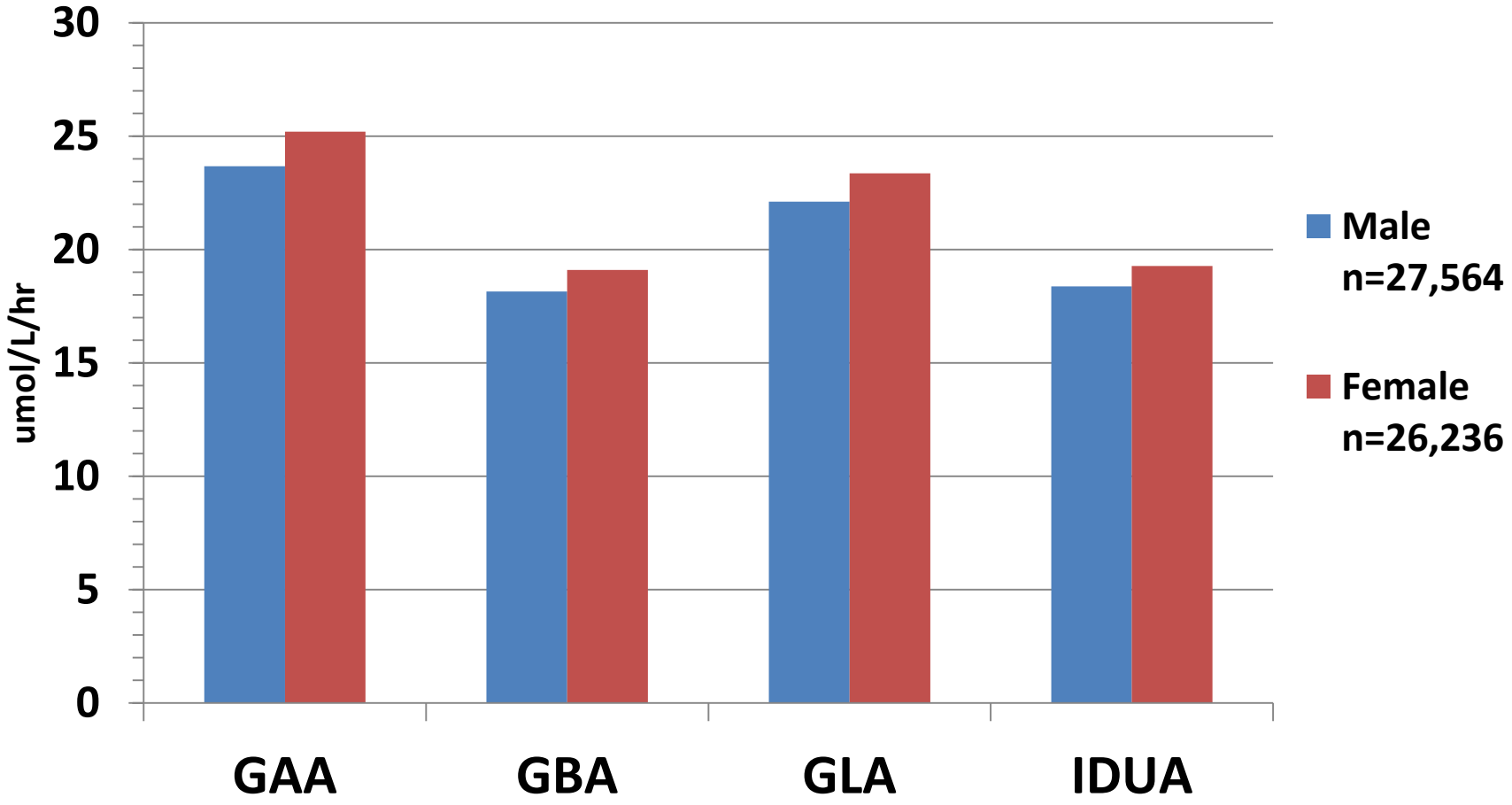
Courtesy of Missouri State Public Health Lab

