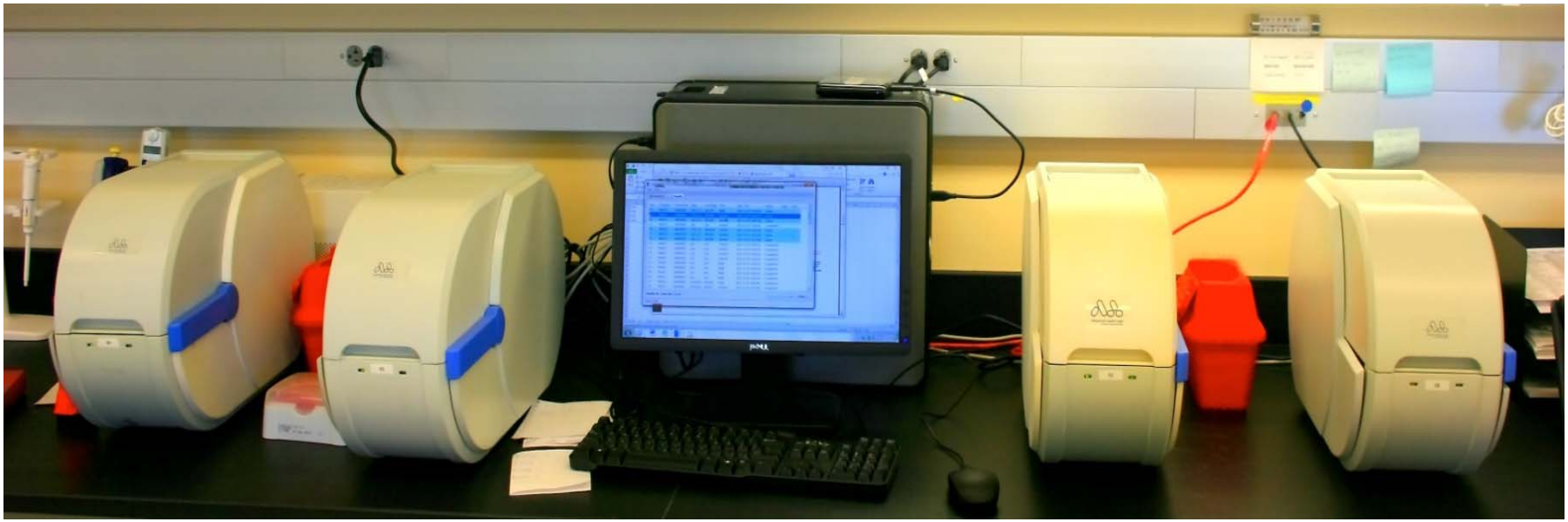
Missouri's Experience with LSD Method Validation and Implementation

to screen for

Pompe, Gaucher, Fabry and Hurler Disorders

Utilizing Digital Microfluidics Technology by Advanced Liquid Logic



Patrick Hopkins, Chief of Missouri NBS Laboratory

LSD Screening in Missouri

- GALC = Krabbe

→ Testing by New York Since August 2012

- GAA = Pompe
- GBA = Gaucher
- GLA = Fabry
- IDUA = Hurler

→ Missouri Testing Since January 15, 2013

- GALC = Krabbe
- ASM = Niemann-Pick
- IDS = Hunter

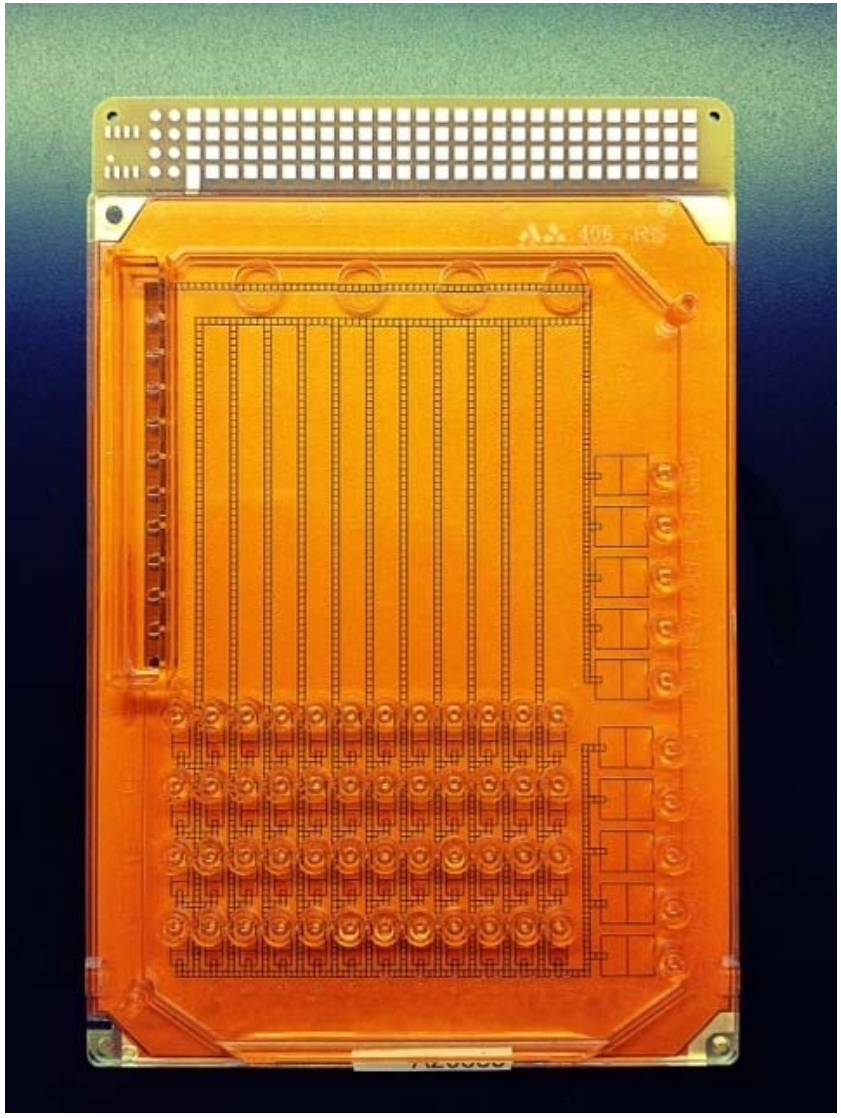
→ Missouri to Add-on Next

Choosing the Testing Method

- Only two choices were obtainable for high throughput:
  MS/MS and Digital Microfluidics
- What are the start-up costs?
- State fiscal climate was extremely limiting:
  - No newly created FTE's allowed
  - A freeze on increasing "fees" except in a few situations (Missouri's Big Government Get Off My Back Act).
  - Fee increase would take over two years to implement and even longer to make a difference.
- Lab space and retrofitting limitations

