Missouri's Experience with Full Population Pilot Screening for Pompe, Gaucher, Fabry and MPS-I Disorders

Utilizing Digital Microfluidics Technology

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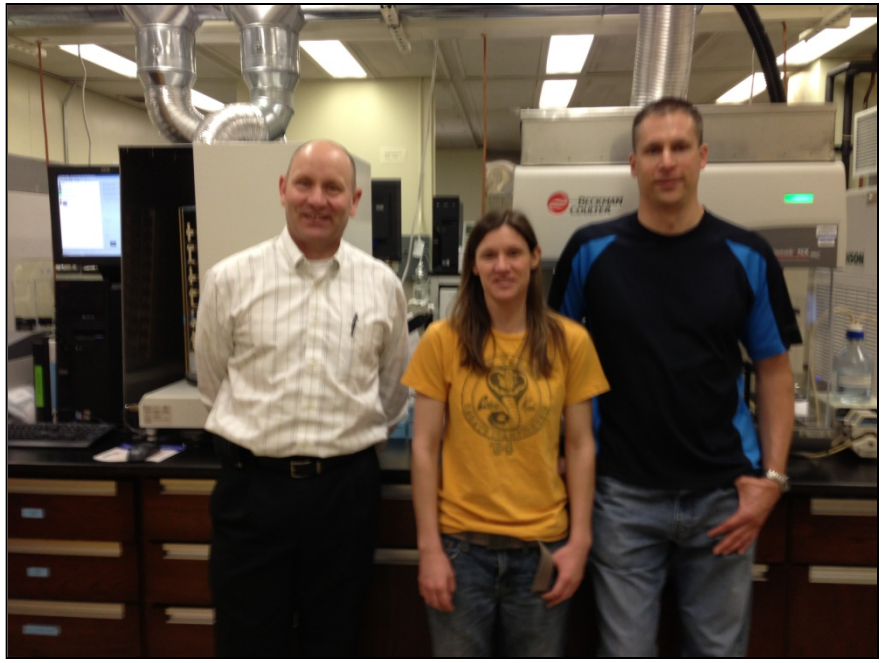
Thank You to New York's NBS Laboratory!



The Krabbe Screening Experts

MO LSD Statewide Pilot Screening

Krabbe (GALC)

→ Testing by New York
Since August 2012
(over 195,000 samples)
(approximately 169,000 births)

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

→ Missouri Testing
Since January 11, 2013
(over 163,000 samples)
(approximately 136,000 births)

Krabbe (GALC)
Niemann-Pick (ASM)

→ Missouri to
Add-on next

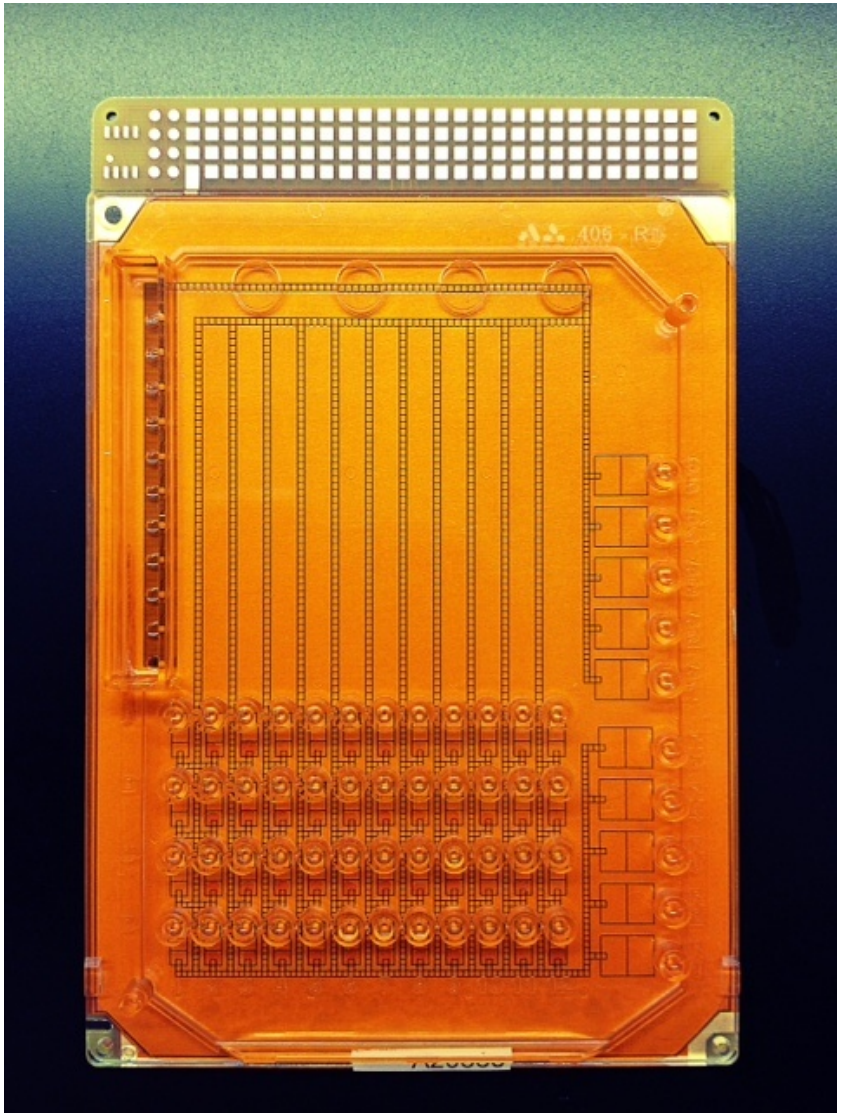
Implementation Process

- Contract procurement (reagent rental)
- Installation and training
- Familiarization
- Validations
- Pre-pilot phase to collect data on de-identified samples for normal ranges and startup cutoffs
- Full population pilot/implementation phase testing with referral and confirmation of positives
- Live testing with reporting on all NBS laboratory reports