Enzyme Median Activities By Age at Collection

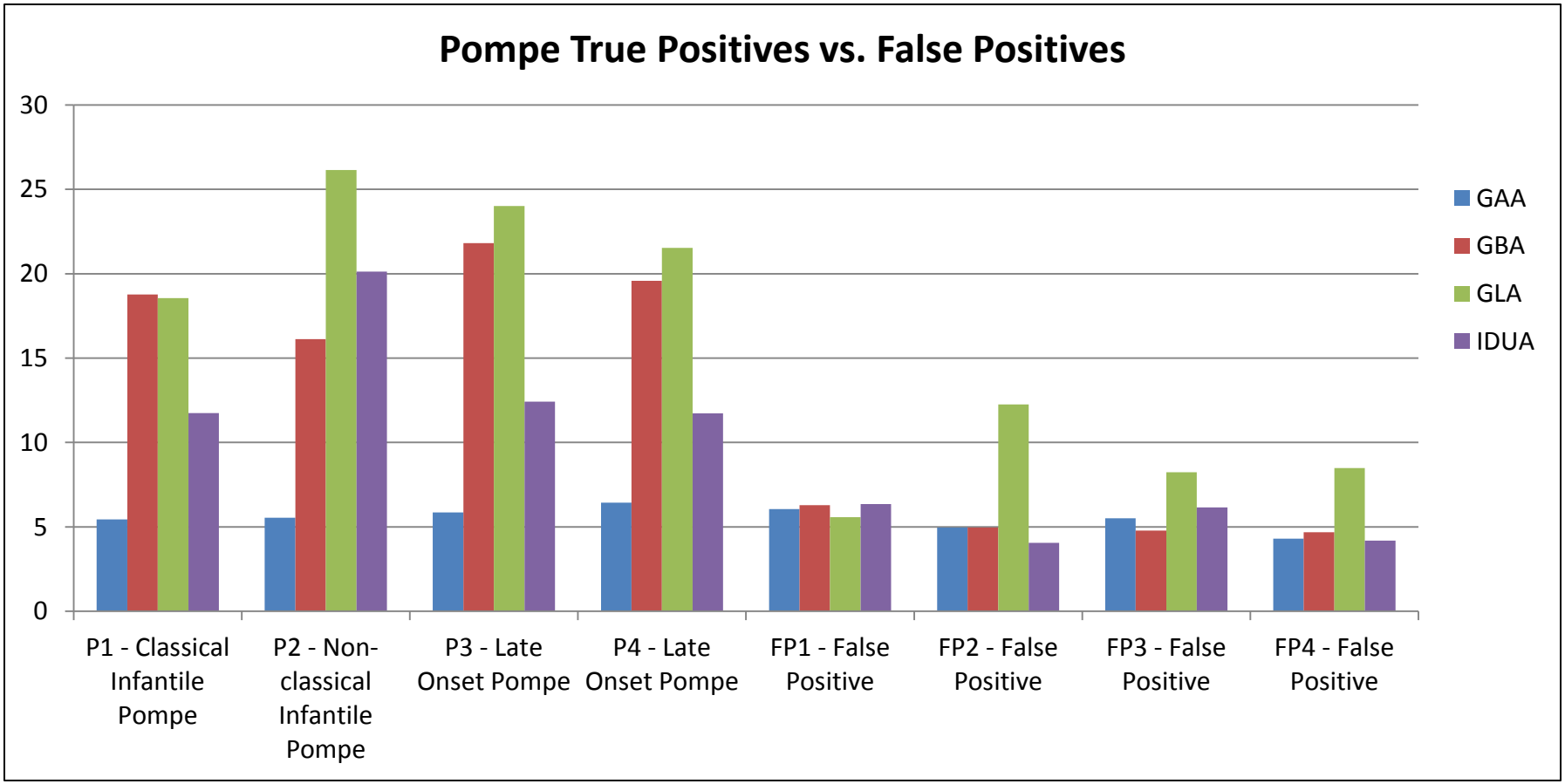


Courtesy of Missouri State Public Health Lab

Median Enzyme Activities
Male vs. Female



The Benefit of Testing Additional LSDs



Note: All eight cases were 37 weeks gestation or greater

Courtesy of Missouri State Public Health Lab

Summary of Important Laboratory Findings

- Enzyme activities drop slightly during the first 2 weeks of age and then stabilize after 14 days-of-age. Need age-related cutoffs for older babies.
- Premature babies can have altered LSD enzyme levels. The repeat screens may be more reliable on these.
- Multiplexing with other enzyme assays greatly helps assess reliability of sample results and risk for referral.
- Some seasonal variation is observed with enzyme activities, similar to GALT assay in that more carriers and pseudo-deficiencies will be detected during higher heat and humidity months (sporadically observed).
- Pleased with the performance of this screening method, the ease at which it can be incorporated into the NBS laboratory, and the ease at which it can be conducted.