2 Work Stations
8 Digital Microfluidics (DMF) Platforms

MS/MS System: 21 Instruments



Enzyme Reactions in DMF Method

ENZYME + SUBSTRATE ⟹ PRODUCT

Gaucher Disease

4MU-ß-D-Glucopyranoside + DBS extract (GBA) —pH 5.2→ 4-MU + Glucose

Pompe Disease

4MU-α-D-Glucopyranoside + DBS extract (GAA) —pH 3.8→ 4-MU + Glucose

Fabry Disease

4MU-α-D-Galactopyranoside + DBS extract (GLA) —pH 4.5→ 4-MU + Galactose

Hurler Disease

4MU-α-L-Iduronide + DBS extract (IDU) —pH 3.5→ 4-MU + Iduronide

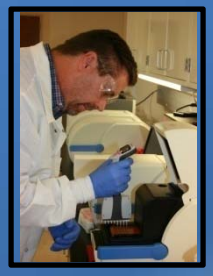
Workflow for LSD Testing in MSPHL

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

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 (6 – 12 months)
- Live Testing with reporting on all NBS lab reports

Pre-Pilot Phase Preparation

We de-identified >13,000 DBS samples after pre-separating 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

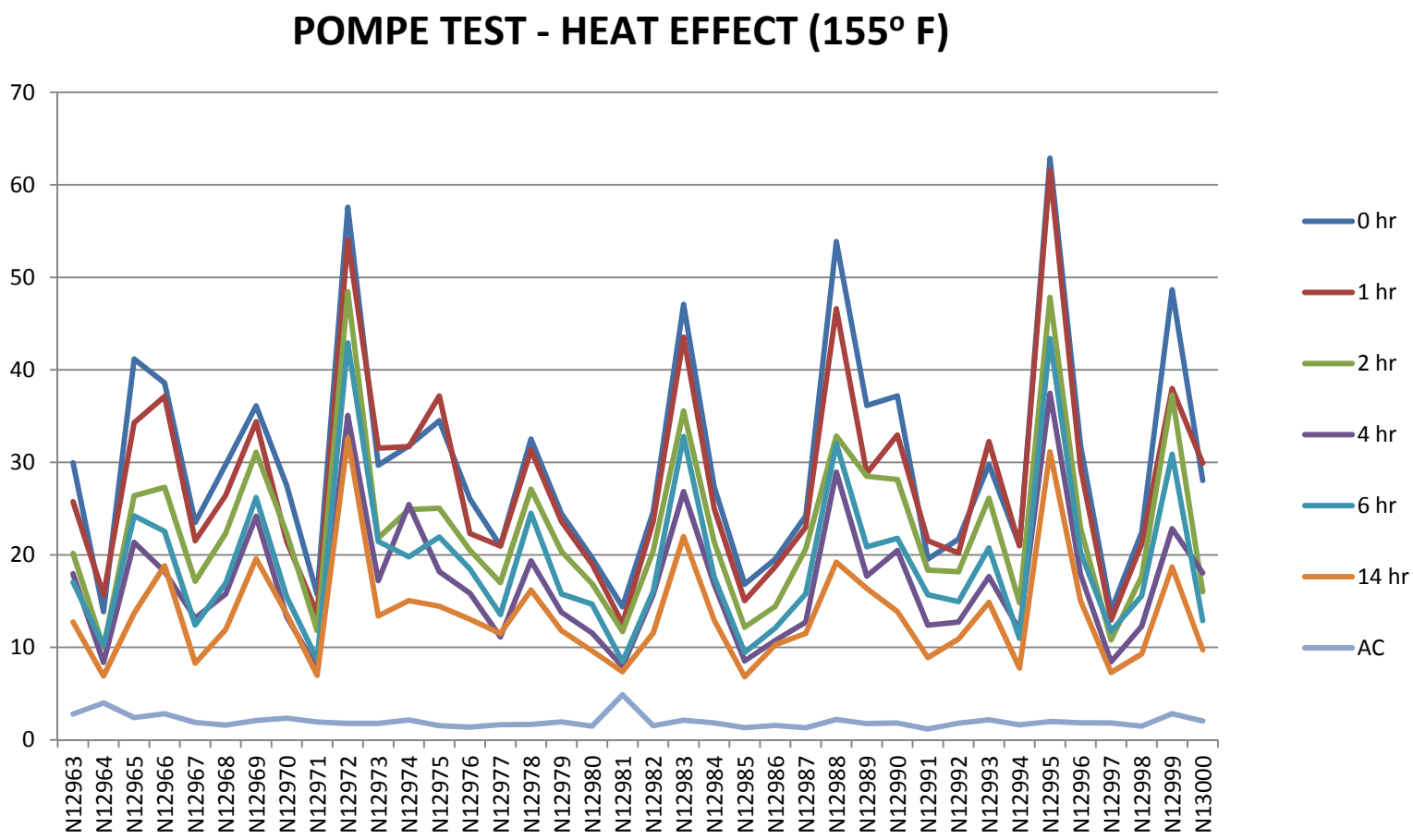
Validation Exercises

- Have conducted many validation experiments and are utilizing StatisPro software from CLSI.
- Have tested around 13,000 QC and CDC samples.
- Have tested over 30,000 de-identified patient samples.
- Have conducted sample exchanges for mini-studies with Mayo and CDC.
- Verified 2 Pompe cases diagnosed clinically and 1 Gaucher carrier during pre-pilot phase.