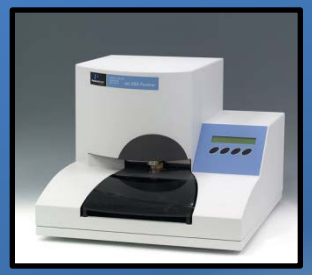
2 Work Stations
8 Digital Microfluidics (DMF) Platforms

Open Platform

48 Well Sample Cartridge



Workflow for LSD Testing in MSPHL



Punch DBS samples

(15 min per 96-well plate)
Single punch for 4-plex assays.

Extraction

(30 min at RT)
Load filler fluid in cartridges. Thaw reagents during extraction.

Loading
(5 min per machine)

Load samples (3.5µL), reagents (12µL) and stop buffer in each cartridge.

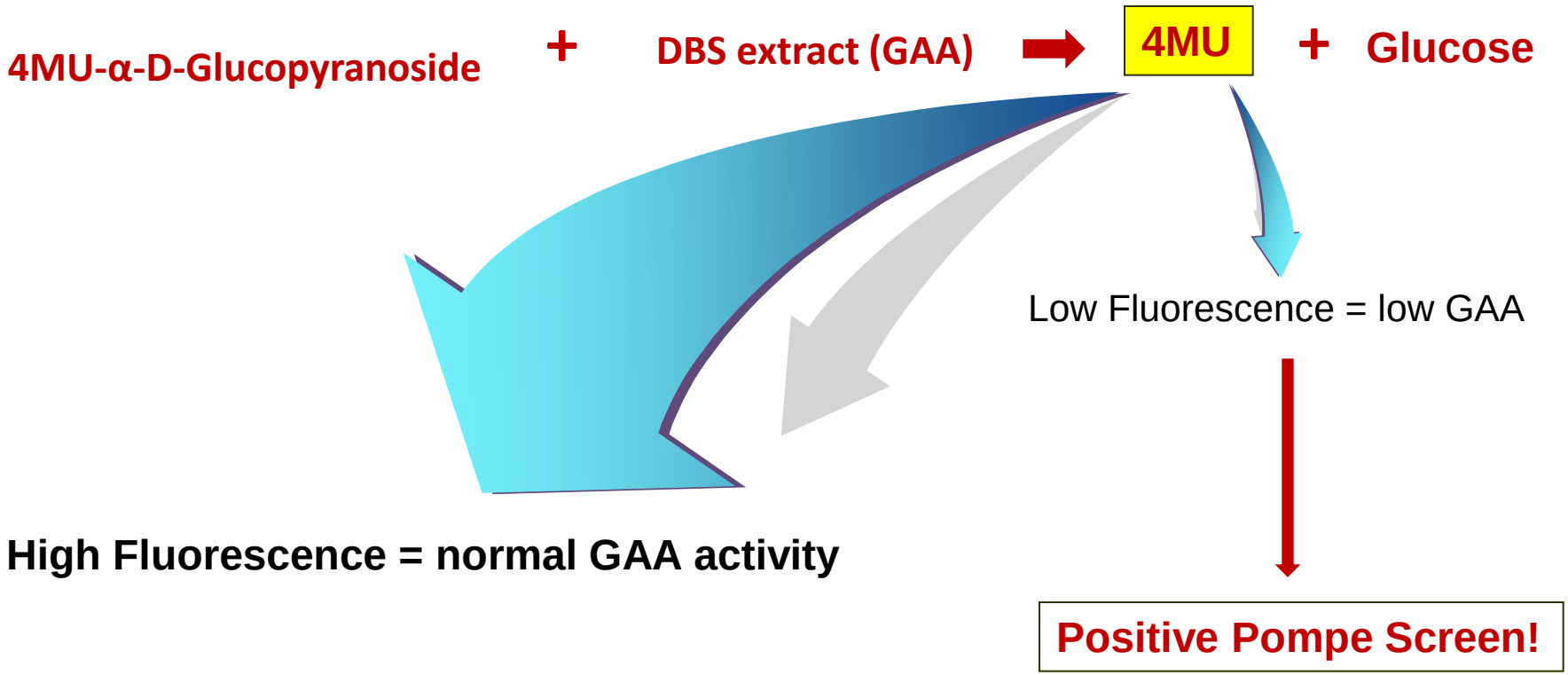
Machine run time
(2.5 h for 4-plex assay)

After 2.5h remove the cartridge from the instrument and get ready for next run.

