



Validating the QSight™ tandem mass spectrometry system for GAA and IDUA enzyme screening in dried blood spots with a 3 hour incubation time

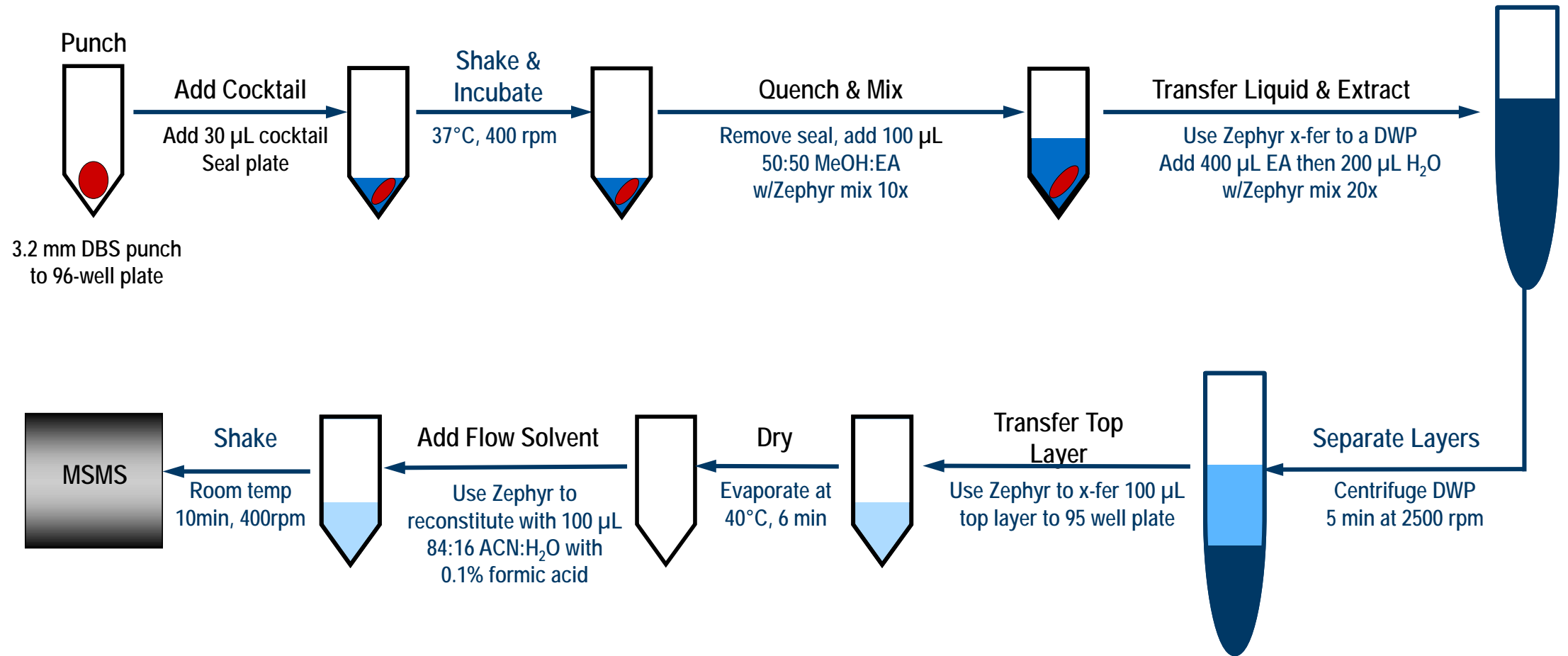
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Pompe/MPS I screening background

- We began screening for Pompe (acid alpha-glucosidase or GAA enzyme deficiency) and MPS I (alpha-L-iduronidase or IDUA enzyme deficiency) on 8/1/2017
- This LDT was initially validated on 2 Waters TQD MS/MS with an 18 hour incubation and PerkinElmer Custom Contract Manufacturing reagents
- We added a PerkinElmer QSight MS/MS to our lab for LSD screening, because we need our TQDs for NeoBase 2
- We had to validate the new instrument, so we decided to validate a 3 hour incubation time too, in order to decrease our turn-around-time



