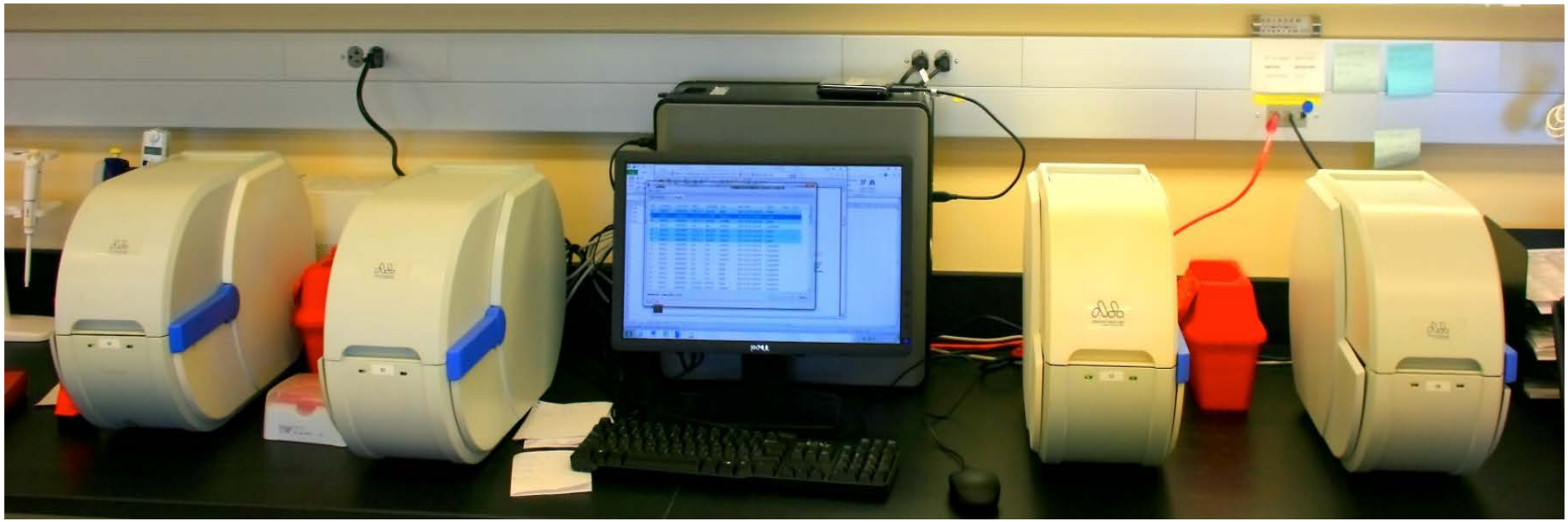
LSD Pilot Screening in Missouri

for

Pompe, Gaucher, Fabry and MPS-I Disorders

Utilizing Digital Microfluidics Technology



Patrick Hopkins, Chief of Missouri NBS Laboratory

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.

3. The department may promulgate rules to implement the provisions of this section. Any rule or portion of a rule, as that term is defined in section 536.010, RSMo, that is created under the authority delegated in this section shall become effective only if it complies with and is subject to all of the provisions of chapter 536, RSMo, and, if applicable, section 536.028, RSMo. This section and chapter 536, RSMo, are nonseverable and if any of the powers vested with the general assembly pursuant to chapter 536, RSMo, to review, to delay the effective date, or to disapprove and annul a rule are subsequently held unconstitutional, then the grant of rulemaking authority and any rule proposed or adopted after August 28, 2009, shall be invalid and void.

4. The department may increase the fee authorized in subsection 6 of section 191.331 to cover the additional cost of the expanded newborn screening test required in this section.

The Power of Advocacy

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

MO LSD Statewide Pilot Screening

Krabbe (GALC)

→ Testing by New York Since August 2012

(over 235,000 samples)
(approximately 204,000 births)

Pompe (GAA)
Gaucher (GBA)
Fabry (GLA)
MPS-I (IDUA)

→ Missouri Testing Since January 11, 2013

(over 201,000 samples)
(approximately 175,000 births)

Krabbe (GALC)
Niemann-Pick (ASM)

→ Missouri to Add-on next

Missouri Newborn Screening

- ~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 4 FTE's in follow-up
- 2 lab FTE's dedicated to LSD screening
- 5 lab staff are trained to conduct LSD testing
- 2 DMF workstations (8 platforms)
- Started full population LSD pilot January 11, 2013

Implementation Plan

- Installation
- Training
- Familiarization
- Validations
- Pre-pilot phase (data collection on de-identified samples)
- Pilot Phase (statewide testing with referrals)
- Live Testing with reporting on all NBS lab reports

Implementation Plan

- Contract procurement (4 – 12 months)
- Installation (2 hours)
- Training (2 days)
- Familiarization (2 weeks)
- Validations (2 months, then ongoing through pilot)
- Pre-pilot phase to collect data on de-identified samples for normal ranges and cutoffs (2 months)
- Pilot/Implementation Phase with statewide testing, referral and confirmation (~14 months)
- Live Testing with reporting on all NBS lab reports

Pre-Pilot Phase Preparation

We used >13,000 de-identified DBS samples that had been stored for 6 months, but first we pre-separated them into specific categories :

- Collection time of > 24 hrs age and < 7 days age with normal health status
- ≥ 7 days-of-age collection, normal health status
- Early collection (< 24 hrs age), not transfused
- Premature, < 7 days of age, not transfused
- Transfused and < 7 days of age
- Transfused and ≥ 7 days of age

Validation Exercises

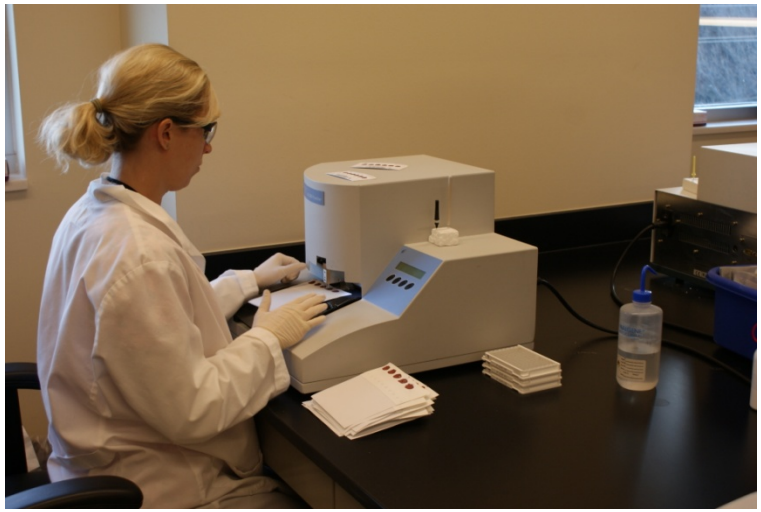
- Conducted extensive validation experiments to support CLIA requirements for Lab developed tests (LDT's).
- Tested around 13,000 QC and CDC samples.
- Tested over 30,000 de-identified DBS samples.
- Conducted sample exchanges for validations with Mayo, CDC and New York.
- Verified 2 Pompe cases diagnosed clinically and 1 Gaucher carrier from our stored identified samples during pre-pilot phase.

Validation of New Methods

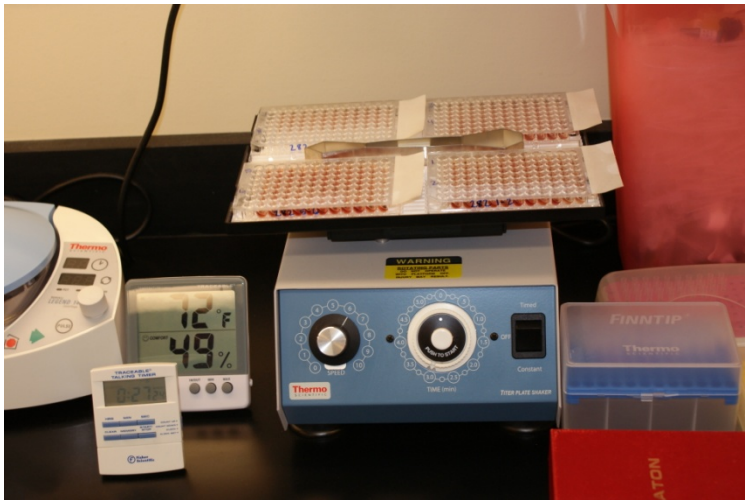
- Accuracy/Sensitivity – Using known positives, quality control and proficiency test samples.
- Precision/Specificity – Within run, between runs, between different reagent lots.
- Linearity/Limit of detection – Consistency from high to low levels of the detection range.
- Instrument matching – to maintain same cutoffs
- Carryover
- Testing interferences – Health status, age of baby, etc.