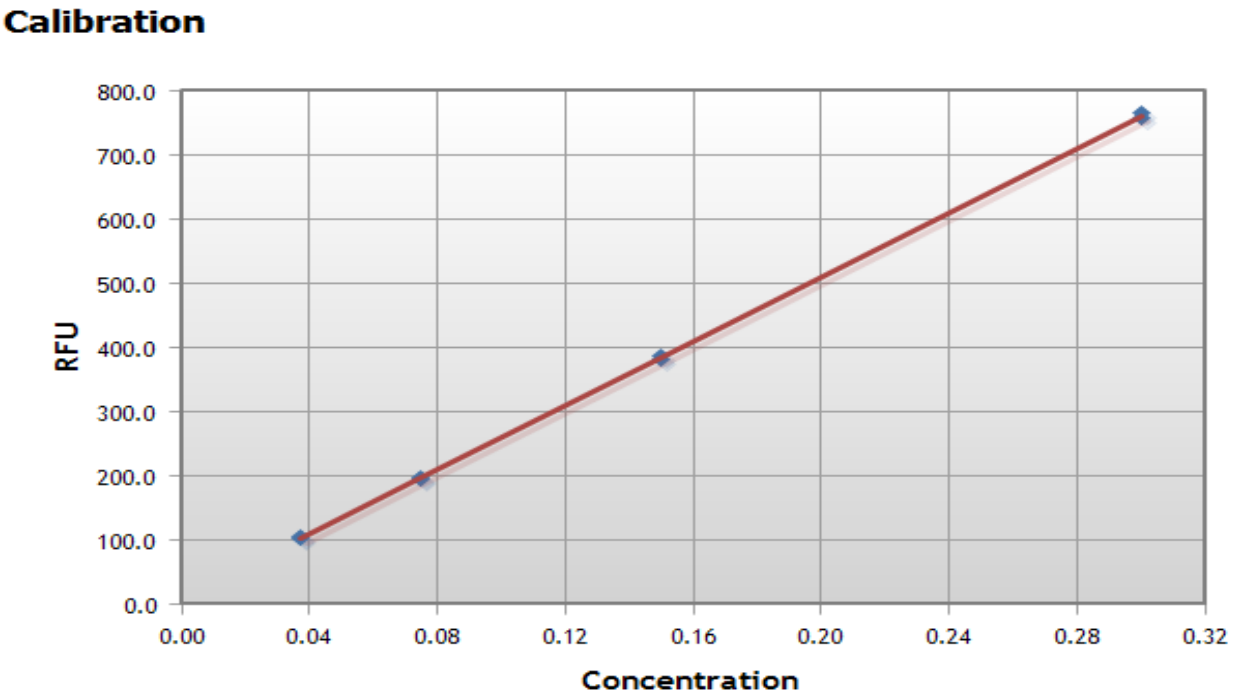
- 2 scientists currently working on 2 work stations of 8 instruments
- 48 sample wells assayed per instrument
  - 10 controls (2 blanks, 4 calibrators, 2 low controls and 2 medium controls)
  - 38 patient samples
- Sample punch to enzymatic activity results in ~4 hrs