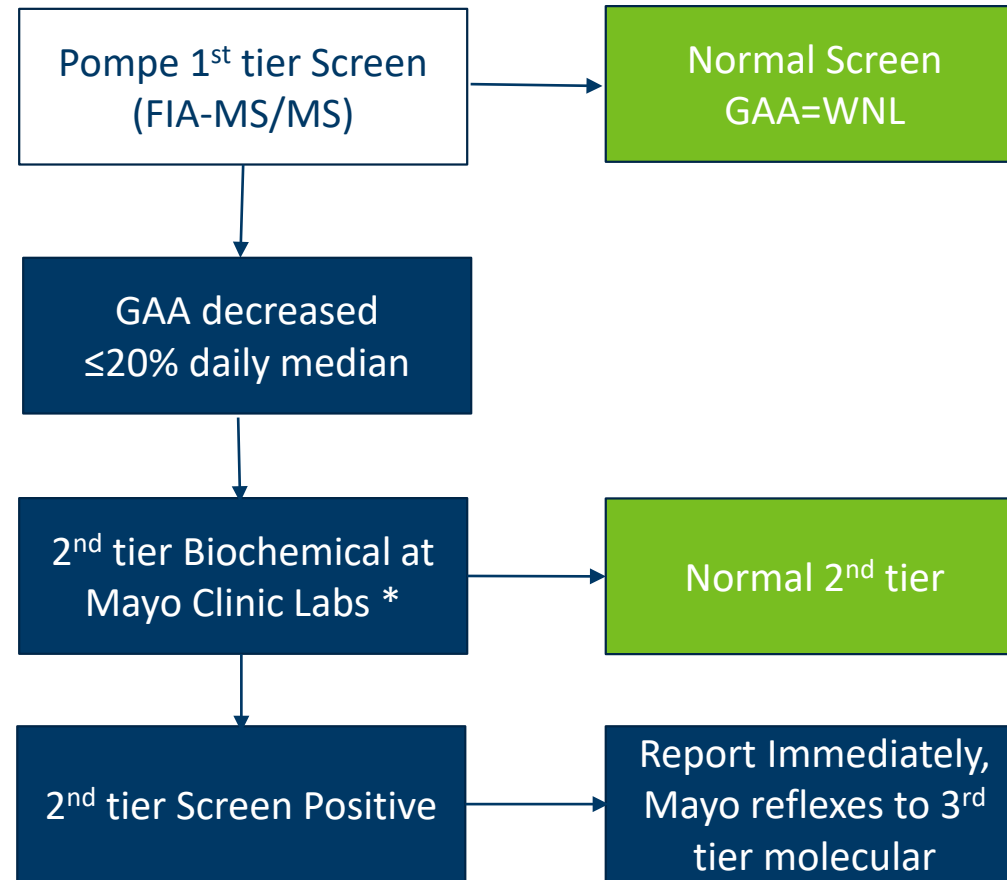
LSD assay



J. Cournoyer, et al, Six-plex FIA-MS/MS method to measure ABG, ASM, GAA, GALC, GLA and IDUA activities in dried blood spots, 2015 WORLDSymposium