New York Krabbe Screening Process

- From August 2012 to July 2015 Missouri sent samples to NY daily via overnight FedEx after all testing was completed (7 days after received by MO).
- NY received MO samples and tested them in the same manner as their own.
  - Retested anything < 20% of the GALC mean
  - Started DNA testing if first analysis is <12%
  - Continued DNA testing if average GALC is < 12%
  - Notified MO for referral if mutation was found.
- NY sent samples and results back to MO

New York Krabbe Screening

- 266,189 Missouri samples screened by **NY** as of 7/31/15 (~230,700 births).
- ~42 with polymorphisms only (these are not at risk for Krabbe disease and are not referred).
- 54 referrals: 6 genotypes of unknown significance, 3 genotypes of unknown onset, 42 with one Krabbe mutation, and 3 refusals to follow-up.
- As of April 2015 Missouri began validation for in-house screening for Krabbe using a Fluorometric Bench Assay and molecular analysis for 30Kb Deletion only.

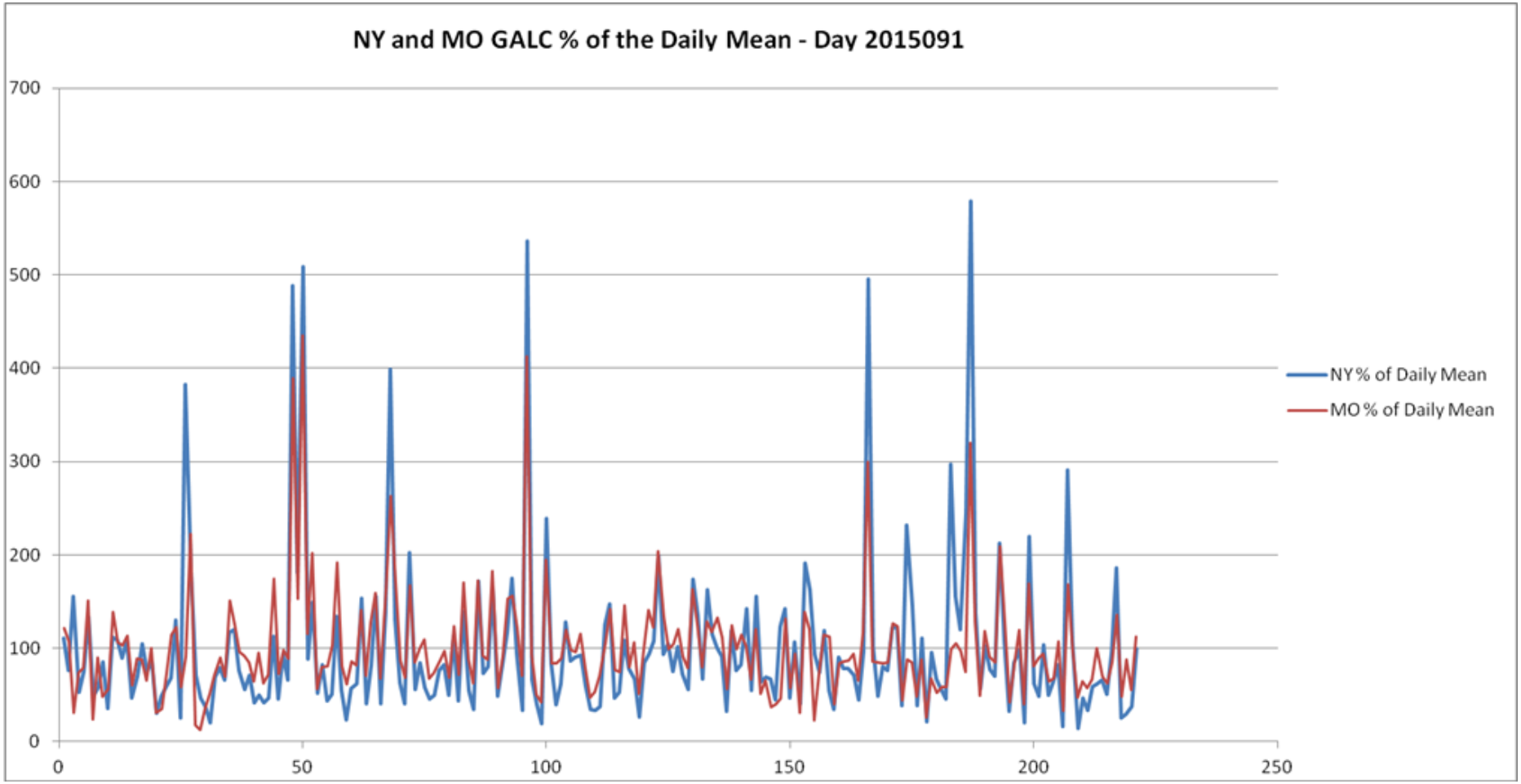
Missouri Krabbe Screening Method

Fluorometric
Bench Method
for GALC activity

Fluorometric Bench Assay for Krabbe in MO

1. Extract DBS (100 ul of extraction solution – 1 hour)
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

First Day of Parallel Testing

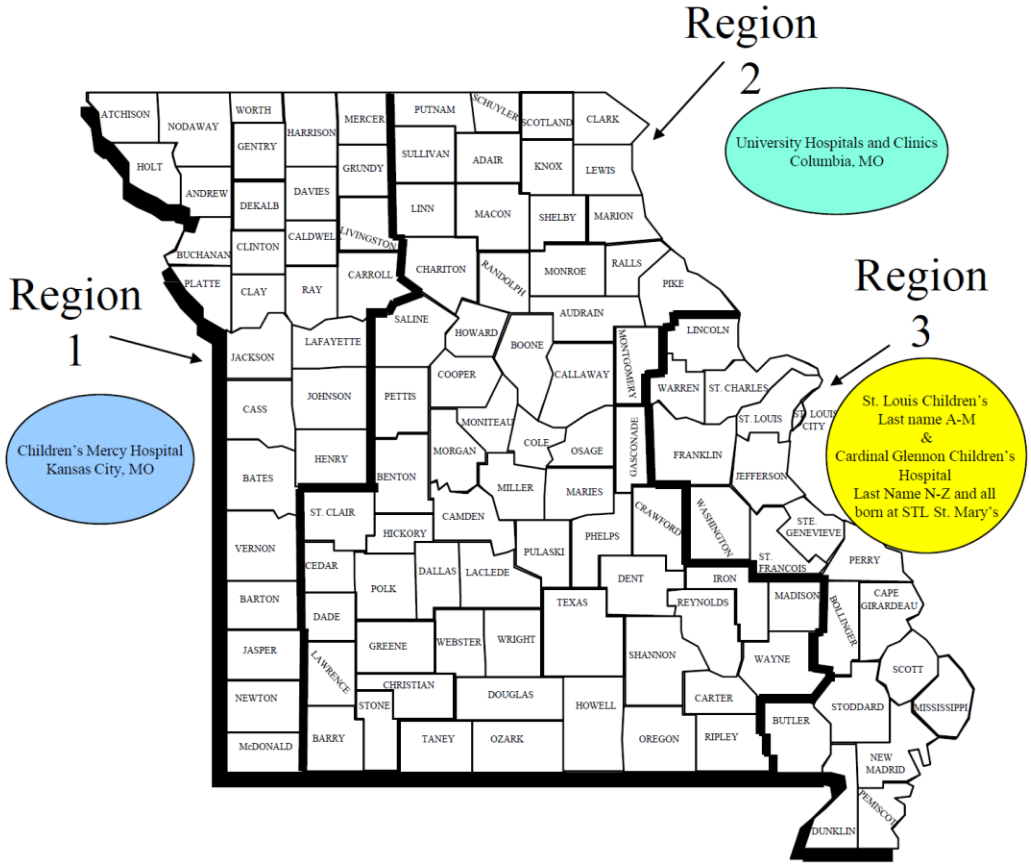


SHORT TERM FOLLOW-UP

Short Term Follow-Up

- Contract with four Genetic Centers in the State
  - Children's Mercy Hospital, Kansas City
  - Children's Hospital, University of Missouri, Columbia
  - St. Louis Children's Hospital, Washington University, St. Louis
  - Cardinal Glennon Children's Hospital, St. Louis