Enzyme Reaction in DMF Method

Artificial Substrate + Enzyme → Product



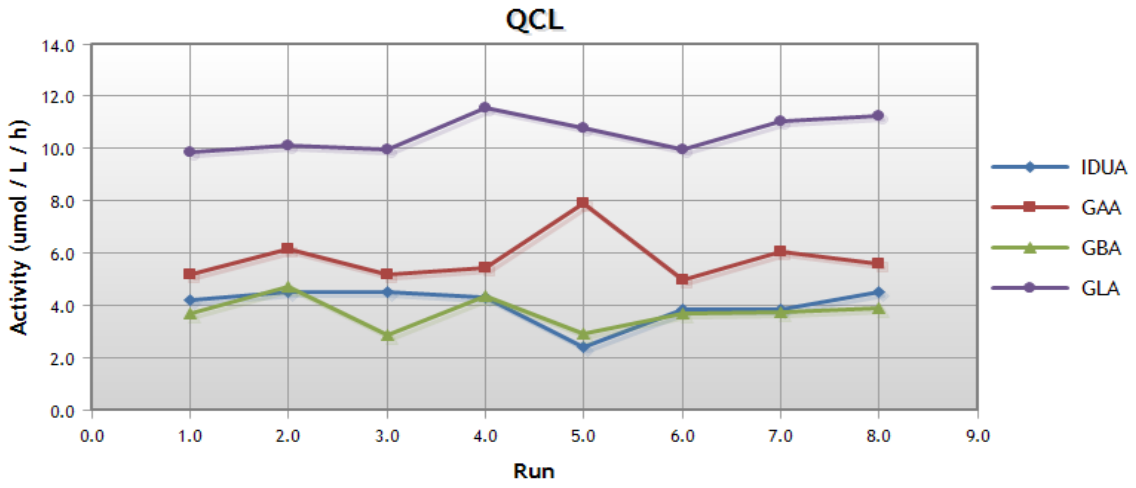
Each Cartridge Has 4 Calibrators

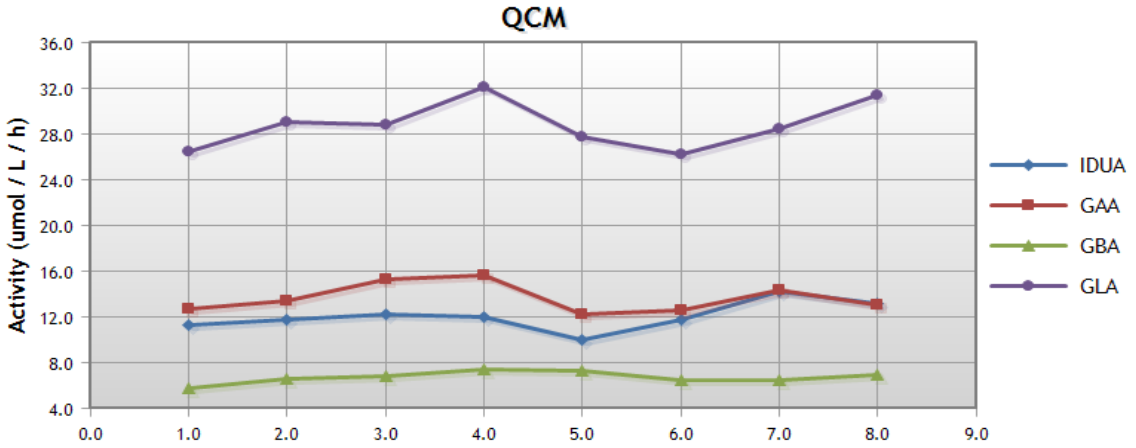


Concentration	A	B
0.0375	104.29	102.23
0.075	196.57	193.99
0.15	386.82	380.51
0.3	763.54	757.61
Slope	**Intercept**	**R^2**
2506.79	8.18	0.9999

Quality Control Monitor for Run

48x4v10 QC Report





Results Screen

Results

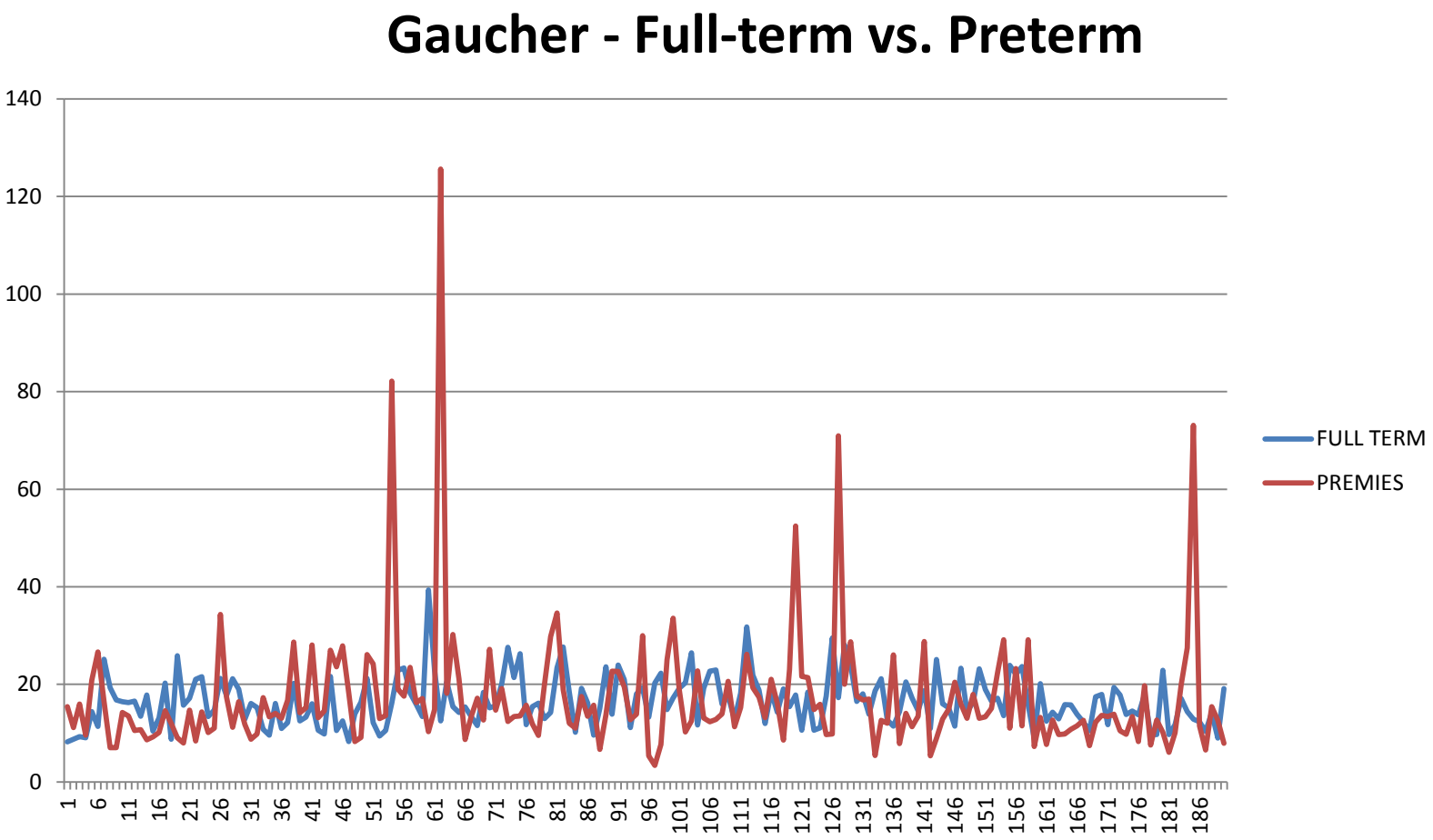
Sample	Location	IDUA (µmol / L / h)	GAA (µmol / L / h)	GBA (µmol / L / h)	GLA (µmol / L / h)
For research use only. Not for use in diagnostic procedures.					
Sample Mean		22.77	23.92	20.80	24.74
CDCBP281	A02	3.51	1.60	2.39	3.87
CDCL282	A03	5.13	3.64	2.72	5.49
CDCM283	A04	24.16	22.73	10.18	37.34
CDCH284	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