2 Work Stations
8 Digital Microfluidics (DMF) Platforms

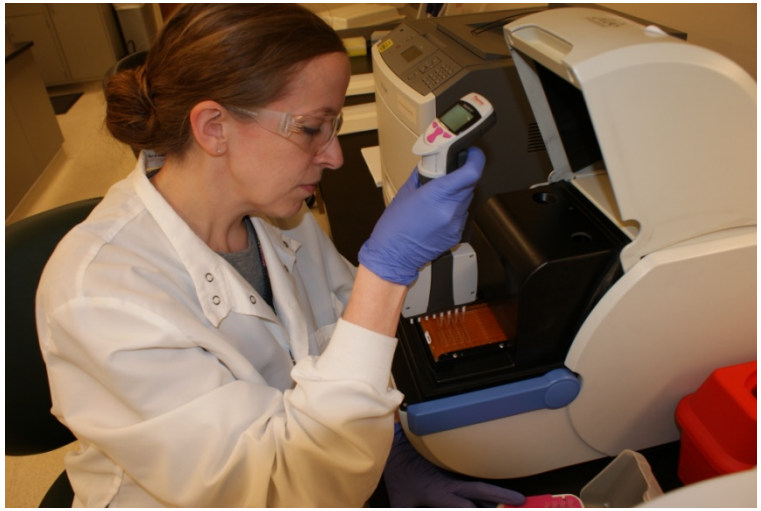
Workflow for LSD Testing at MSPHL



Punch samples (15 min per 96-well plate)

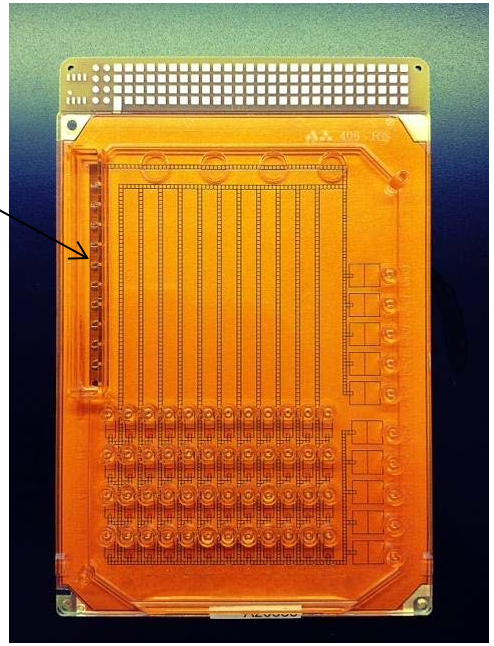


Extract samples (30 min at room temp)



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

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



Enzyme Reactions in DMF Method

ENZYME + SUBSTRATE ⟹ PRODUCT

Gaucher Disease

4MU-ß-D-Glucopyranoside + DBS extract (GBA) $\xrightarrow{\text{pH 5.2}}$ 4-MU + Glucose

Pompe Disease

4MU-α-D-Glucopyranoside + DBS extract (GAA) $\xrightarrow{\text{pH 3.8}}$ 4-MU + Glucose

Fabry Disease

4MU-α-D-Galactopyranoside + DBS extract (GLA) $\xrightarrow{\text{pH 4.5}}$ 4-MU + Galactose

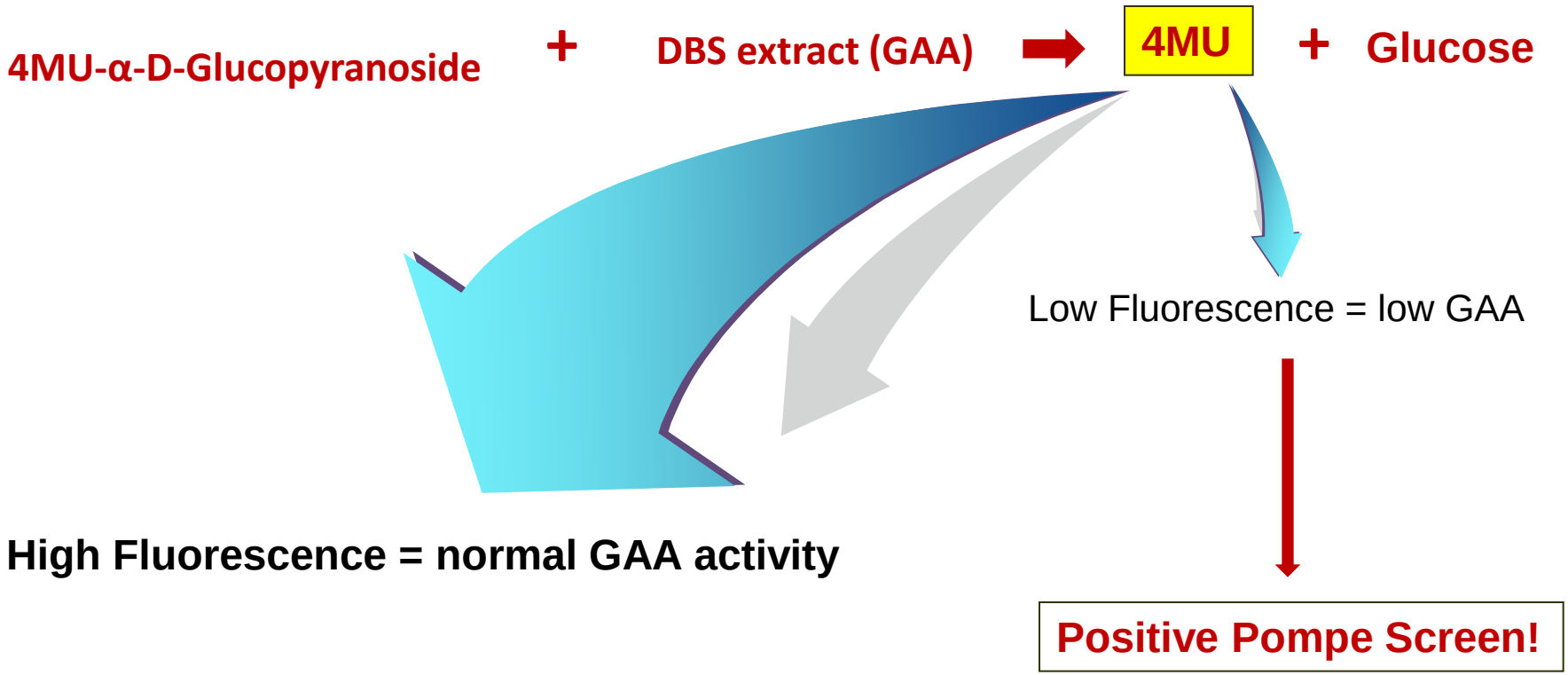
Hurler Disease

4MU-α-L-Iduronoside + DBS extract (IDU) $\xrightarrow{\text{pH 3.5}}$ 4-MU + Iduronide

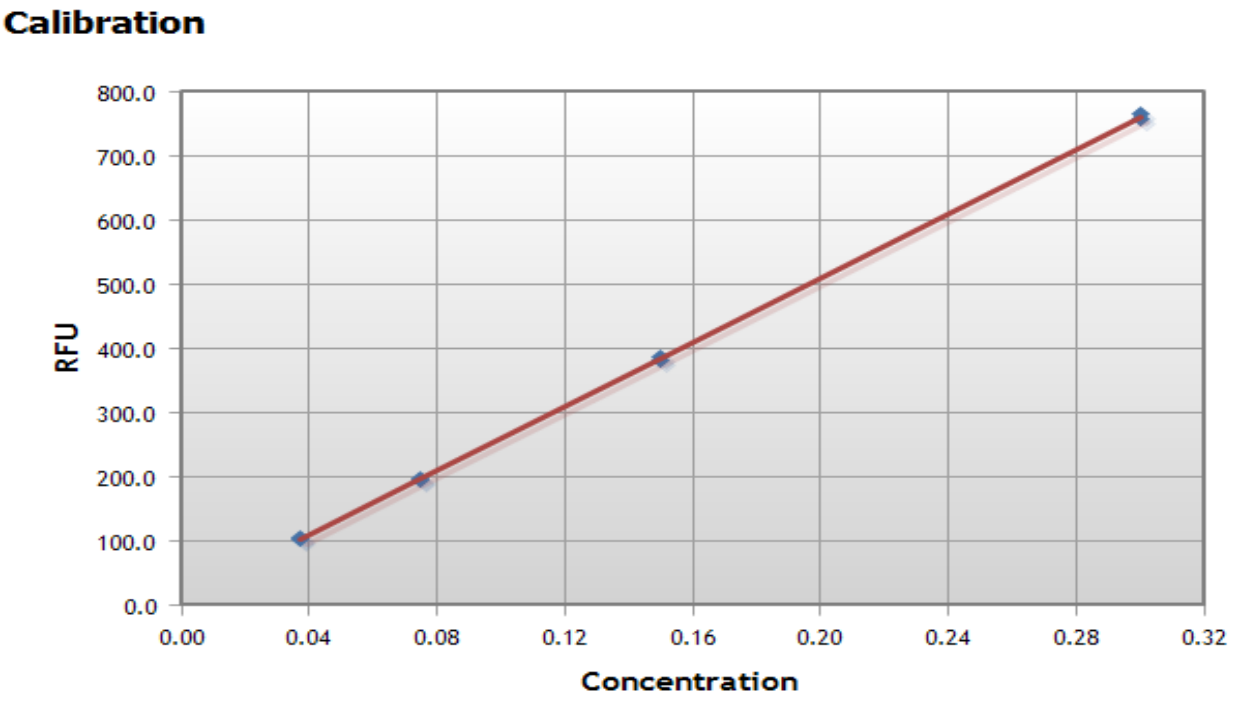
Low 4-MU fluorescence means low enzyme activity in bloodspot sample