Follow-Up Centers Coverage



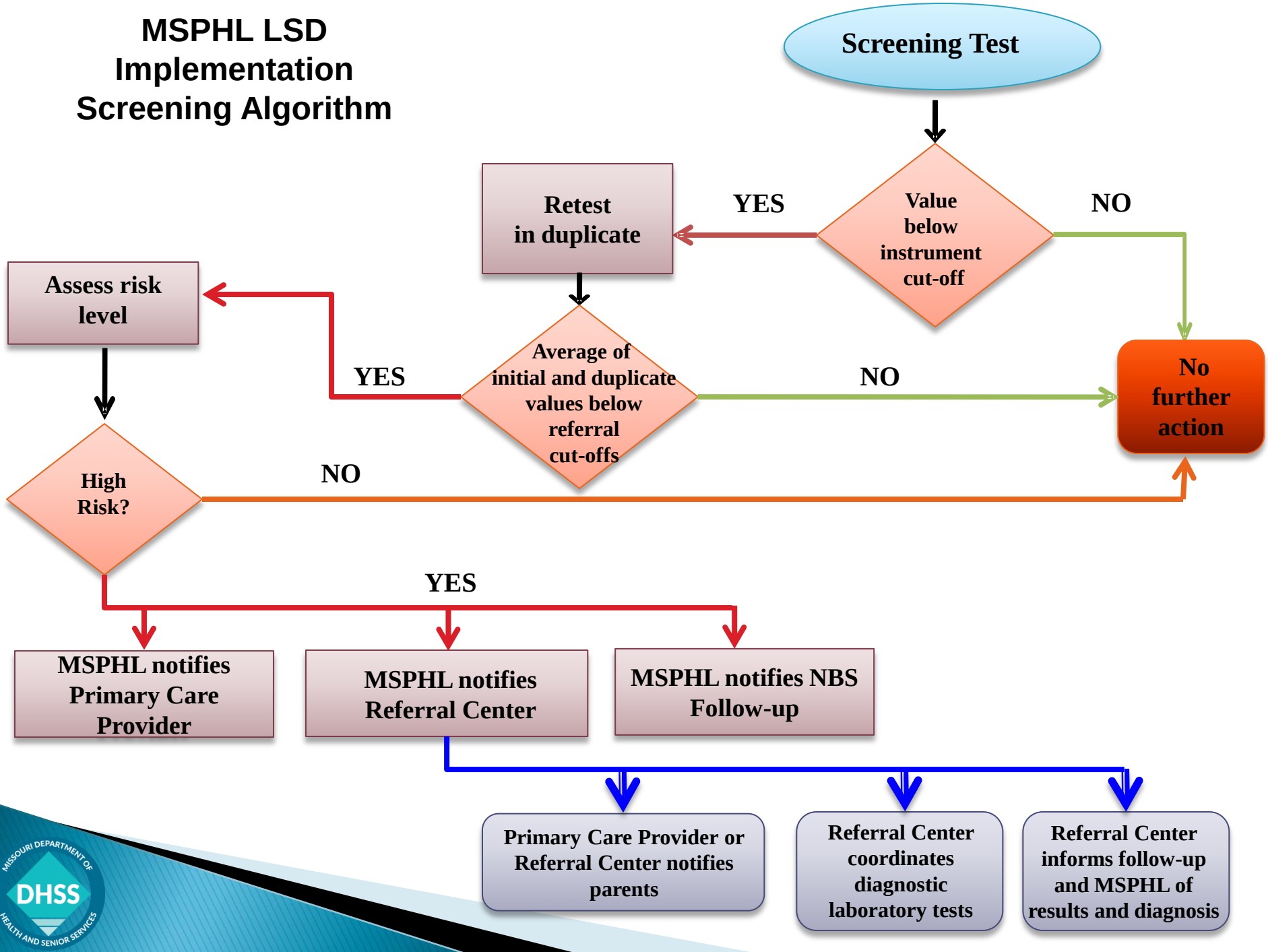
Missouri Newborn Screening Follow-Up Centers