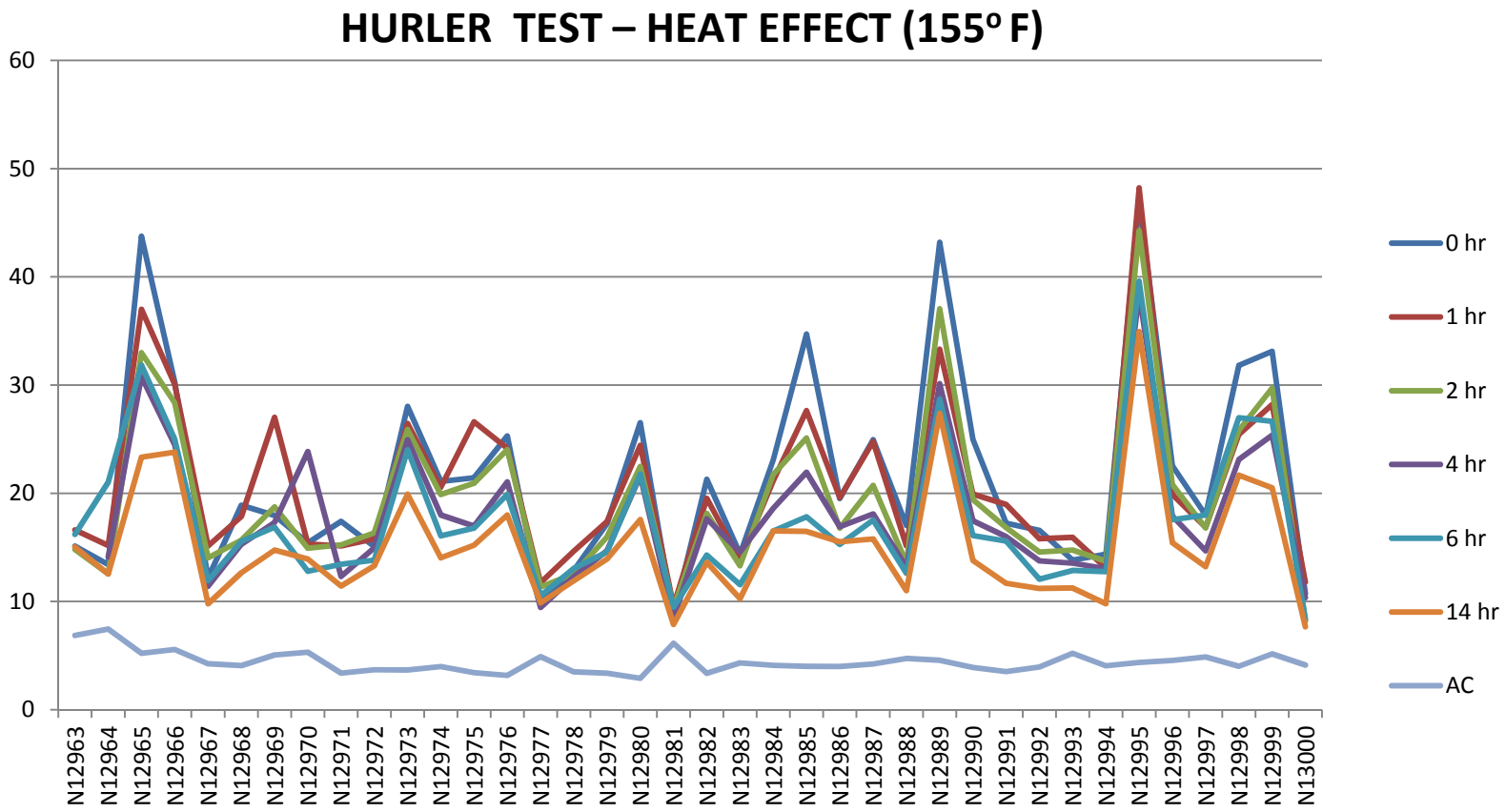
Validation of New Methods

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

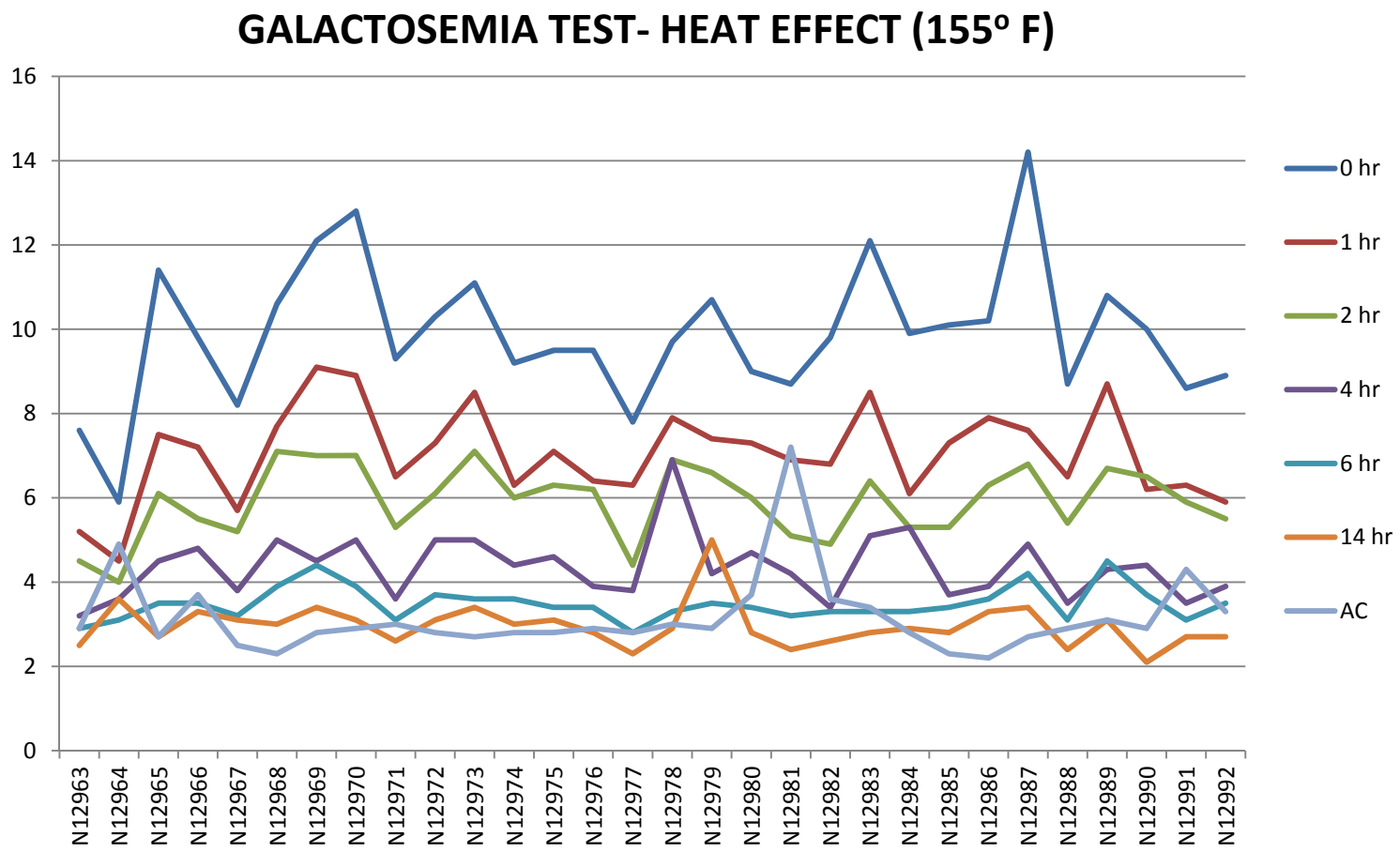
Sample Stability Study



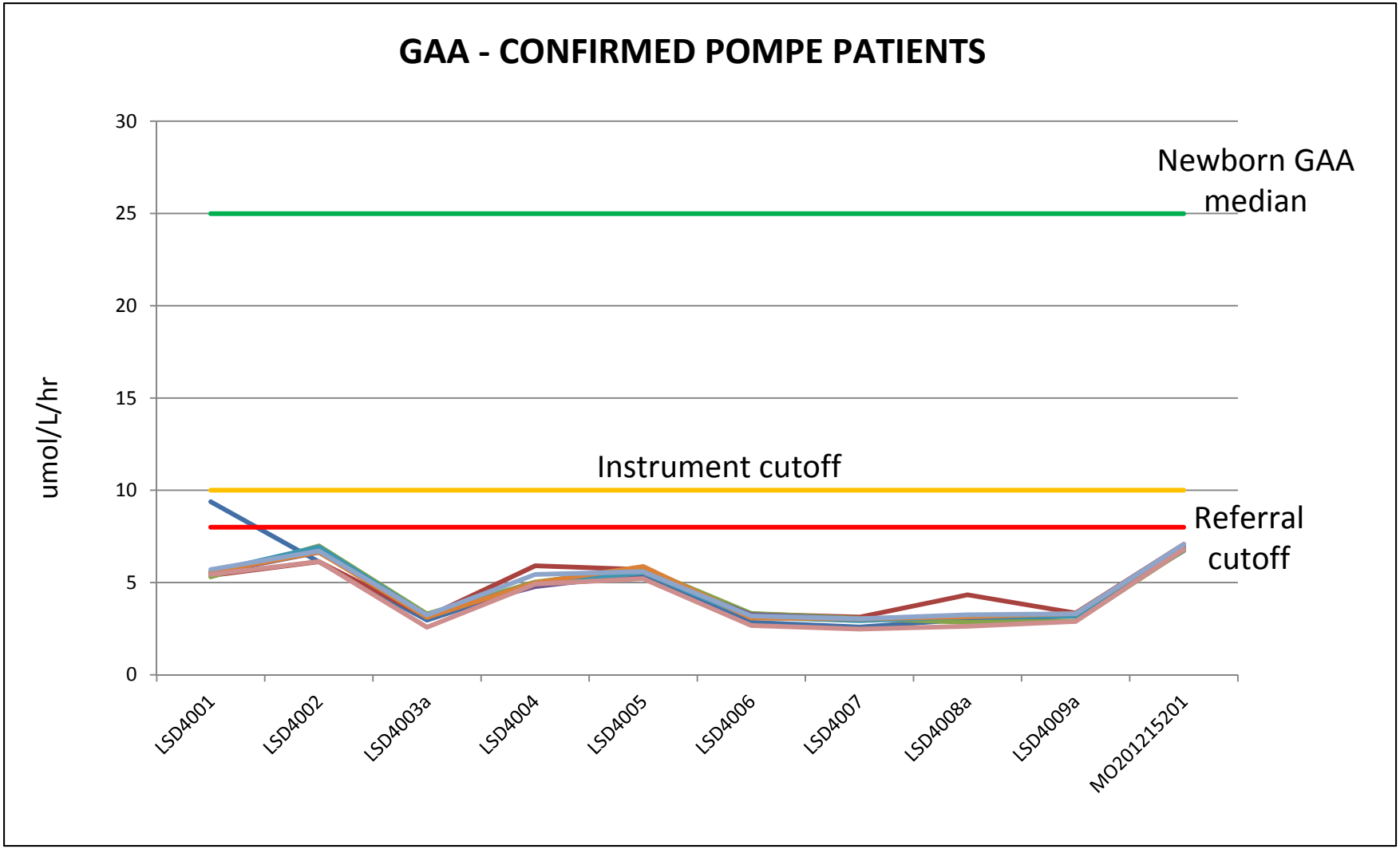
Sample Stability Study



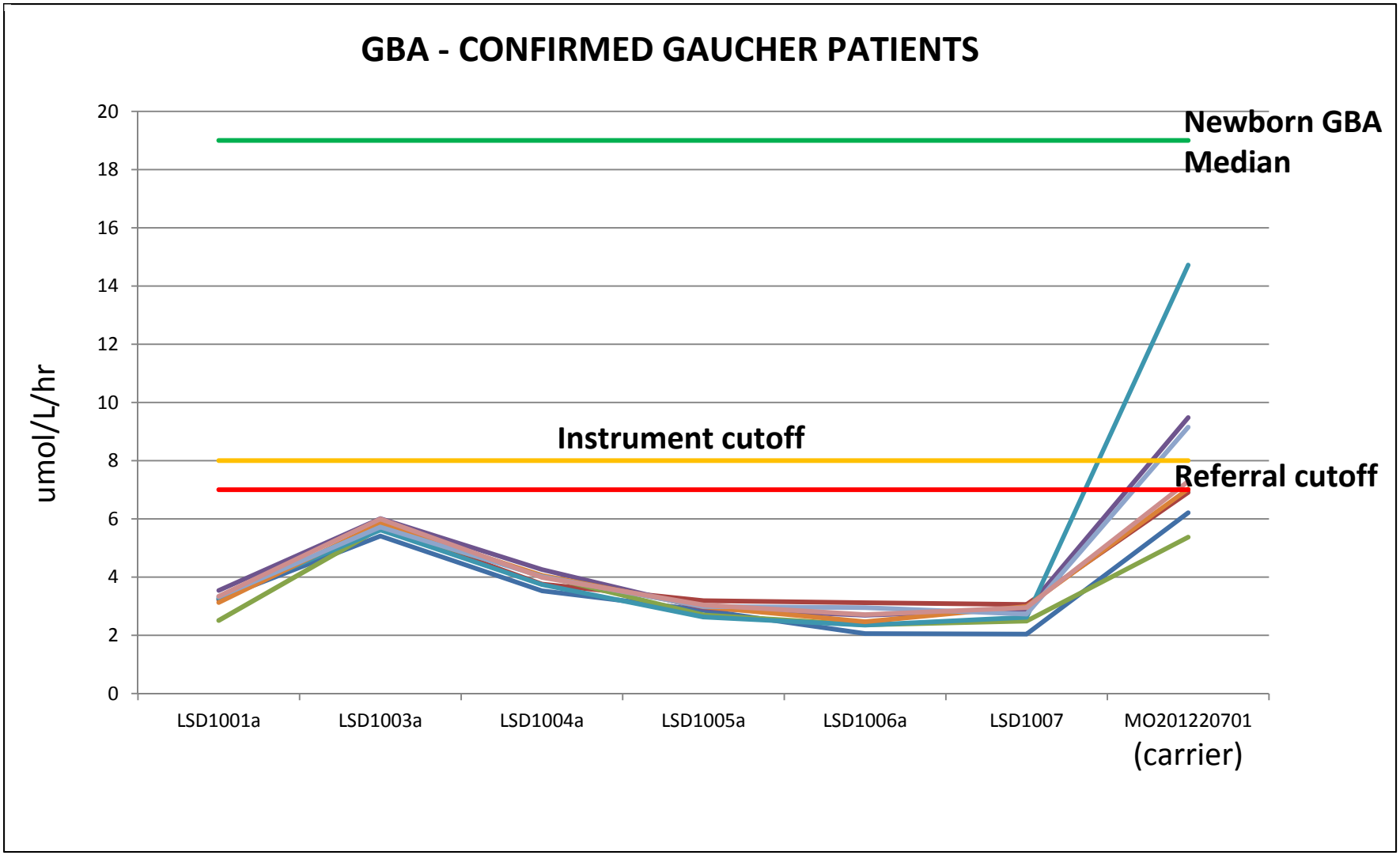
Sample Stability Study



Testing Known Positives



Testing Known Positives



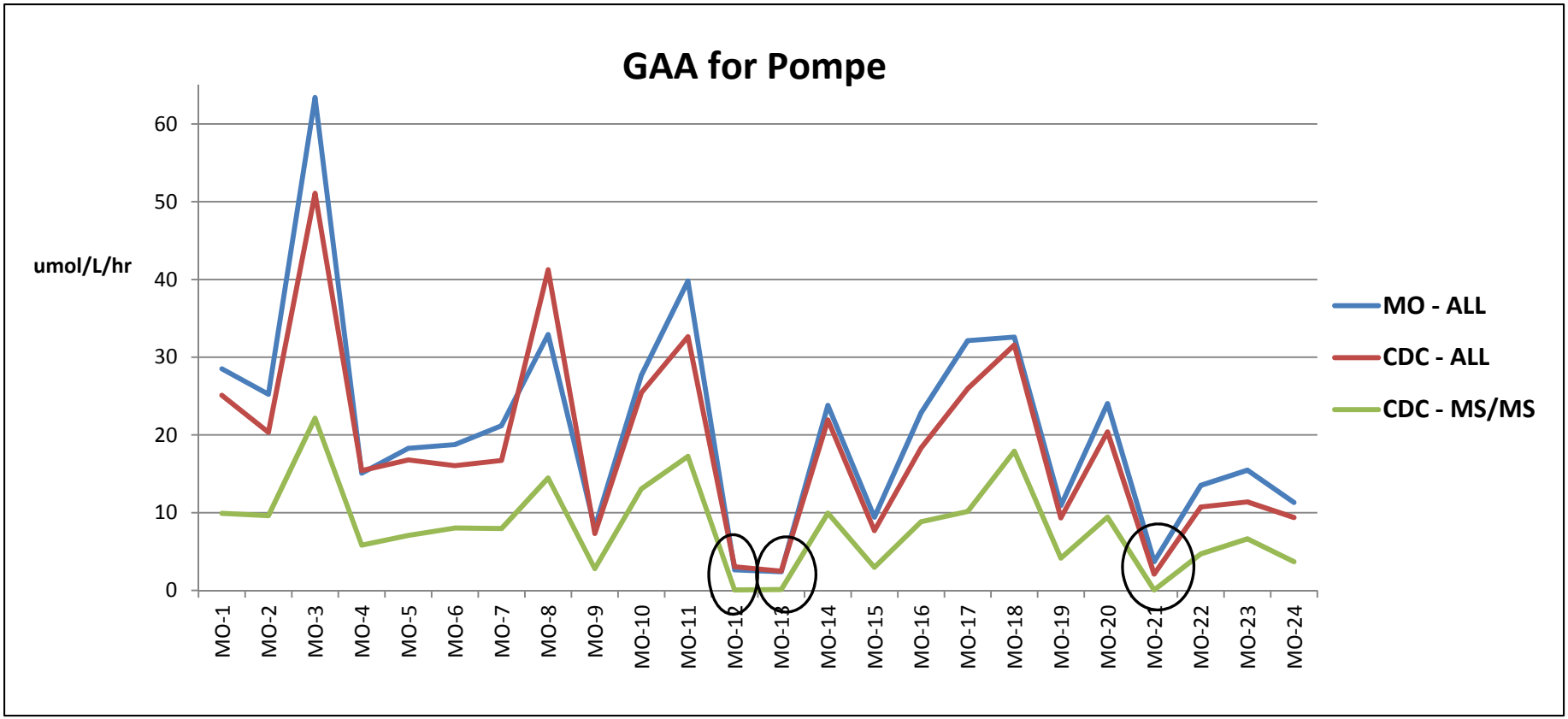
Missouri Blind Abnormal Samples Tested at Mayo