Enzyme Reaction in DMF Method

Artificial Substrate + Enzyme → Product



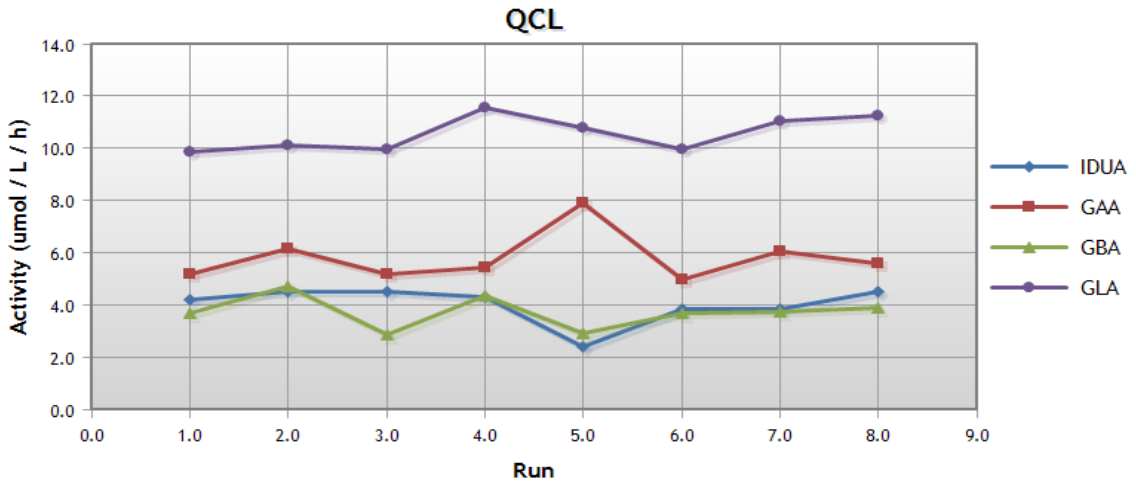
Each Cartridge Has 4 Calibrators

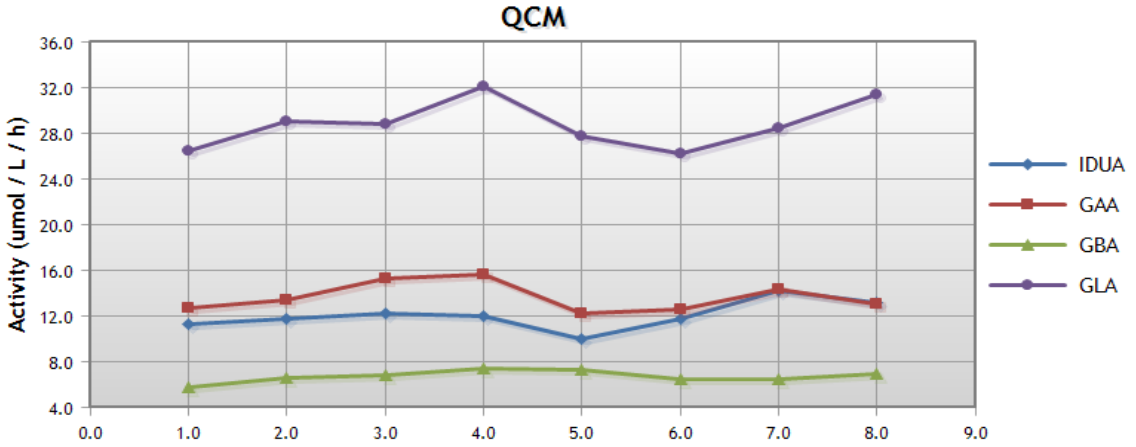


Concentration	A	B
0.0375	104.29	102.23
0.075	196.57	193.99
0.15	386.82	380.51
0.3	763.54	757.61
Slope	**Intercept**	**R^2**
2506.79	8.18	0.9999

Quality Control Monitor for Run

48x4v10 QC Report





Results Screen

Results

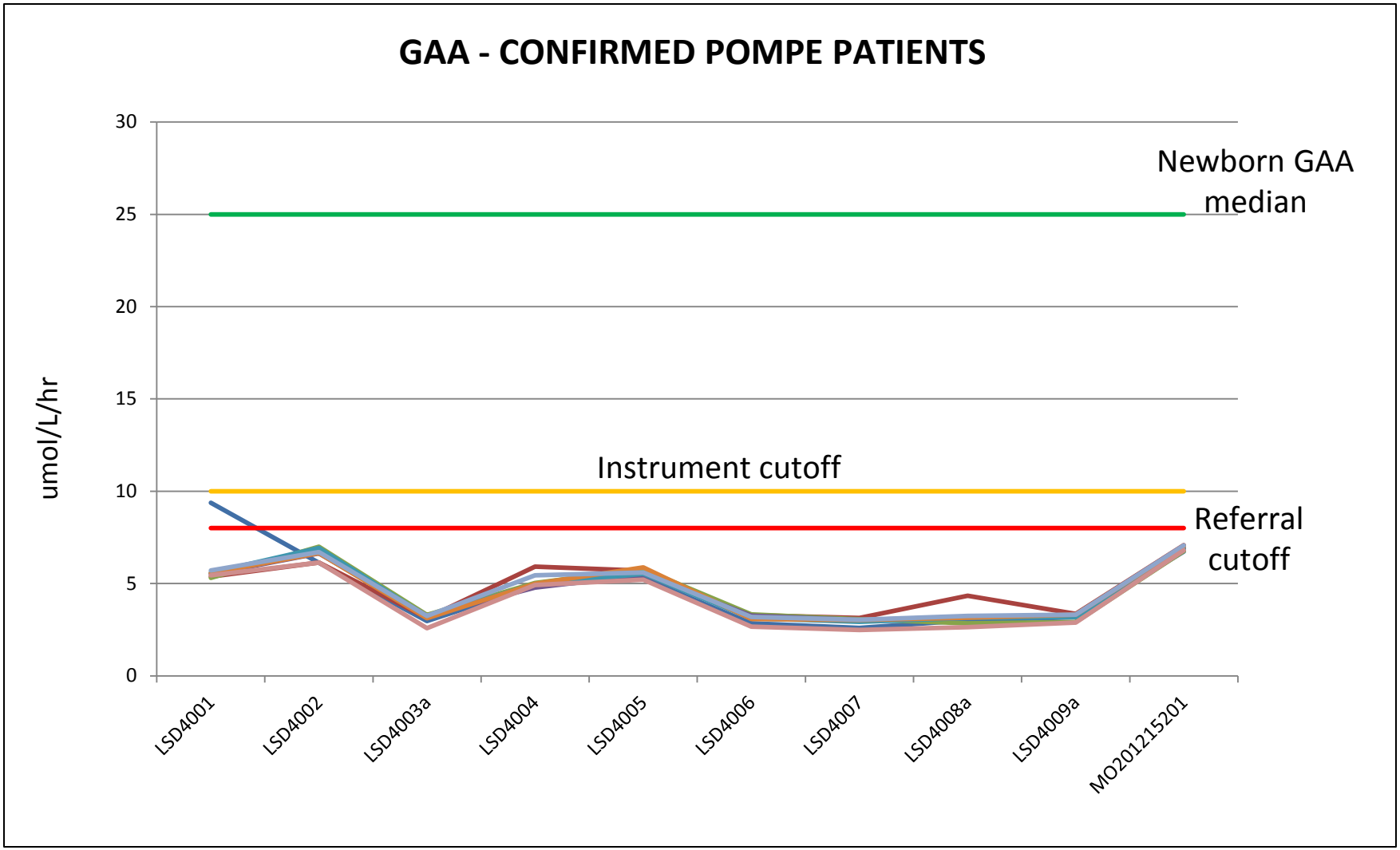
Sample	Location	IDUA (µmol / L / h)	GAA (µmol / L / h)	GBA (µmol / L / h)	GLA (µmol / L / h)
For research use only. Not for use in diagnostic procedures.					
Sample Mean		22.77	23.92	20.80	24.74
CDCBP281	A02	3.51	1.60	2.39	3.87
CDCL282	A03	5.13	3.64	2.72	5.49
CDCM283	A04	24.16	22.73	10.18	37.34
CDCH284	A05	39.48	33.50	12.92	54.95
14036	A06	37.31	9.91	35.37	94.67
14036	A07	28.92	8.33	35.84	86.73
14036	A08	7.60	8.35	9.88	7.32
14036	A09	9.09	10.60	11.93	9.46
QCM	A10	12.62	12.22	6.29	31.67
QCL	A11	5.71	5.87	4.00	10.83
14037	B10	17.84	22.80	20.31	11.81
14037	B11	23.80	33.63	20.43	14.29
14037	B12	21.91	33.57	20.52	9.83
14037	C02	19.67	6.20	18.58	22.28
14037	C03	18.38	5.55	17.93	20.06
14037	C04	12.15	12.77	12.39	8.59
14037	C05	12.08	11.59	10.59	7.90
14037	C06	21.74	19.23	32.69	11.43
14037	C07	12.15	14.52	20.72	8.10
14037	C08	23.08	29.23	25.47	27.50