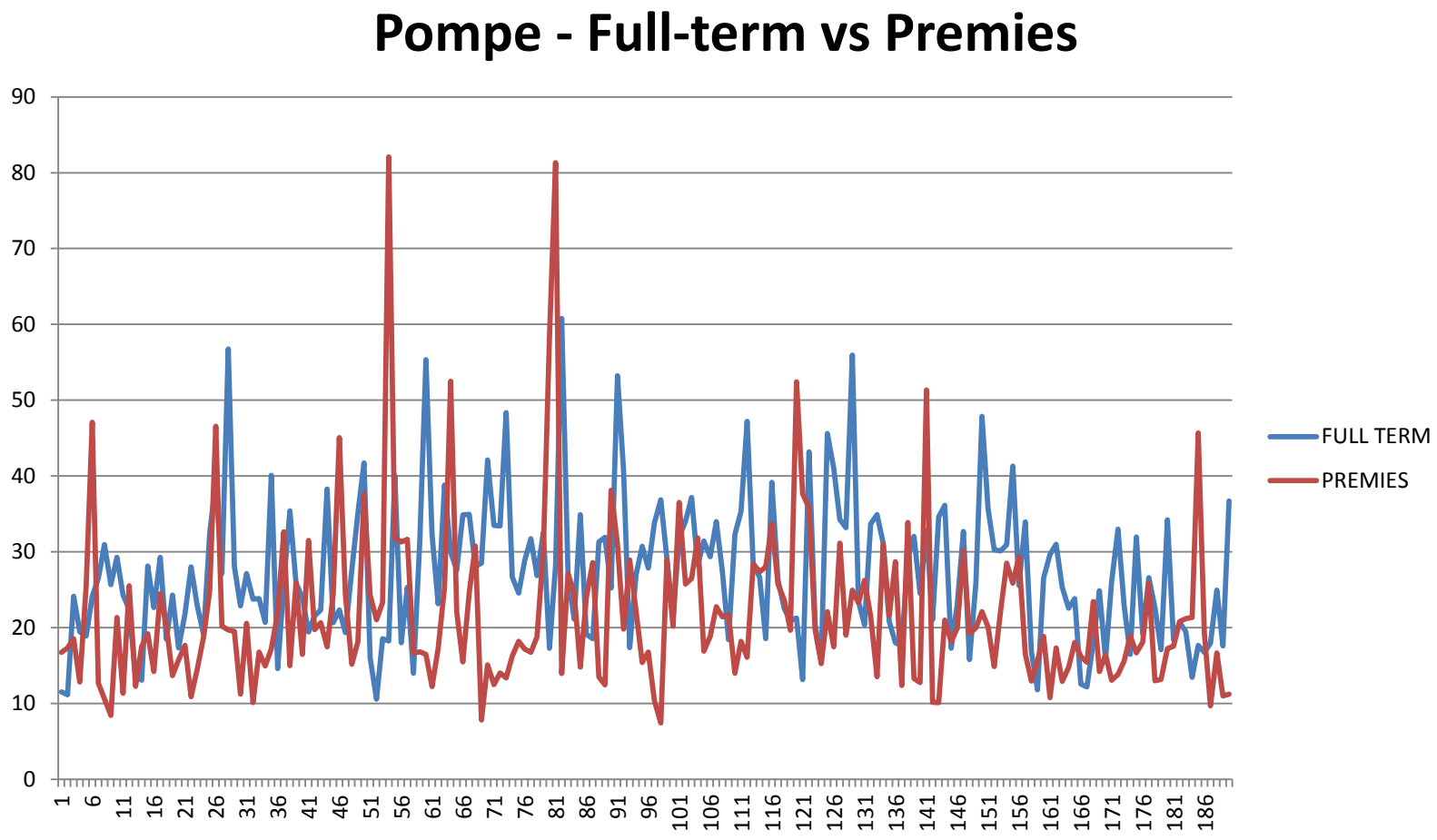
Yellow = Instrument Cutoff

Red = Referral Cutoff

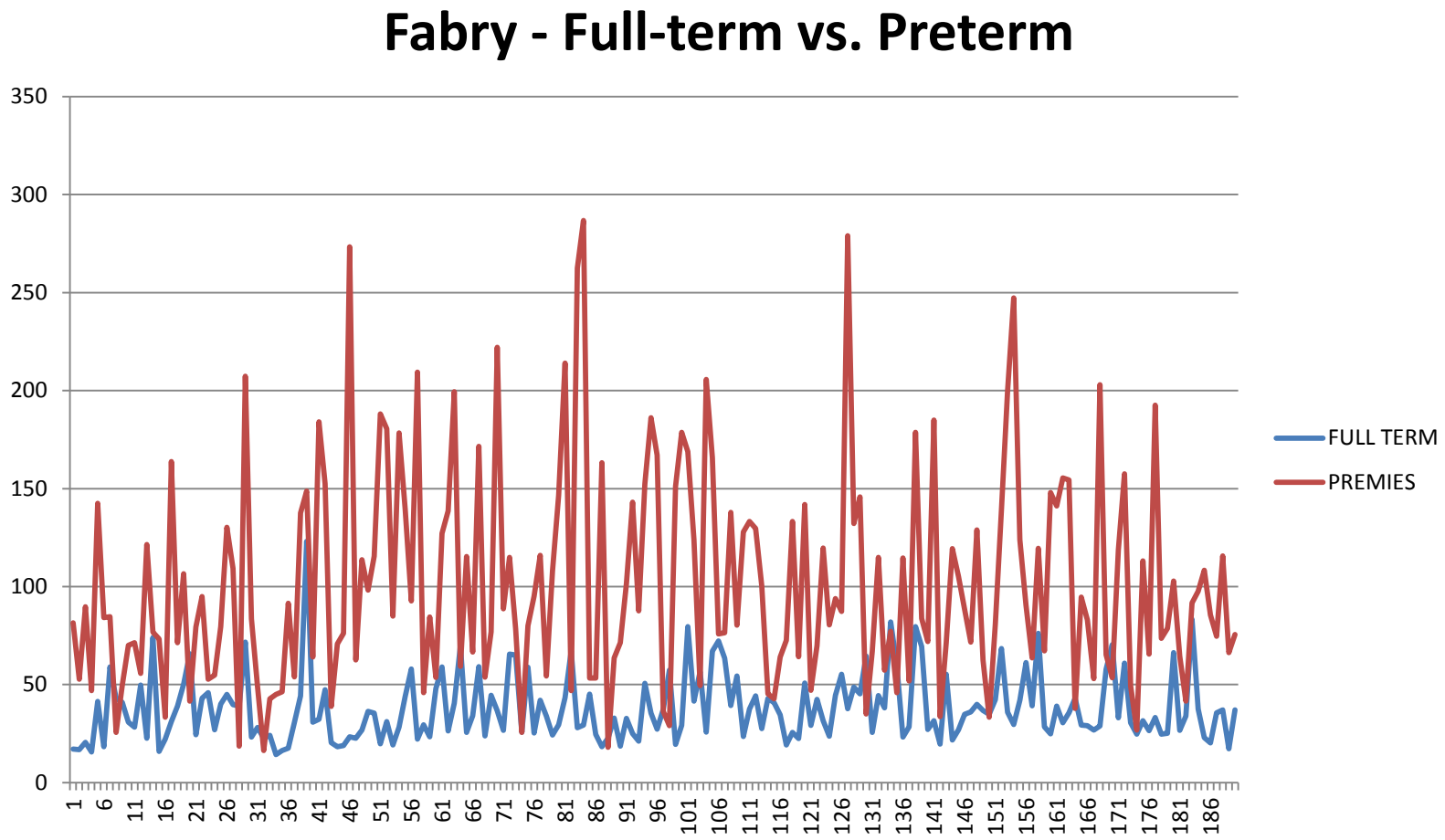
Health Status Effect



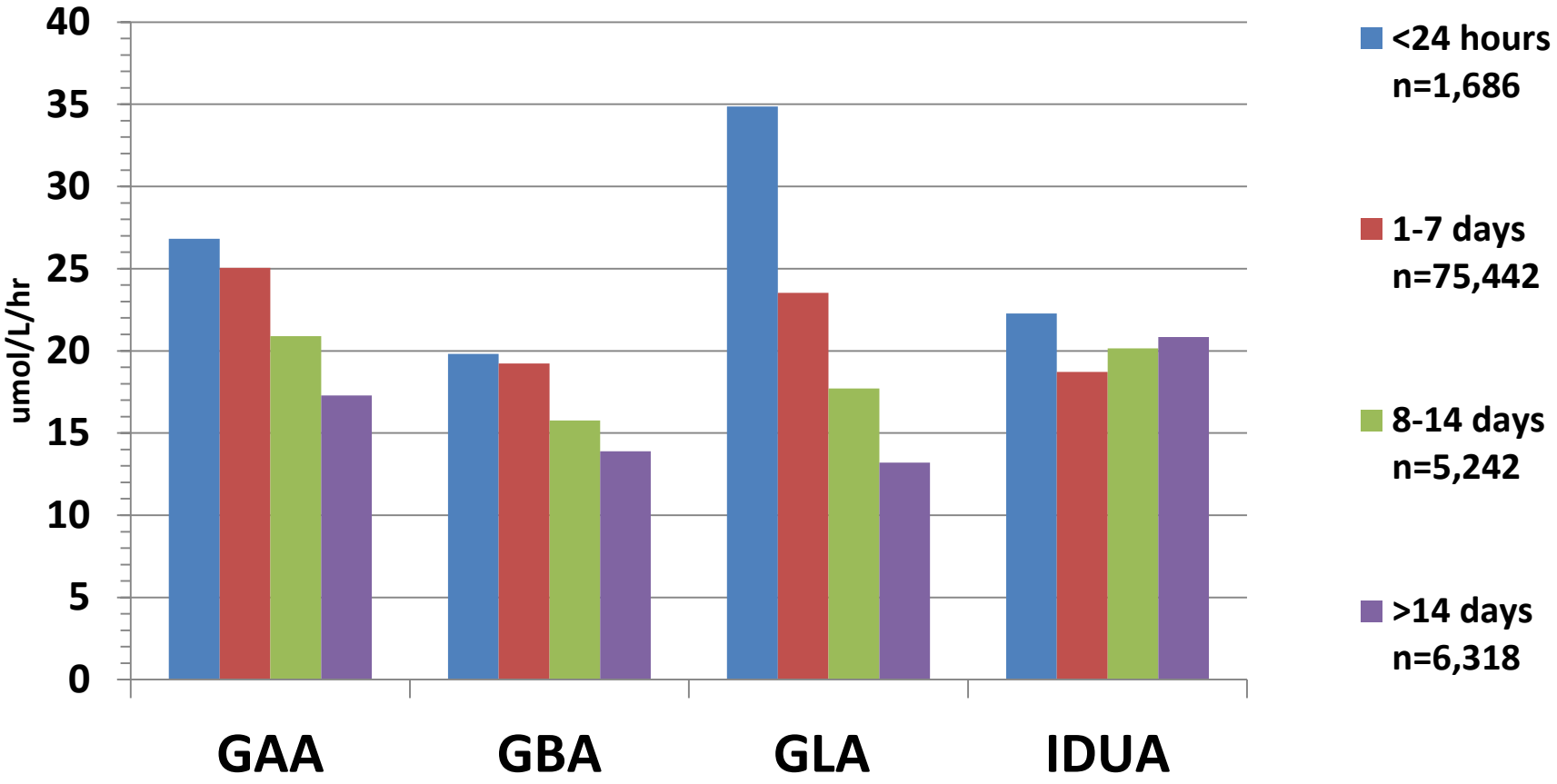
Health Status Effect



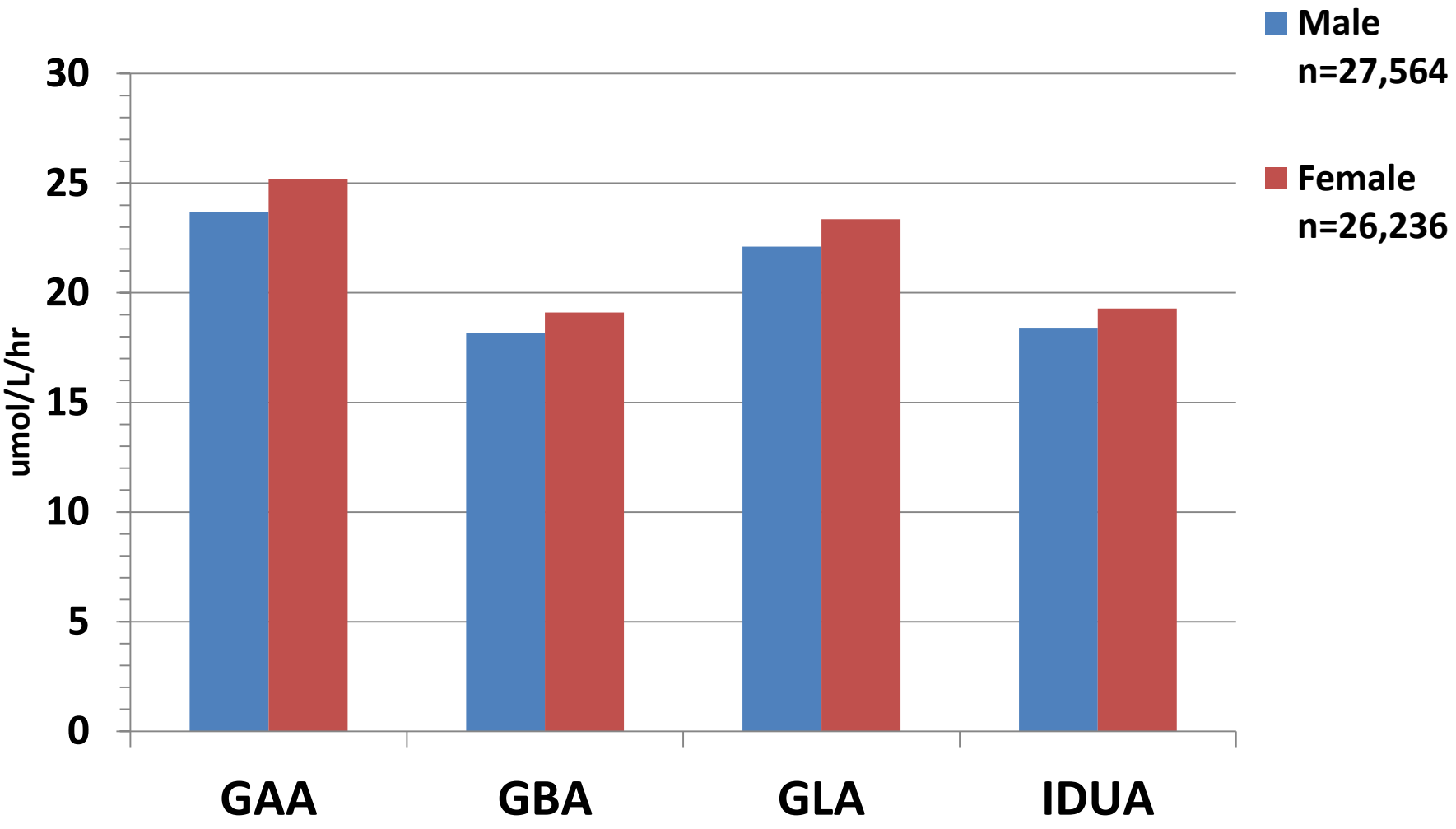
Health Status Effect



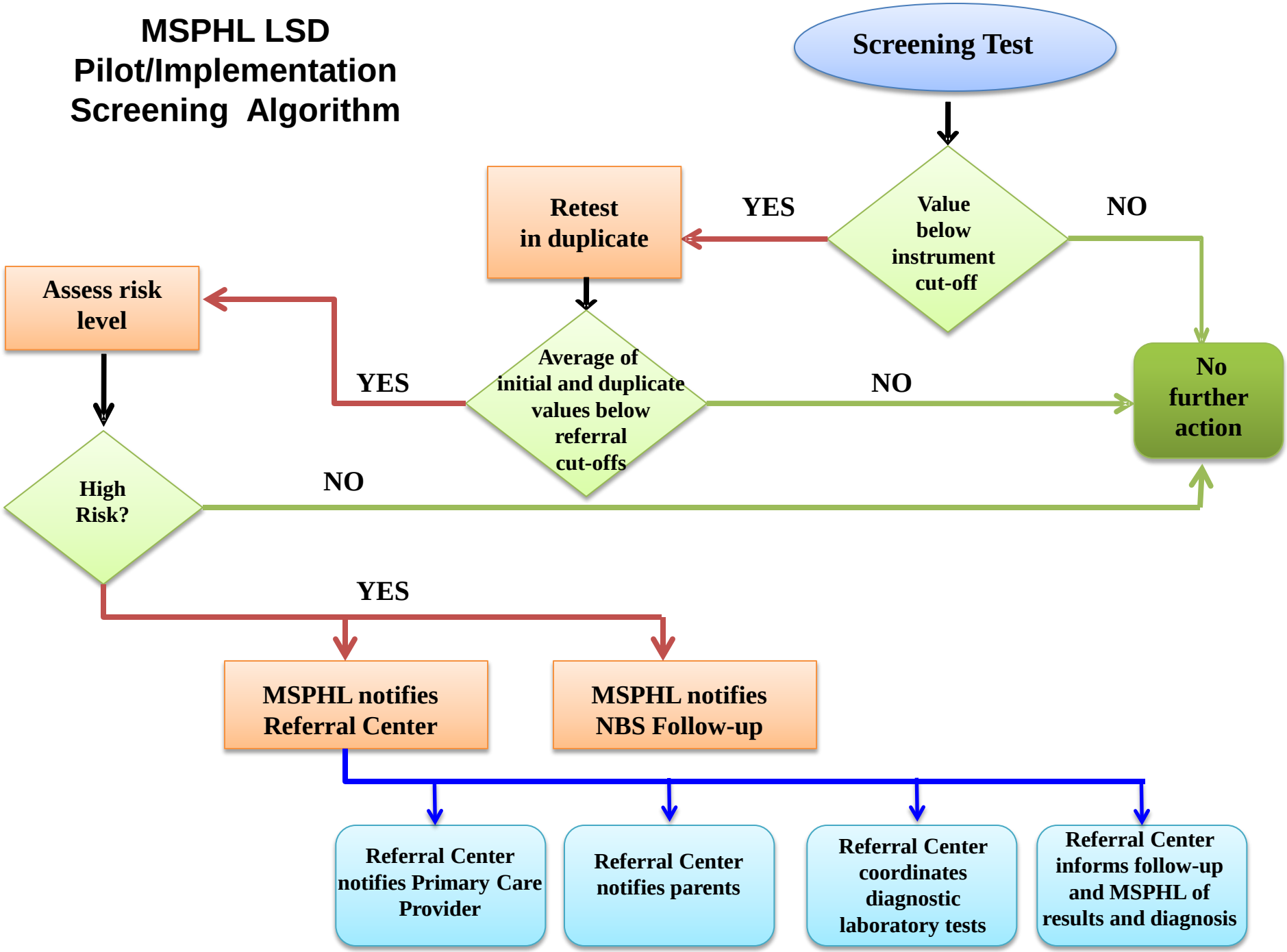
Enzyme Median Activities By Age at Collection



Median Enzyme Activities



MSPHL LSD Pilot/Implementation Screening Algorithm



Missouri LSD Pilot/Implementation Phase Totals

10/21/14

Disorder	Screen Positives	Confirmed Disorders	Conditions of ??? Significance or ??? Onset	Pseudo-deficiencies	Carriers	False Positives	Pending	Lost to Follow-up	PPV