	GAA	GBA	GLA	IDUA	Method
N180	19	3	24	7	Luminex
	4.8	1.6	3.4	1.9	MS/MS
	7.38	5.42	8.97	12	Mayo ALL
	9.82	5.87	10.8	8.76	Missouri ALL
N372	46	5	88	5	Luminex
	14	7.9	16.1	2.7	MS/MS
	18.37	8.84	62.11	4.45	Mayo ALL
	27.63	15.83	42.66	4.06	Missouri ALL
N1234	15	3	57	12	Luminex
	4.1	4.4	12.2	4	MS/MS
	5.39	6.98	20.55	13.15	Mayo ALL
	6.81	8.81	23.28	12.68	Missouri ALL
N1750	29	4	168	16	Luminex
	12.7	8.2	113.4	6.8	MS/MS
	17.8	10.64	199.02	34.09	Mayo ALL
	21.26	13.29	193.54	40.08	Missouri ALL
N1752	4	2	19	6	Luminex
	3.2	2.6	4.2	3.1	MS/MS
					Mayo ALL
	7.42	6.72	8.9	6.76	Missouri ALL
N2255	5	5	35	13	Luminex
	3	2.5	8.8	3.9	MS/MS
	6.79	6.7	16.85	27.44	Mayo ALL
	6.1	5.83	19.41	28.89	Missouri ALL
N2329	15	2	18	10	Luminex
	5.9	1.4	3.4	3.2	MS/MS
	8.27	3.4	9.89	9.46	Mayo ALL
	12.48	4.19	13.07	14.24	Missouri ALL
N2595	64	6	318	4	Luminex
	17	7.5	23.2	1.5	MS/MS
	18.51	9.57	43.7	4.42	Mayo ALL
	26.22	12.3	44.49	4.14	Missouri ALL