Positive Pompe in duplicate

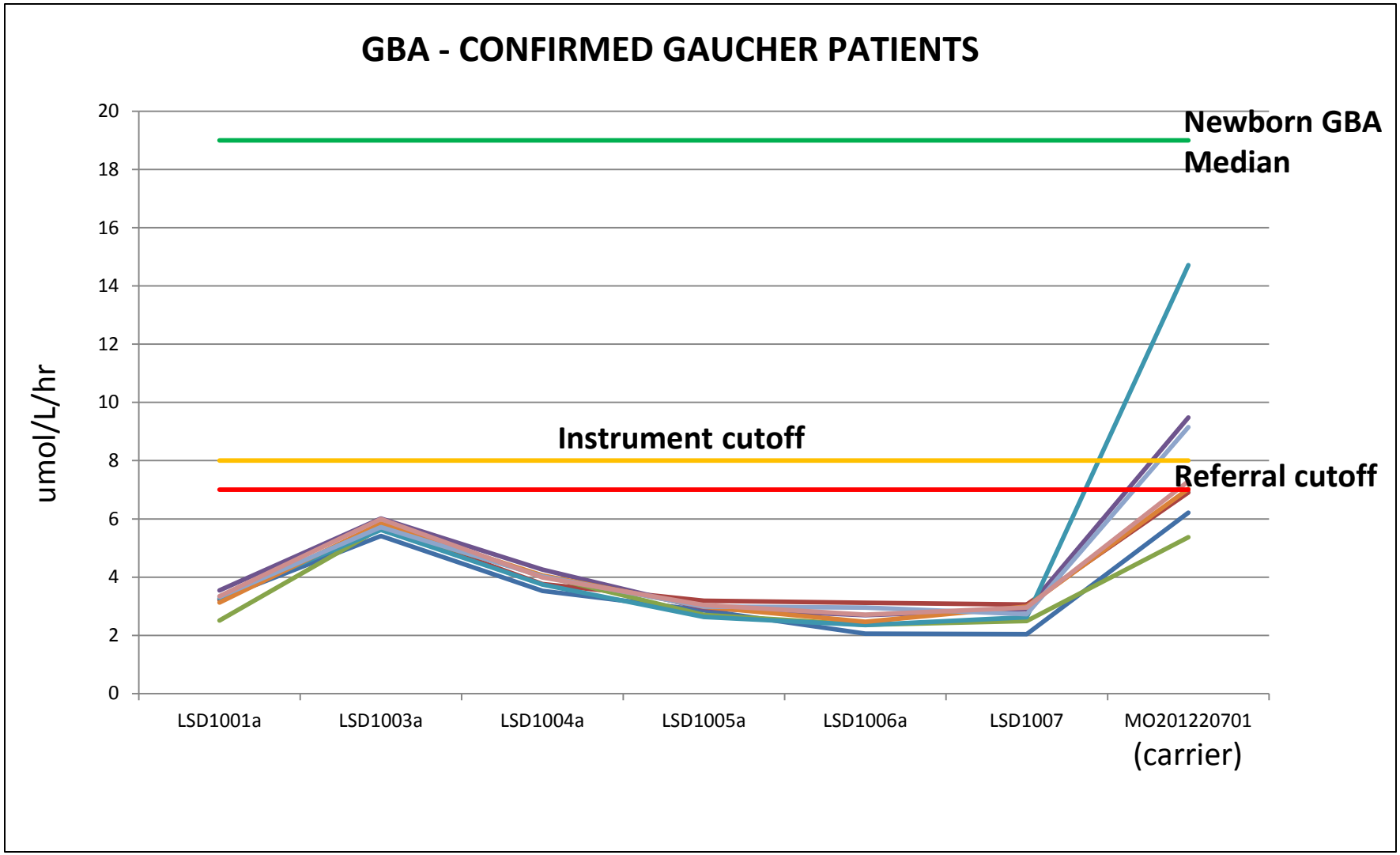
Yellow = Instrument Cutoff

Red = Referral Cutoff

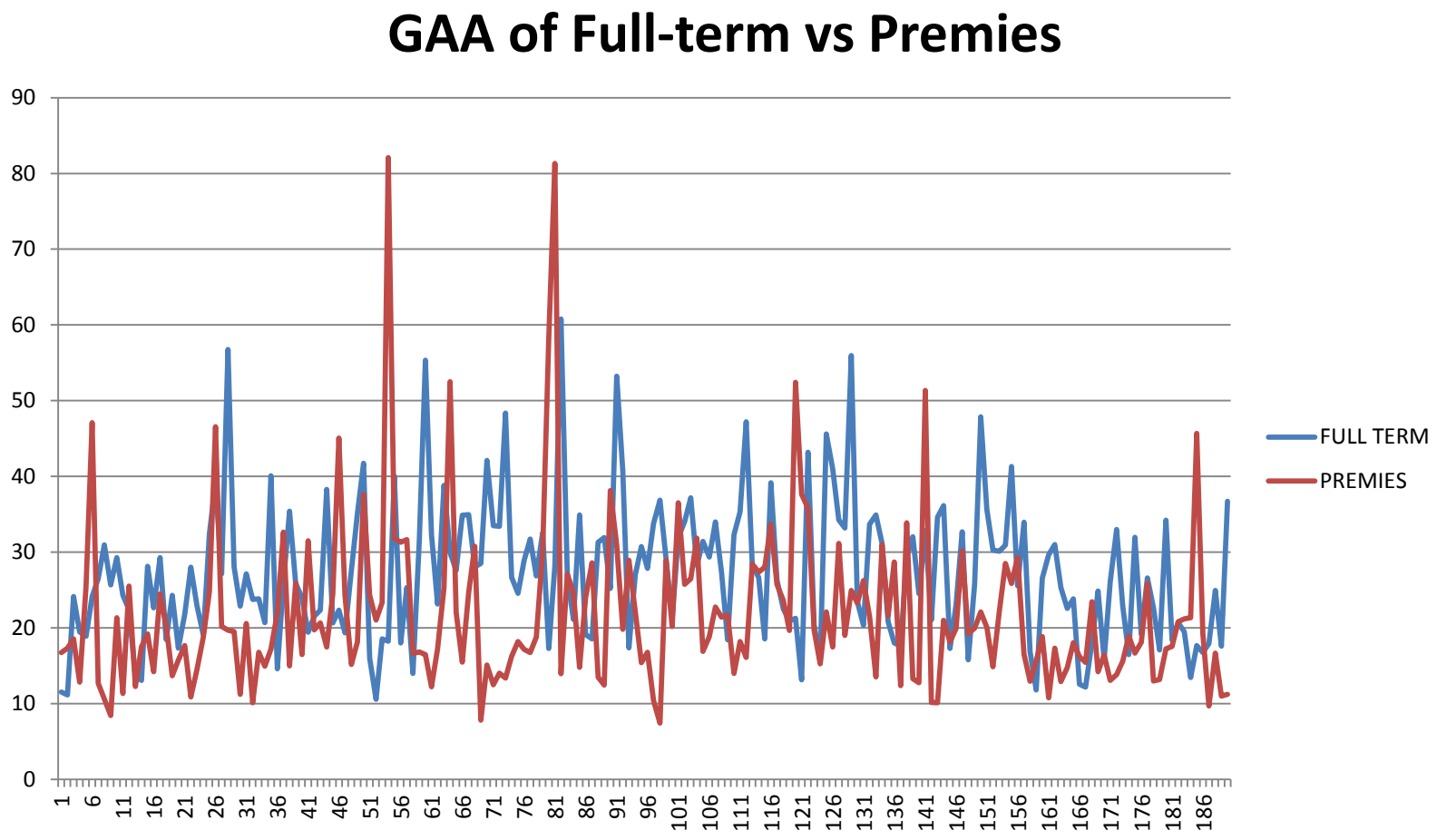
Testing Known Positives