MISSOURI DEPARTMENT OF DHSS HEALTH AND SENIOR SERVICES

Guidelines for Follow-Up

- During the implementation phase, the primary care physician was not notified of a negative result.
- Designated referral center depending on the location of the infant received a phone and fax screen-positive test result.
- Centers contacted the PCP to coordinate care
- Newborn Screening Program Follow-Up Manager also received a fax result.
- Once the program went live, the PCP were notified at the same time as the referral centers.

MSPHL LSD Implementation Screening Algorithm



Pompe

- Confirmatory testing includes:
  - GAA enzyme if GAA enzyme level is low- reflexed to DNA mutational analysis
  - Urine Hex4 CPK, CKMB, LFTs, LDH (local)

- Cardiology evaluation
  - Echocardiogram and EKG
  - Optional chest x-ray

Gaucher

- Confirmatory testing includes:
  - GBA enzyme
  - if GBA enzymes are low–DNA mutational analysis

Fabry

- Confirmatory testing includes:
  - GLA enzyme and for males only if low, reflex to DNA
  - GLA enzyme and DNA testing on all referred females
- Clinical evaluation and molecular testing on mother
- Evaluate other family members at risk through a pedigree analysis

Hurler (MPS1)

- Confirmatory testing includes:
  - IDUA enzyme, if IDUA enzyme level is low-reflexed to DNA mutational analysis
  - Urine GAG screen (quantitative and qualitative GAGs) If consistent with MPS1 mutation testing is required

Krabbe (NY screening)

- Confirmatory GALC enzyme
- Repeat NBS
- Parental carrier testing
- Additional studies if Neurological exam is abnormal include:
  - LP for CSF protein
  - Brain MRI (no contrast)
  - NCV
  - Brain stem auditory evoked response

◦ If GALC enzyme is low and only one mutation/Variant of Unknown Significance is identified with or without polymorphisms

- Baby is seen as soon as possible by Genetic Center (Neurology is not consulted)

Krabbe (Missouri Screening)

- NBS utilizing GALC enzyme screening with limited DNA (30Kb Deletion) testing
  - Child is seen within 24 hours for assessment with Genetics and Neurology
    - Confirmatory GALC enzyme
    - Complete DNA sequencing (with del/dup if 30 kb deletion not assessed through NBS) of GALC gene
    - Parental carrier testing
    - Additional studies if Neurological exam is abnormal include:
      - LP for CSF protein
      - Brain MRI (no contrast)
      - NCV
      - Brain stem auditory evoked response

Confirmatory Results

Missouri LSD Screening and Confirmatory Totals						
Disorders	Screen positive	Confirmed positive	Pseudo-Deficiencies	Carriers	Normal	Pending/ LTF/RFT
Pompe	137	34	25	29	42	6/1/0
Gaucher	30	5	0	4	19	1/0/1
Fabry	157	86	0	0	58	7/2/4
MPS 1	117	3	55	8	39	9/3/0
Krabbe	81	10	0	53	4	10/0/4
Multiple LSD	1	0	0	0	1	0/0/0
Total	523	139	80	93	163	33/6/9

Total Samples Screened for LSDs in MO as of 7/15/16 = (~276,000 births)
Total Samples Screened for Krabbe in NY as of 7/31/15 = 266,189 (~ 230,700 births)

LFT-Loss to Follow-up
RFT-Refused further Testing

Pompe Confirmed

- 34 confirmed positive
- 7 closed out as infantile – 6 CRIM positive and 1 CRIM Negative all were started on ERT
- 19 closed out as late onset – NO RX just F/U
- 6 closed out as Genotype of Unknown Significance –F/U
- 2 closed out as Unknown Onset–F/U
- 25 Pseudodeficiencies