= Abnormal suspected by Missouri

= Abnormal with Mayo's cutoffs

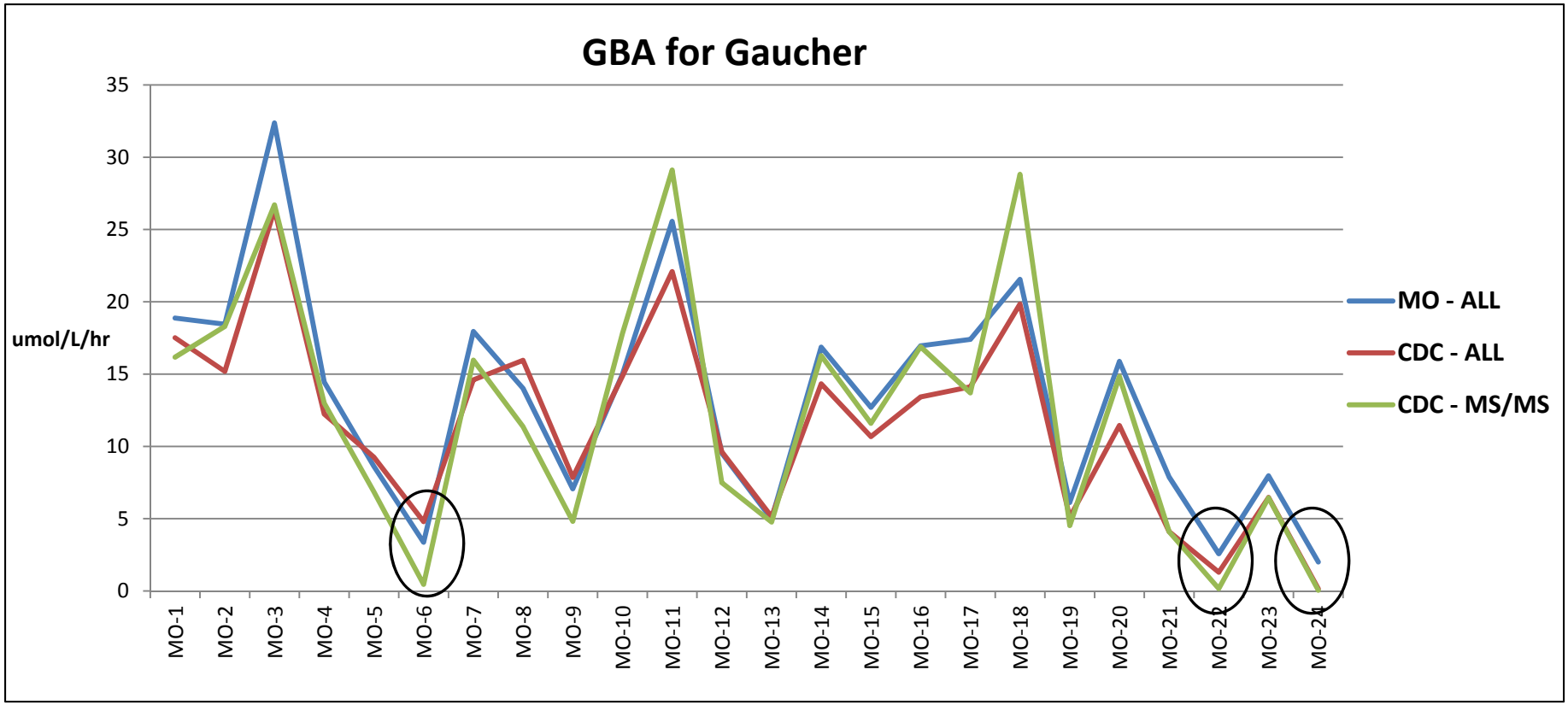
Missouri Blind Samples Tested at CDC



3 True Pompe Samples

Missouri GAA cutoff = 7.0 umol/L/hr

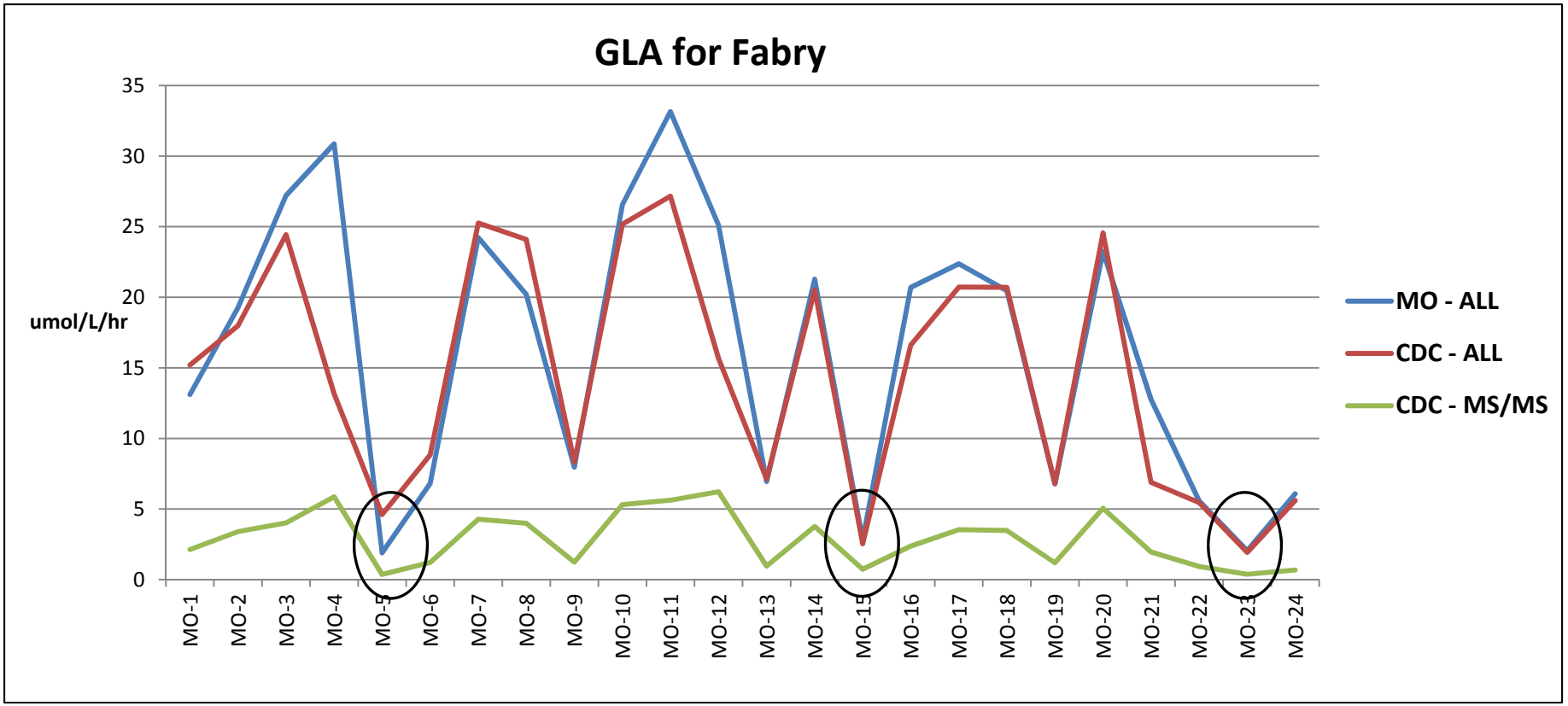
Missouri Blind Samples Tested at CDC



3 True Gaucher Samples

Missouri GBA cutoff = 7.0 umol/L/hr

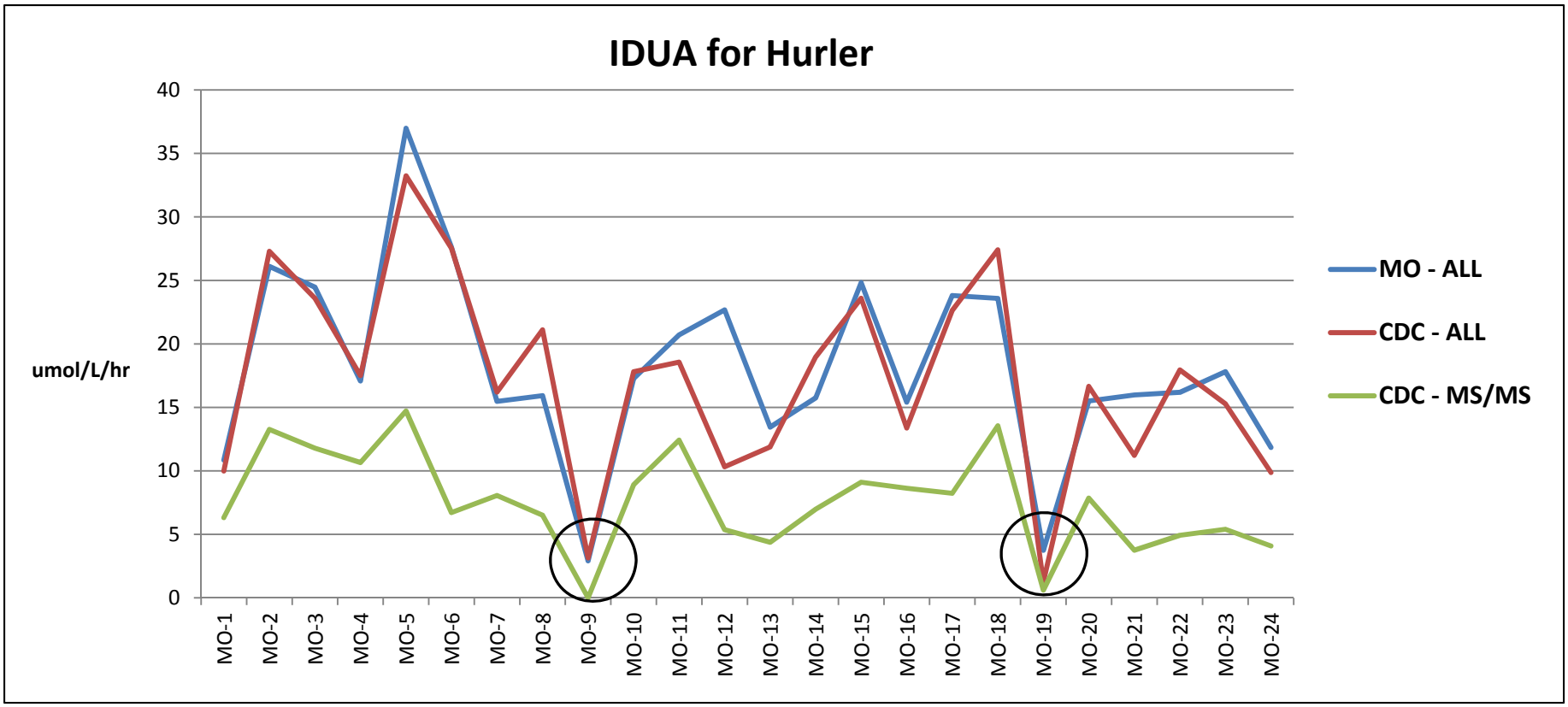
Missouri Blind Samples Tested at CDC



3 True Fabry Samples

Missouri GLA cutoff = 6.2 umol/L/hr

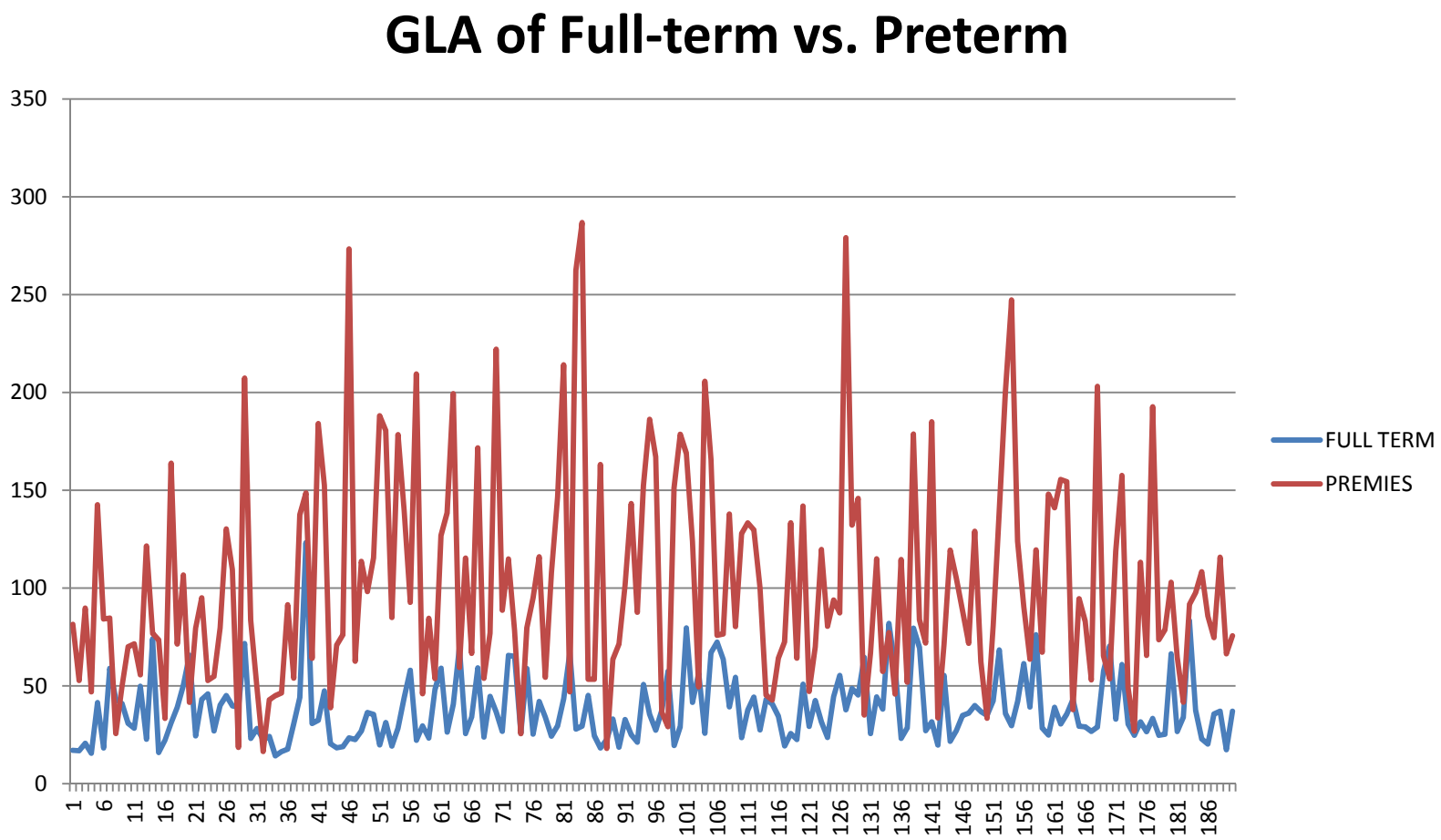
Missouri Blind Samples Tested at CDC



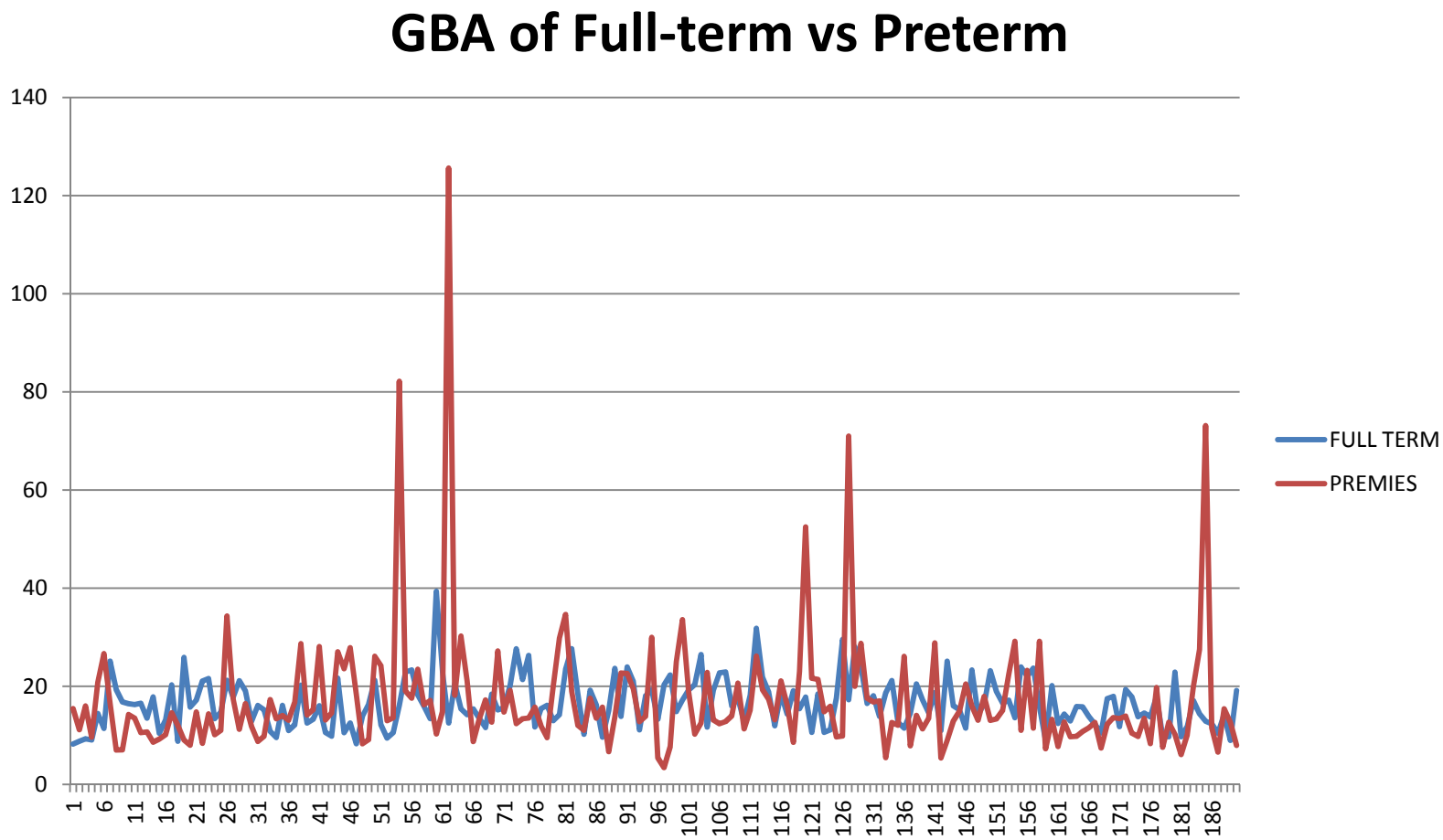
2 True Hurler Samples

Missouri IDUA cutoff = 4.0 umol/L/hr

Health Status Effect



Health Status Effect



Summary of Progress to Date