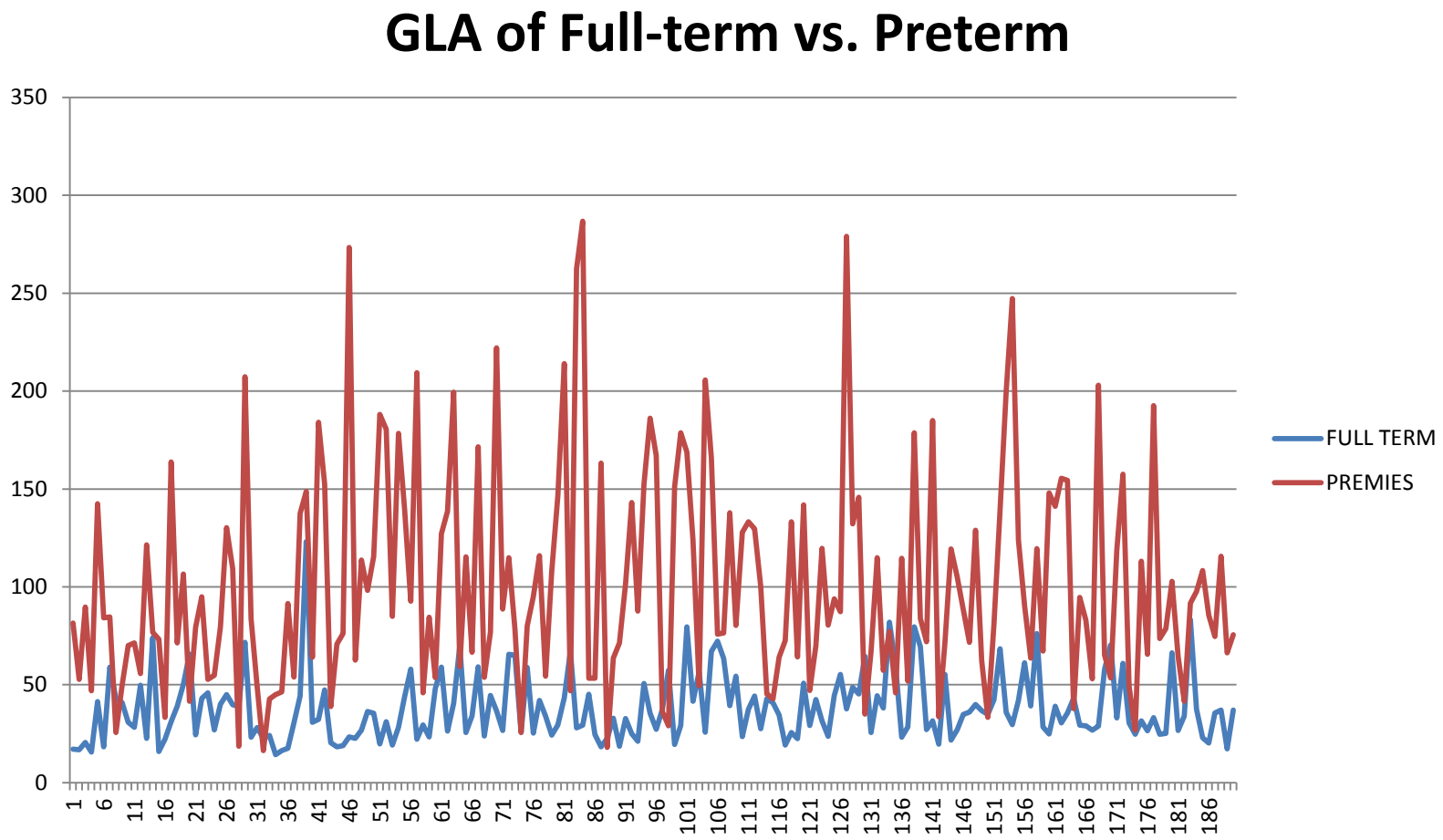
Testing Known Positives



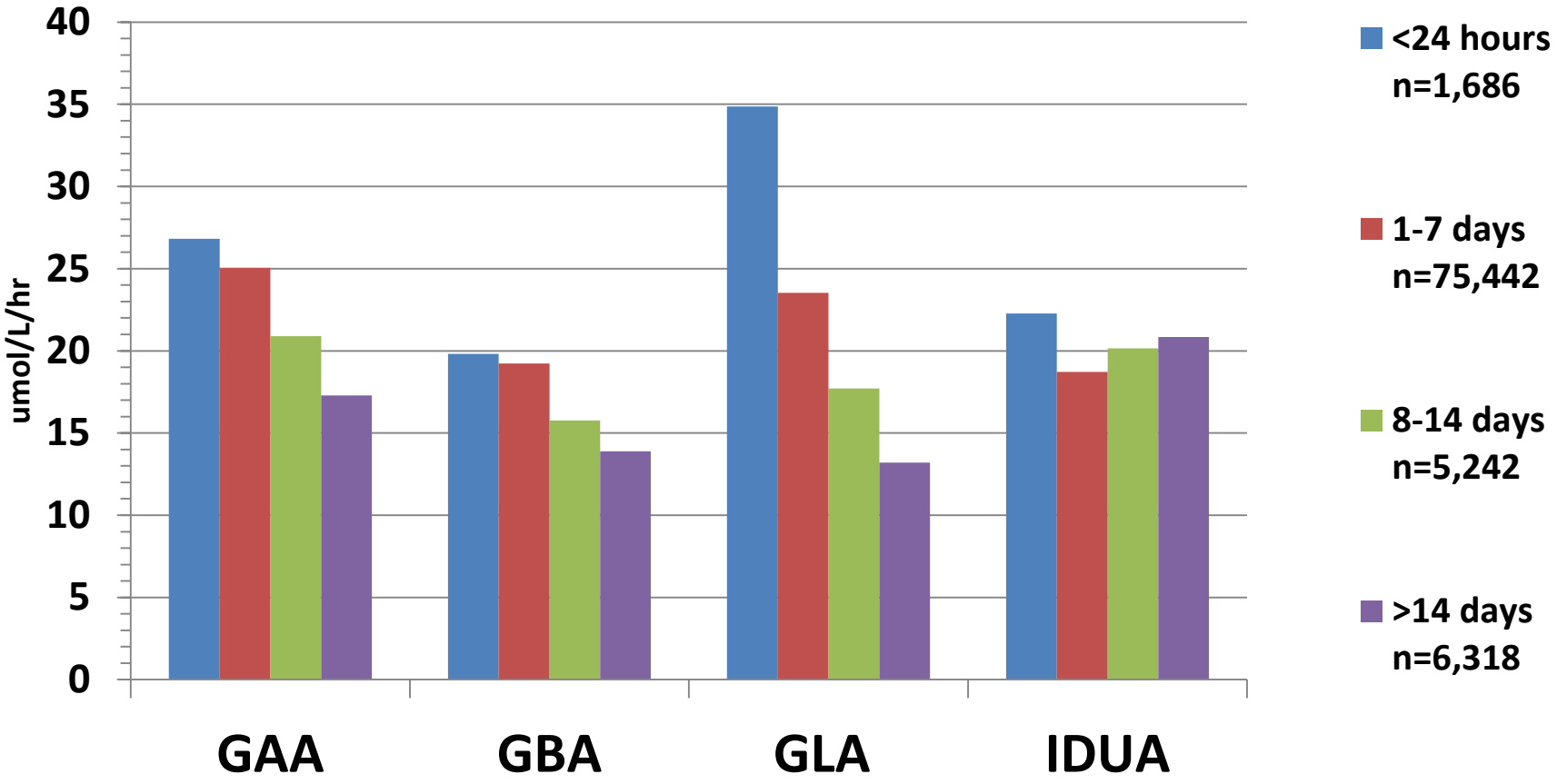
Health Status Effect



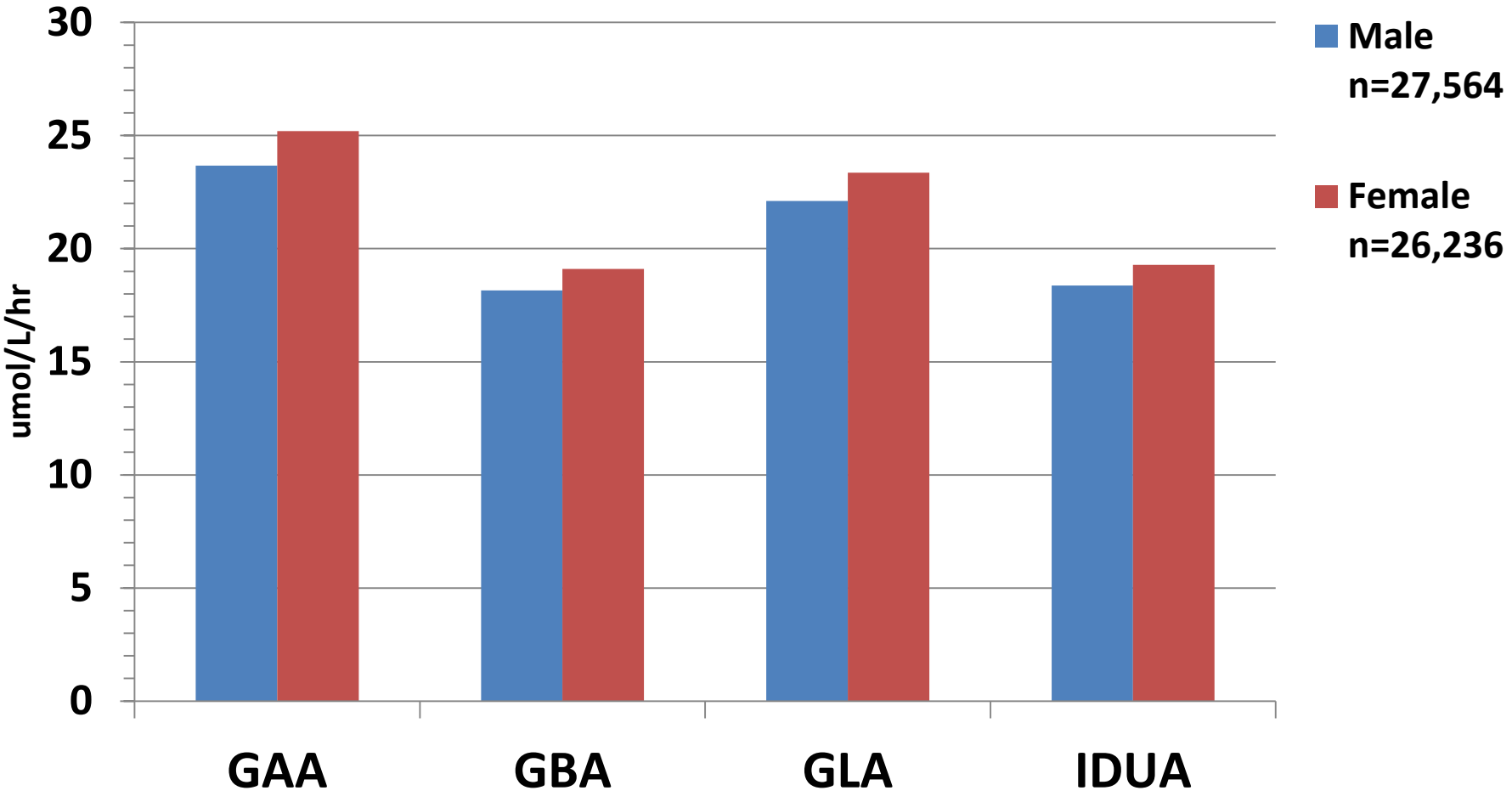