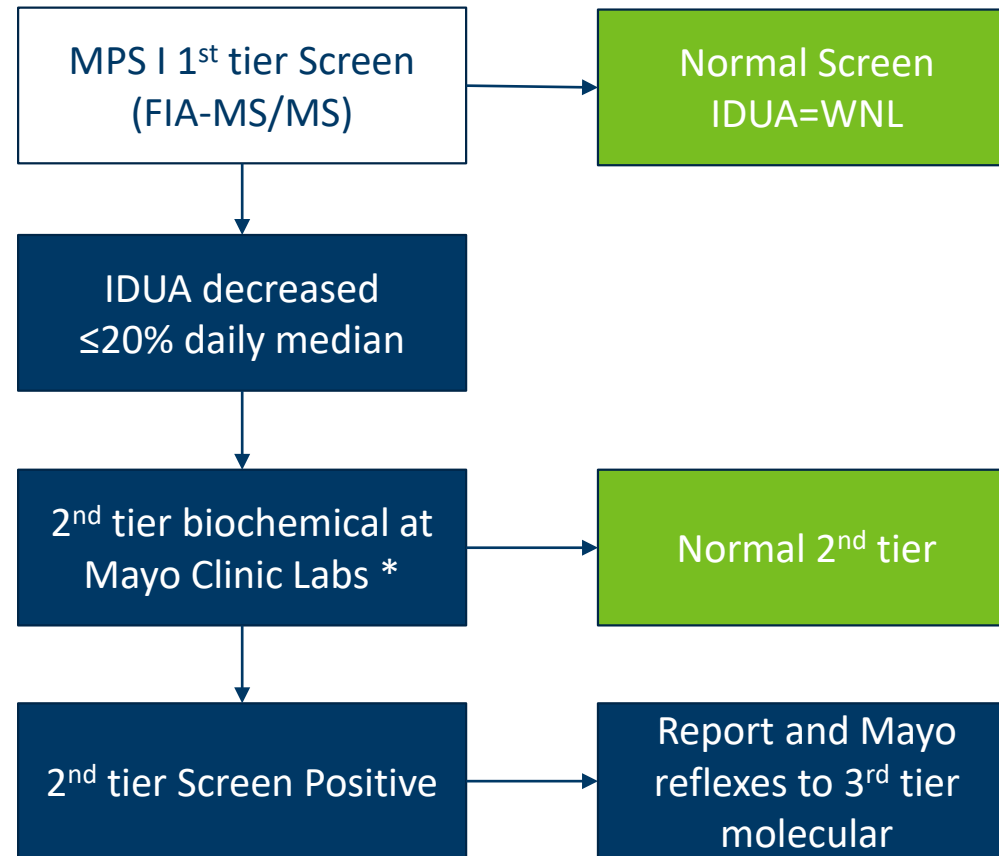
Pompe 3-tier screening algorithm



*GAA activity, (creatine/creatinine)/GAA ratio, CLIR 12-plex



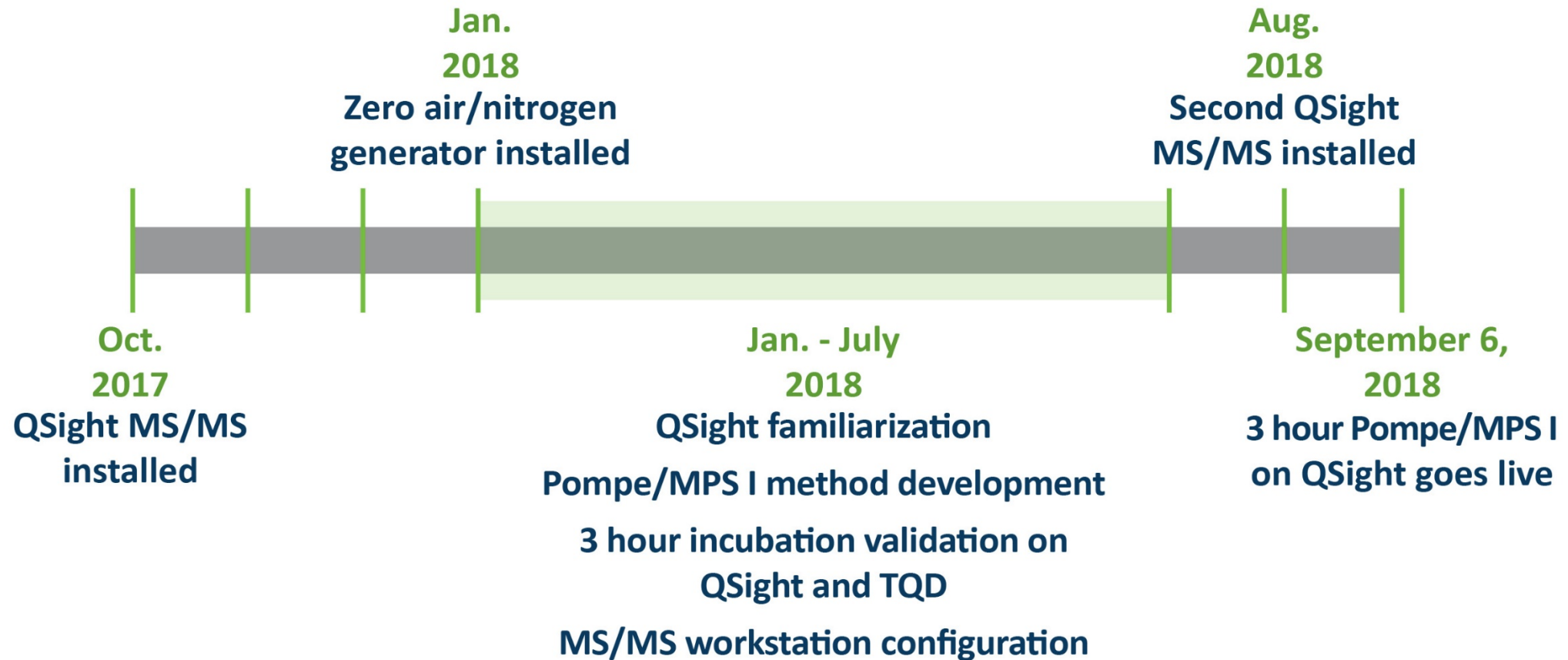
MPS I 3-tier screening algorithm



*DS is dermatan sulfate and HS is heparan sulfate



Timeline for implementing QSight and 3 hr incubation



Sensitivity in $\mu\text{mol/L/hr}$

	QS GAA 3h	TQD GAA 18h	QS IDUA 3h	TQD IDUA18h
Limit of Blank Filter Paper Average	0.029	0.019	0.013	0.00
Limit of Detection	0.15	0.044	0.090	0.020
*Average Internal Standard	0.37	0.048	0.25	0.12
*Standard Deviation Internal Standard	0.070	0.0089	0.10	0.0059

*These values have not been background subtracted
 $\text{LoD} = \text{LoB} + 1.645 * \text{Stdev of CDC's Base/Low Control}$





Accuracy for Pompe

- Accuracy was tested using 9 specimens with reduced GAA activity
- 3 previously graded PT panels from CDC's NSQAP were also tested
- 100% concordance between the 3hr on QSight and the 18hr on TQD
- 2 Confirmed infantile onset Pompe disease cases were correctly identified
 - GAA activity $<0.6 \mu\text{mol/L/hr}$ and $< 5\%$ daily median
- Accuracy testing did indicate cutoffs should be raised from 15 to 20% daily median in order to reflex the same number of specimens for 2nd tier biochemical testing