Gaucher Confirmed

- 5 confirmed positive
- 4 closed out as Gaucher
  - 3 Non Neuronopathic
  - 1 Neuronopathic
- 1 closed out as Genotype of Unknown Significance
- All on treatment

Fabry Confirmed

- 86 confirmed positive
- 83 closed out as Fabry
- 3 closed with Genotype of Unknown Significance
- 7 confirmed babies were female
- The p.A143T allele is very common and was found in 61% of cases identified
- Identified multiple family members with Fabry

Hurler Confirmed

- 2 confirmed positive
- Both severe form
- 1st child had multiple abnormalities, died with complications from transplant
- 2nd underwent a transplant and is doing well
- 55 Pseudodeficiencies

Krabbe Confirmed

- 10 confirmed positive
- 0 closed out as Infantile Onset
- 4 closed out as Unknown Onset
- 6 closed out as Genotype of Unknown Significance

All are being f/u either by PCP or Neurology

Incidence of LSDs in Missouri

	Incidence in MO	Published Incidence
Fabry	1:3,300	1:40,000
Pompe	1:39,000	1:40,000
Gaucher	1:69,000	1:100,000
MPS1	1:138,000	1:100,000
Krabbe	0:305,000	1:300,000

CHALLENGES

Patient Volume and Building Infrastructure

- More referrals than anticipated
- Recommend a dedicated person to follow-up on NBS and coordinate appointments to ensure timely assessments
- Clinical evaluations may need to occur immediately
- Assemble a team in advance of other specialists
  - Neurology
  - Cardiology
- Plan educational sessions with other specialists to ensure everyone has the same goal

Courtesy of Linda Manwaring at St. Louis Children's Hospital

Laboratory Reporting and Interpretation

- Utilize a lab that has experience with NBS follow-up
- Differences exist in how results are reported and interpreted
- Results are not always conclusive
- Poor genotype/phenotype correlations
- High number of variants of unknown significance on DNA testing
- Previously unreported pseudodeficiencies
  - 2-3% of AA for MPS1
  - 4% of Asians for Pompe

Courtesy of Linda Manwaring at St. Louis Children's Hospital

Clinical Follow-up and Management

- Lack of medical management guidelines/practices for the asymptomatic patient
  - How often should we see these patients and when do we consider starting treatment?
  - Is there a risk for those with a pseudodeficiency allele and a known mutation to be at risk of developing disease? Should these children be followed and how often?
  - Need for better biomarkers
  - Are we treating a lab value vs. clinical symptoms?
  - Are we doing harm to the patient by making them medically fragile when they are otherwise in good health by ordering too many evaluations too soon?
  - Concern for patient becoming lost to follow-up is more likely if patient is healthy for many years before onset of symptoms

Courtesy of Andrea Atherton, Children's Mercy Hospital

A Word on Fabry Disease

- NBS patients and their "affected" family members have tripled our Fabry population in almost 3 years
- 61% have the A143T allele
  - Few symptomatic adult relatives
  - How do you manage these cases?
- How do you plan to test asymptomatic at-risk relatives?
  - See in clinic vs ordering testing without a clinic visit
  - Can they be seen by a Genetic Counselor alone?
  - Number of patients can be overwhelming

Courtesy of Linda Manwaring at St. Louis Children's Hospital

Ethical Dilemmas

- Identifying carriers and diagnosing late onset conditions in the newborn period
- Loss of the parent/child bond when there is a diagnosis made in infancy before signs and symptoms are present
- Inability to get life insurance, long term care insurance, disability insurance
- Parents and patients do not “fit in” to the support groups

Courtesy of Andrea Atherton, Children’s Mercy Hospital

Lessons Learned

- Screening and confirmation for Lysosomal Storage disorders are complex
- Create a task force
- Follow-up guidelines for long term follow-up is critical but need to be flexible
- Frequent communication between the specialists has been helpful
- Regular communication with centers

Overall Summary

- Screening for the LSD's have been going smoothly
- To date the incidences are at or higher than the published incidences
- The false positive rates are similar to other NBS tests
- No reported undetected cases presented clinically to date
- Still many unanswered questions

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THANK YOU

QUESTIONS?