Health Status Effect



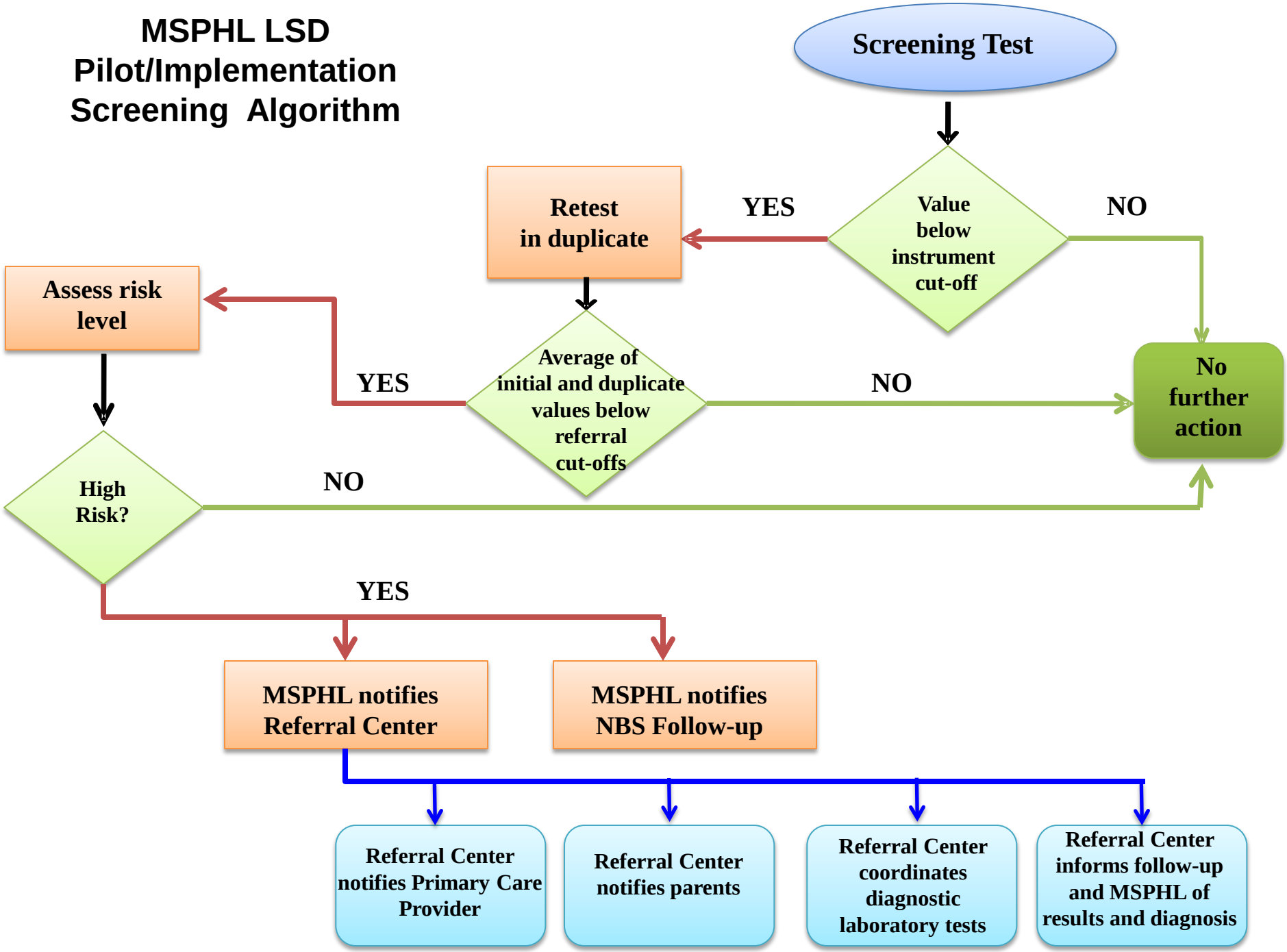
Enzyme Median Activities By Age at Collection



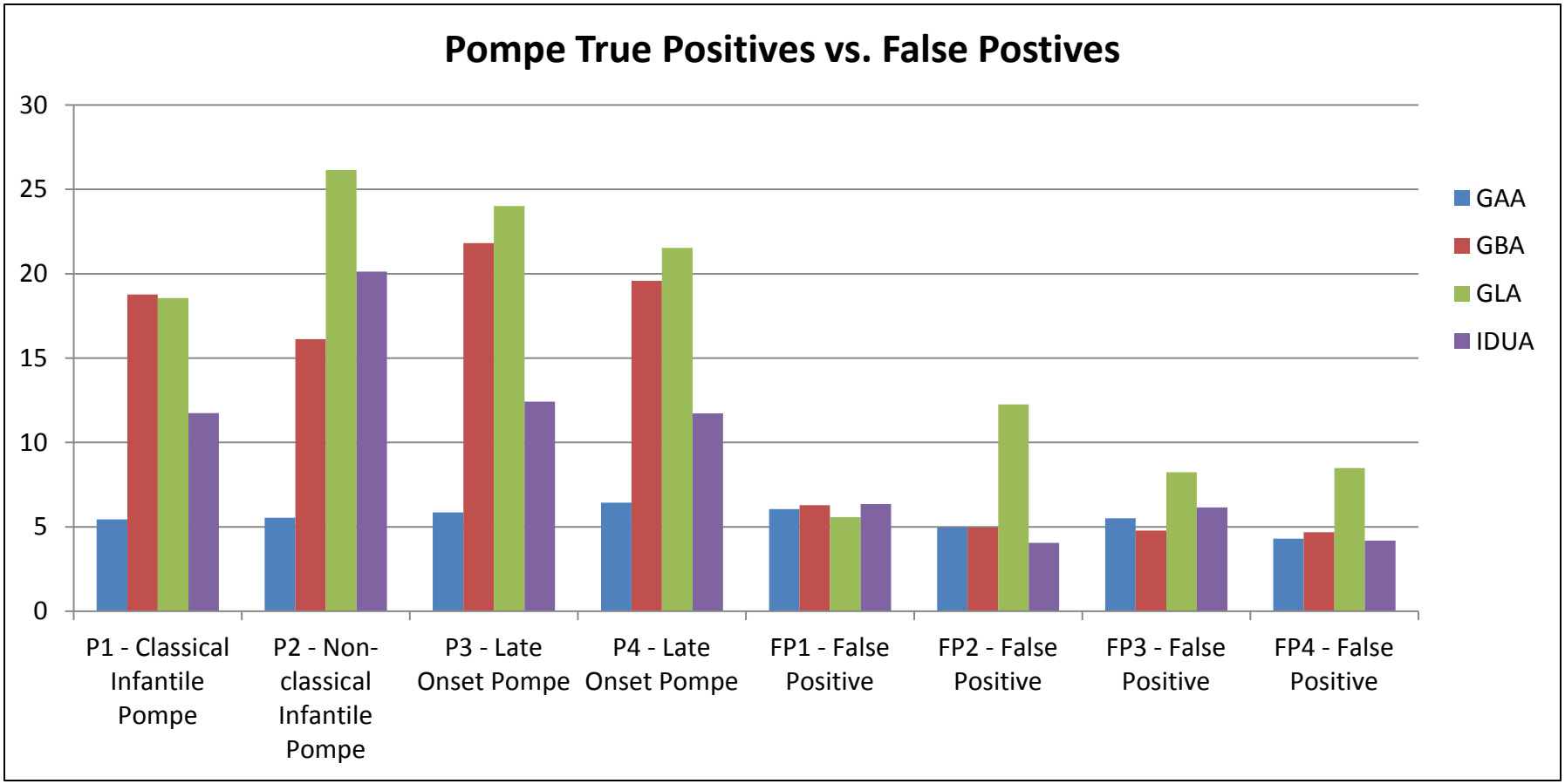
Median Enzyme Activities Male vs. Female