Pompe	68	12 (5 infantile, 7 late)	3	10	11	16	15	1	29%
Gaucher	19	1	2	0	2	13	1	0	17%
Fabry	95	35	7	0	0	39	8	6	51%
MPS-I	65	1	0	23	4	28	8	1	2%
Krabbe	32	0	8	0	24	0	0	0	25%
Aggregate	279	49	20	33	41	96	32	8	29%

Total Samples Screened for LSDs in MO NBS lab as of 10/21/14 = 163,528 (~ 136,500 births)

Total Samples Screened for Krabbe via NY as of 10/21/14 = 195,595 (~ 169,000 births)

PPV = CP / TR - Pending & Lost
PPV (Positive Predictive Value)
CP (Confirmed Positive)
TR (Total Referred)

False Positive Rate

- Pompe = 0.02%
- Gaucher = 0.01%
- Fabry = 0.02%
- MPS-I = 0.03%
- Krabbe = 0.01%

Total Samples Screened for LSDs in MO NBS lab as of 10/21/14 = 163,528 (~ 136,500 births)

Total Samples Screened for Krabbe via NY as of 10/21/14 = 195,595 (~ 169,000 births)

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 quality of sample 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.
- 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

- 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

Follow-Up Lessons Learned

- Screening and confirmation for Lysosomal disorders are complex
- Follow-up guidelines may need to be flexible
- Frequent communication between the specialists has been helpful
- Having a close relationship with the appropriate pediatric specialists is key

Follow-Up Challenges

- How to communicate to parents the unknown onset or unknown risk diagnosis
- How to follow patients with unknown onset and unknown risk diagnoses
- What are the implications of testing unaffected siblings for the variants of unknown risk?
- Detection of Pseudo-deficiency and Carriers
- Lost to follow-up

Detected on 2nd Day of Pilot

NBS for Pompe has been recommended by the DACHDNC to be added to the core panel of screening disorders.

Gavin's Story is on the Save Babies Through Screening Foundation website.

Acknowledgements

- Dr. Joe Orsini and the NY Krabbe screening team.
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