- We are very pleased with this screening method, the ease at which it can be incorporated into the NBS laboratory, and the ease at which it's conducted.
- During Pre-pilot we confirmed with 2 Pompe cases diagnosed clinically and 1 Gaucher carrier.
- During the Pilot/Implementation Phase we detected a true Pompe on the second day of testing!
- We have detected and referred an unexpectedly large number of cases, especially Hurlers.
- We are very pleased with our Positive Predictive Value results seen thus far from over 27,000 screens.

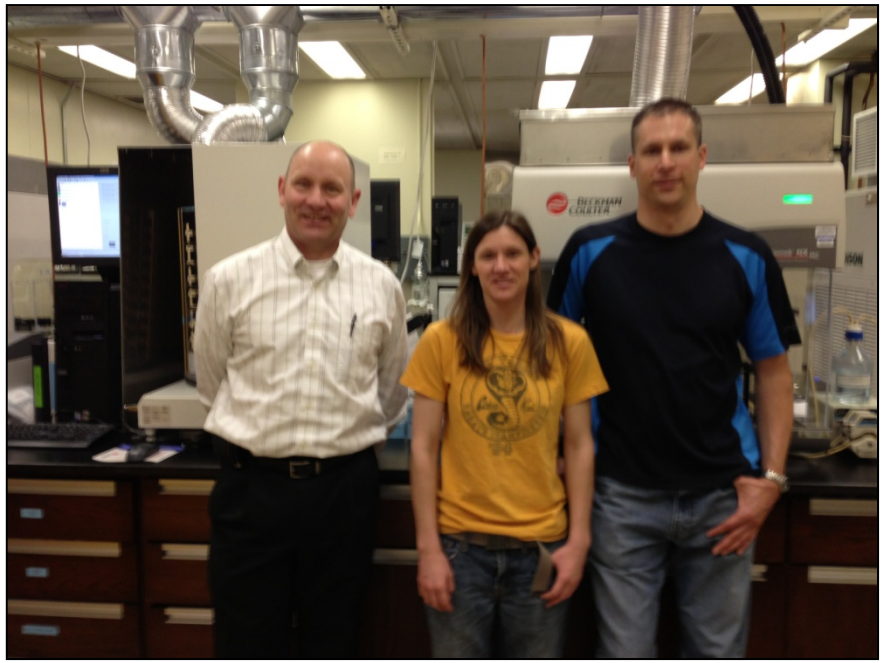
Statistics to Date

Disorder	Screen Positive	Confirmed	Pseudo-deficiency	Carrier	False Positive	Pending
Pompe	8	3	1	2	2	0
Gaucher	2	0	0	0	0	2
Fabry	10	3	0	0	0	7
Hurler	16	2	0	0	2	12
Multiple	1	0	0	0	1	0
Aggregate	37	8	1	2	5	21

Total Specimens Screened in Missouri as of 5/3/13 = 27,037

“Obstacles are those frightful things you see when you take your eyes off your goals.” — Henry Ford

Thank You to New York's NBS Laboratory!



The Planet's Krabbe Screening Experts

Acknowledgements

- Dr. Joe Orsini and the NY Krabbe screening team.
- Carlene Campbell, Tracy Klug, Darla Eiken, Dennis Schmitz and the Missouri LSD screening team.
- Dr. Sharmini Rogers, Julie Raburn-Miller, Jami Kiesling and the Missouri NBS follow-up team.
- Dr. Robert Vogt, Dr. Hui Zhou, and the CDC LSD quality assurance support team.
- Dr. Dietrich Matern and the Mayo LSD team.
- The Advanced Liquid Logic Team.