MSPHL LSD Pilot/Implementation Screening Algorithm



The Benefit of Testing Additional LSDs



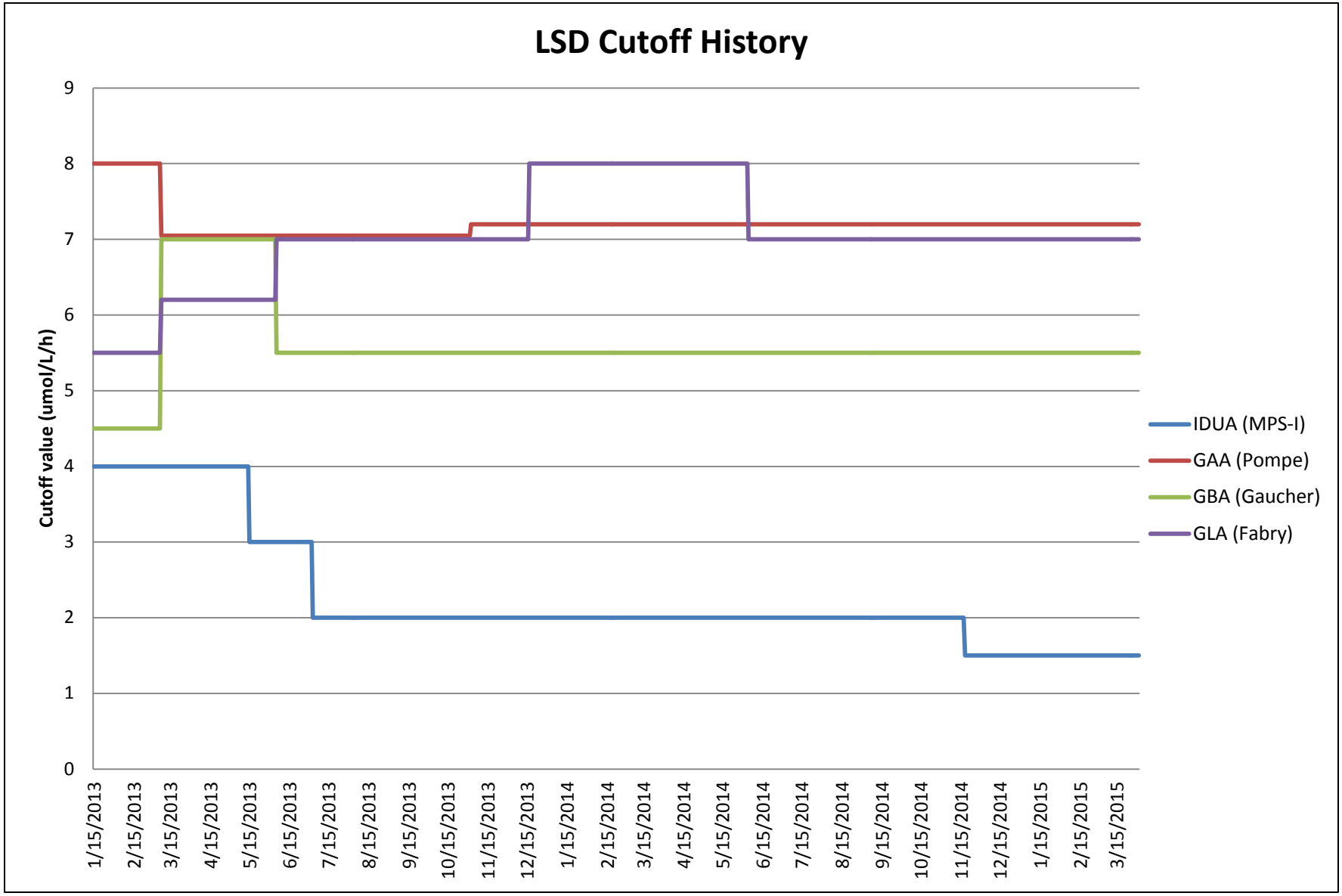
Note: All eight cases were 37 weeks gestation or greater

Current Referral Cutoffs
(< 7 Days-of-age)

- GAA (Pompe) cutoff = 7.20 umol/L/hr (0.25% ‘ile)
- GBA (Gaucher) cutoff = 5.50 umol/L/hr (0.15% ‘ile)
- GLA (Fabry) cutoff = 7.00 umol/L/hr (0.52% ‘ile)
- IDUA (MPS1) cutoff = 1.5 umol/L/hr (0.07% ‘ile)

Cutoffs for < 7 Days-of-age

- Pompe < 7.2 (referral); < 10.0 (instrument)
- Gaucher < 5.5 (referral); < 7.0 (instrument)
- Fabry < 7.0 (referral); < 9.0 (instrument)
- Hurler < 1.5 (referral); < 4.0 (instrument)


LSD Cutoff History
Cutoff value (umol/L/h)
0
1
2
3
4
5
6
7
8
9
1/15/2013
2/15/2013
3/15/2013
4/15/2013
5/15/2013
6/15/2013
7/15/2013
8/15/2013
9/15/2013
10/15/2013
11/15/2013
12/15/2013
1/15/2014
2/15/2014
3/15/2014
4/15/2014
5/15/2014
6/15/2014
7/15/2014
8/15/2014
9/15/2014
10/15/2014
11/15/2014
12/15/2014
1/15/2015
2/15/2015
3/15/2015
IDUA (MPS-I)
GAA (Pompe)
GBA (Gaucher)
GLA (Fabry)

Age Related Cutoffs

7-13 days of age	*14+ days of age*
Pompe < 4.50	Pompe < 4.50
Gaucher < 4.00	Gaucher < 4.00
Hurler < 2.0	Hurler < 2.0
Fabry < 5.0	Fabry < 3.0

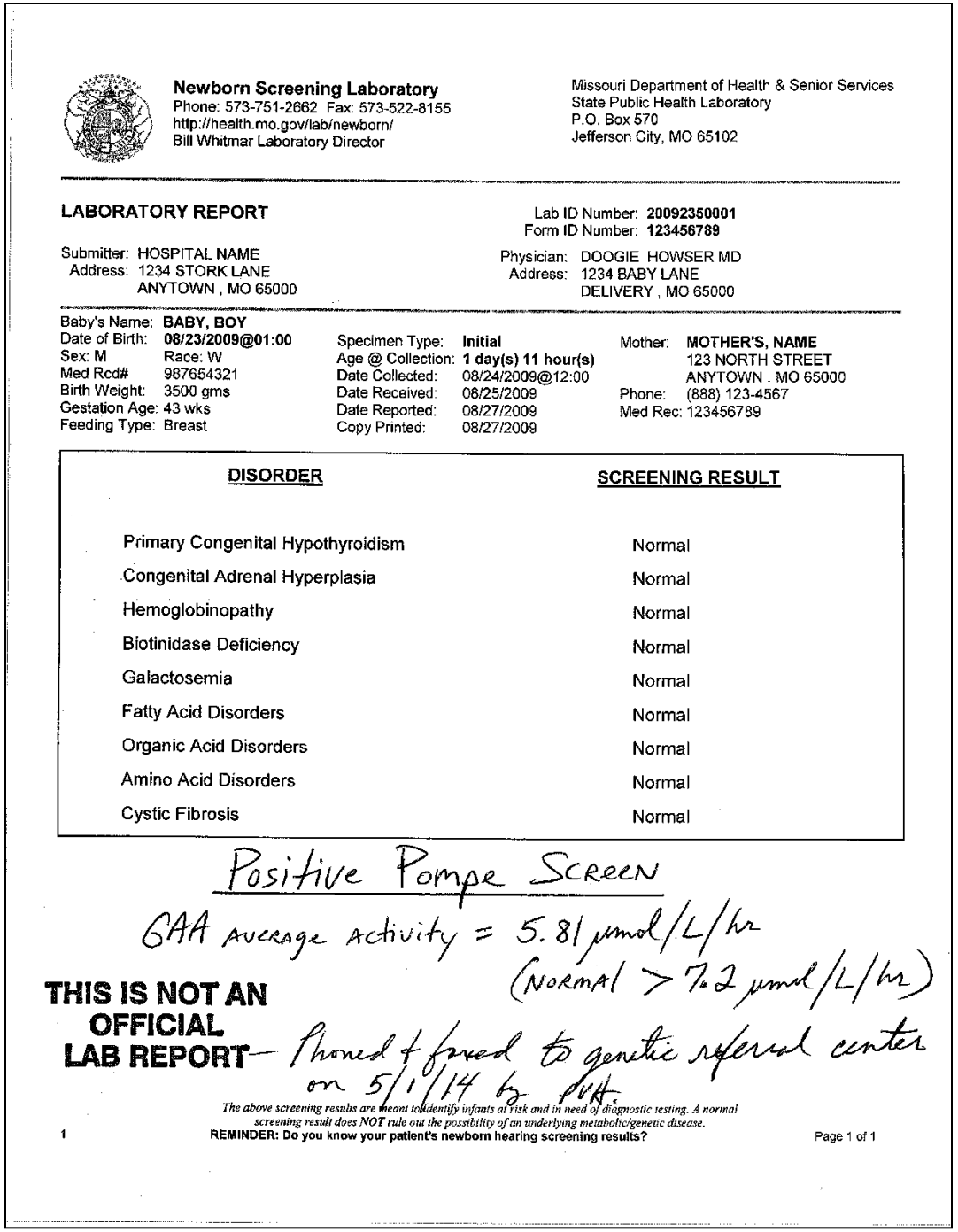
"Hit Rate"
Flagging Rate for All LSD Combined

February 2015 Total Samples Received = 6,716

Samples Flagged = 192 (2.9%)

- Referred = 9
- Borderline = 33
- Inconclusive = 14
- Preemie = 3
- Early Collect = 3
- Transfused = 2
- > 7 days-of-age (normal) = 59
- > 14 days-of-age (normal) = 7
- Normal = 62

Positive Pompe referral notification during the pilot phase



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

LABORATORY REPORT

Lab ID Number: **20092350001**
Form ID Number: **123456789**

Submitter: HOSPITAL NAME
Address: 1234 STORK LANE
ANYTOWN , MO 65000

Physician: DOOGIE HOWSER MD
Address: 1234 BABY LANE
DELIVERY , MO 65000

Baby's Name: **BABY, BOY**
Date of Birth: **08/23/2009@01:00**
Sex: M
Race: W
Med Rcd# 987654321
Birth Weight: 3500 gms
Gestation Age: 43 wks
Feeding Type: Breast

Specimen Type: **Initial**
Age @ Collection: **1 day(s) 11 hour(s)**
Date Collected: 08/24/2009@12:00
Date Received: 08/25/2009
Date Reported: 08/27/2009
Copy Printed: 08/27/2009

Mother: **MOTHER'S, NAME**
123 NORTH STREET
ANYTOWN , MO 65000
Phone: (888) 123-4567
Med Rec: 123456789

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

Positive Pompe Screen

GAA Average Activity = 5.81 μmol/L/hr
(Normal > 7.2 μmol/L/hr)

THIS IS NOT AN OFFICIAL LAB REPORT

Phoned & faxed to genetic referral center on 5/1/14 by PVH.

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

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

Page 1 of 1

Missouri Newborn Screening Follow-Up Data Report

Pompe Disease

Fax completed form to *573-751-6185, Bureau of Genetics and Health Childhood, Jami Kiesling, RN*

Missouri State Public Health Laboratory Screening Data

Name of Baby:_____ Date of Birth:_____

MO State Lab ID #:_____ MO Barcode #:_____

Physician of Record:_____ Physician's Phone:_____

Referral Center:_____ Date of Referral: _____

Pompe (GAA) results: _____ High Risk Cutoff: _____

Fabry (GLA) results: normal High Risk Cutoff: _____

Gaucher (GBA) results: normal High Risk Cutoff: _____

Hurler (IDUA) results: normal High Risk Cutoff: _____

Confirmatory Testing Results:

Date of initial clinic visit/consultation:_____

Acid alpha-glucosidase (GAA) activity:_____ Reference Range: _____

HEX4: _____ Creatine kinase:_____

Other labs:_____

Chest x-ray: ☐ normal ☐ abnormal ☐ not indicated
Electrocardiogram: ☐ normal ☐ abnormal ☐ not indicated
Echocardiogram: ☐ normal ☐ abnormal ☐ not indicated

Mutation analysis:_____

CRIM status: ☐ positive ☐ negative

Skin and/or muscle biopsy: _____

Current confirmatory diagnosis:

☐ Normal ☐ Carrier ☐ Pompe–Infantile Onset ☐ Pompe–Late Onset ☐ Other: _____

Confirmed date:_____ Treatment date:_____ ☐ Treatment is not indicated for this diagnosis.

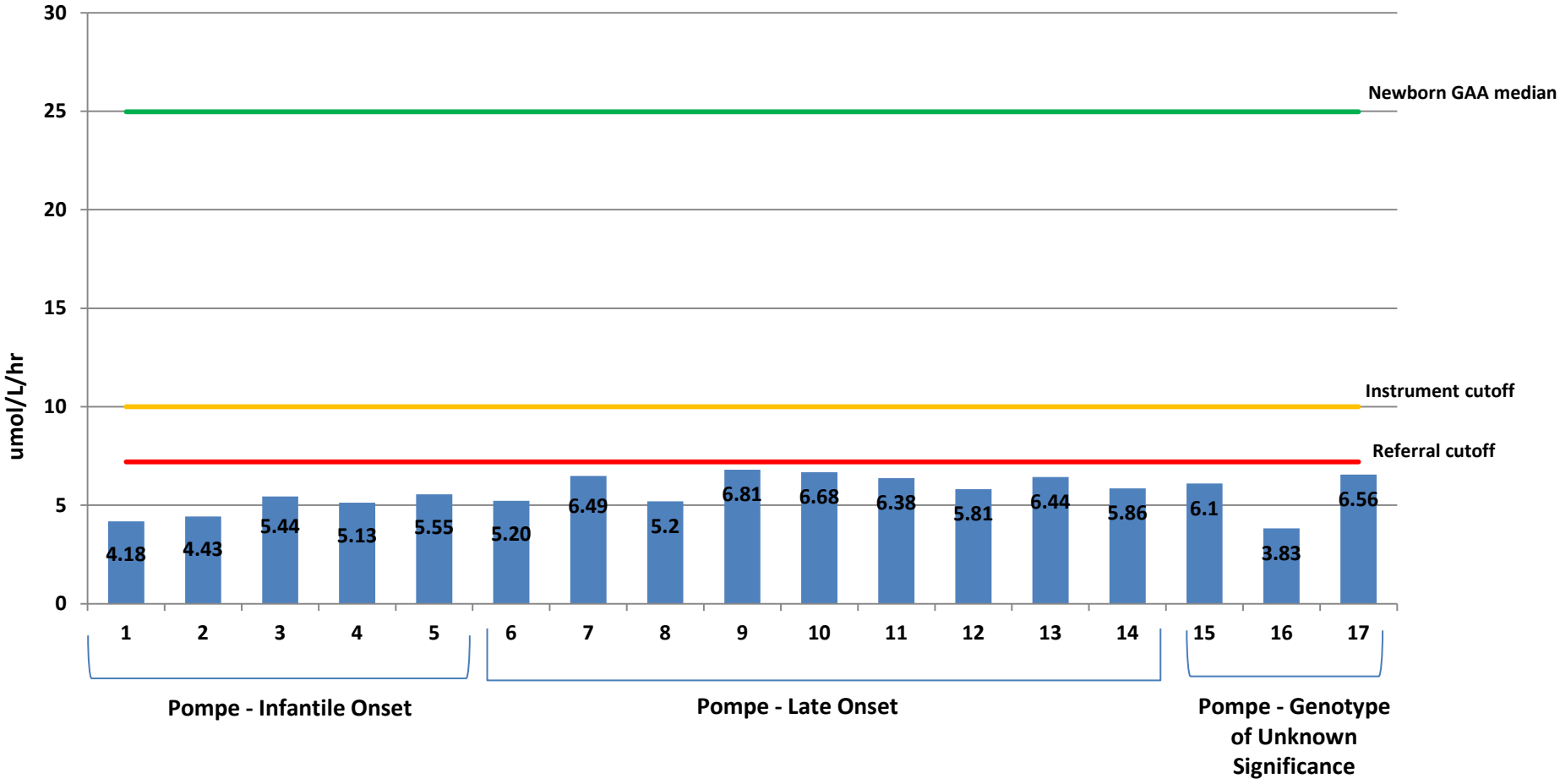
Follow-up plans:_____

_____

_____

Signature of Physician or Designee: _____ **Date**:_____

Confirmed Pompe Cases from ~ 156,000 Births



Missouri LSD Pilot/Implementation Phase Totals to Date

3/31/15

Disorder	Screen Positives	Confirmed Disorders	Conditions of ??? Significance or ??? Onset	Pseudo-deficiencies	Carriers	False Positives	Pending	Lost to Follow-up	PPV
Pompe	84	14 (5 infantile, 9 late)	3	14	15	32	5	1	21%
Gaucher	20	2	2	0	2	13	0	1	21%
Fabry	110	48	3	0	0	43	11	5	54%
MPS-I	78	1	0	36	5	31	3	2	2%
MPS-I*	51	1	0	29	4	15	1	1	2%

Total Samples Screened for LSDs in Missouri as of 3/31/15 = 201,764 (~ 175,000 births)

*** Totals with the current MPS-I cutoff applied retrospectively to the start of pilot**

False Positive Rates

- Pompe = 0.02%
- Gaucher = 0.01%
- Fabry = 0.02%
- MPS-I = 0.03% (0.02% with current cutoff)

Note:

•Multiplexing the four LSDs has been beneficial in reducing false positives for the LSD's individually as it aids in revealing compromised samples.

•Premature babies (especially < 34 wks gestion) and early collected screens can display unreliable results.

•Seasonal influence from heat and humidity can bring about sporadic false positives.

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).
- We are very 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 conducted.

Missouri's Follow-up

- Missouri has four contracted referral centers
- The designated referral center contacts the primary care physician
- A plan is developed and appointments made with a genetic disease specialist and other related pediatric specialists
- Confirmatory testing is completed and treatment/management started based on developed guidelines

Acknowledgements

- Carlene Campbell, Tracy Klug and the Missouri NBS LSD lab team
- Dr. Sharmini Rogers, Julie Raburn, Jami Kiesling and the Missouri NBS follow-up team
- The Missouri LSD Task Force
- Dr. Robert Vogt, Dr. Hui Zhou and CDC LSD team
- Dr. Dietrich Matern and the Mayo LSD team
- Dr. Joe Orsini and the NY LSD team
- The Baebies Inc. team