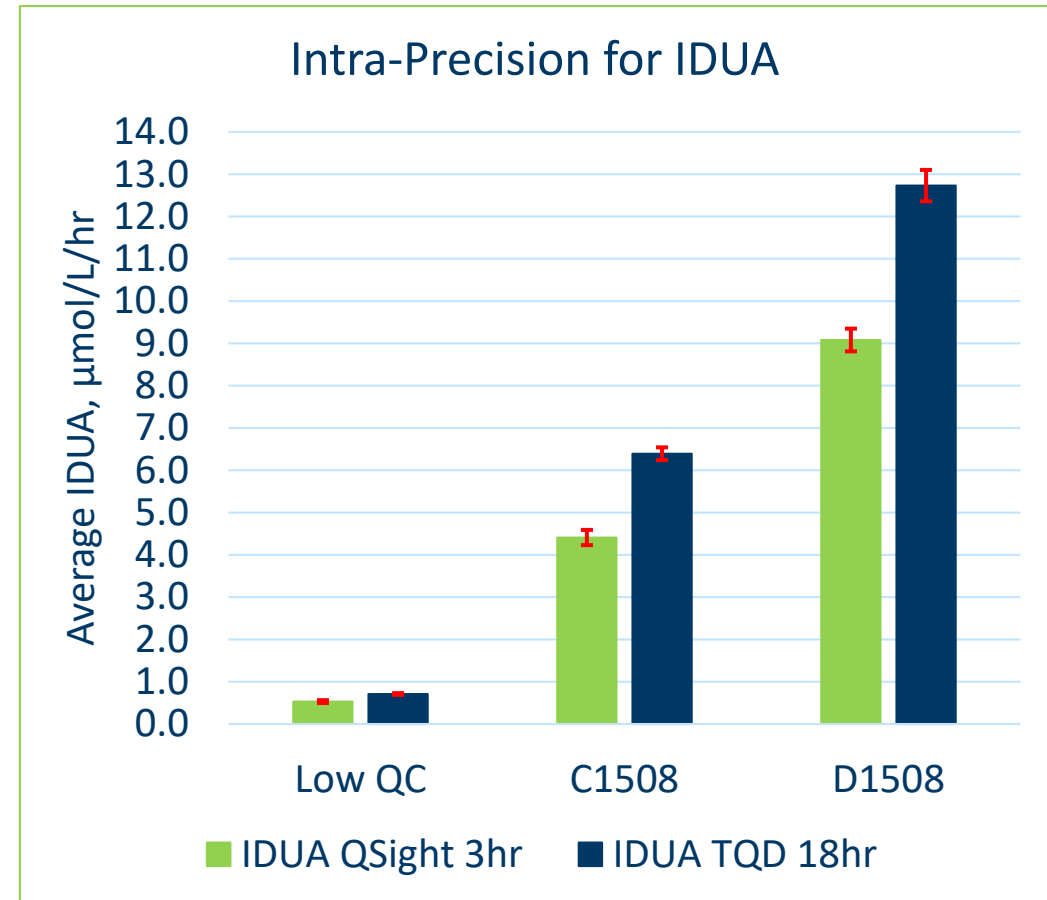
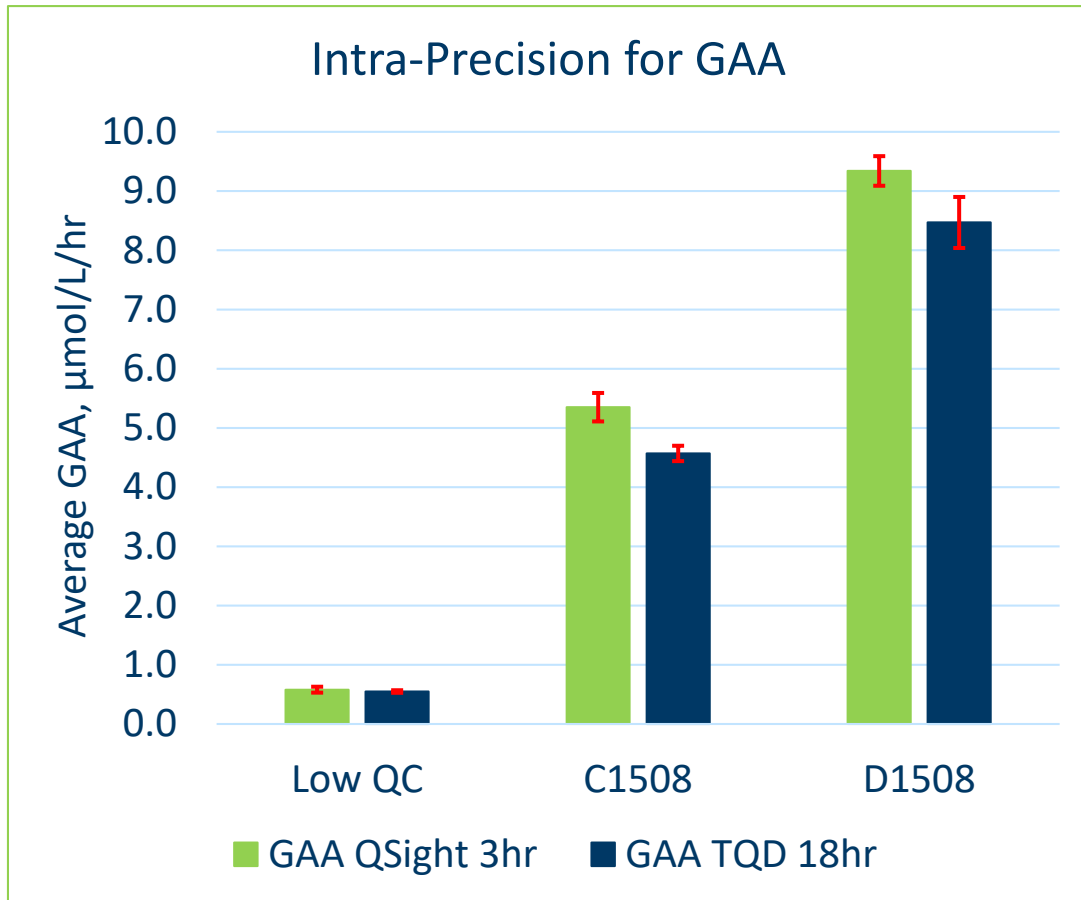


Accuracy for MPS I

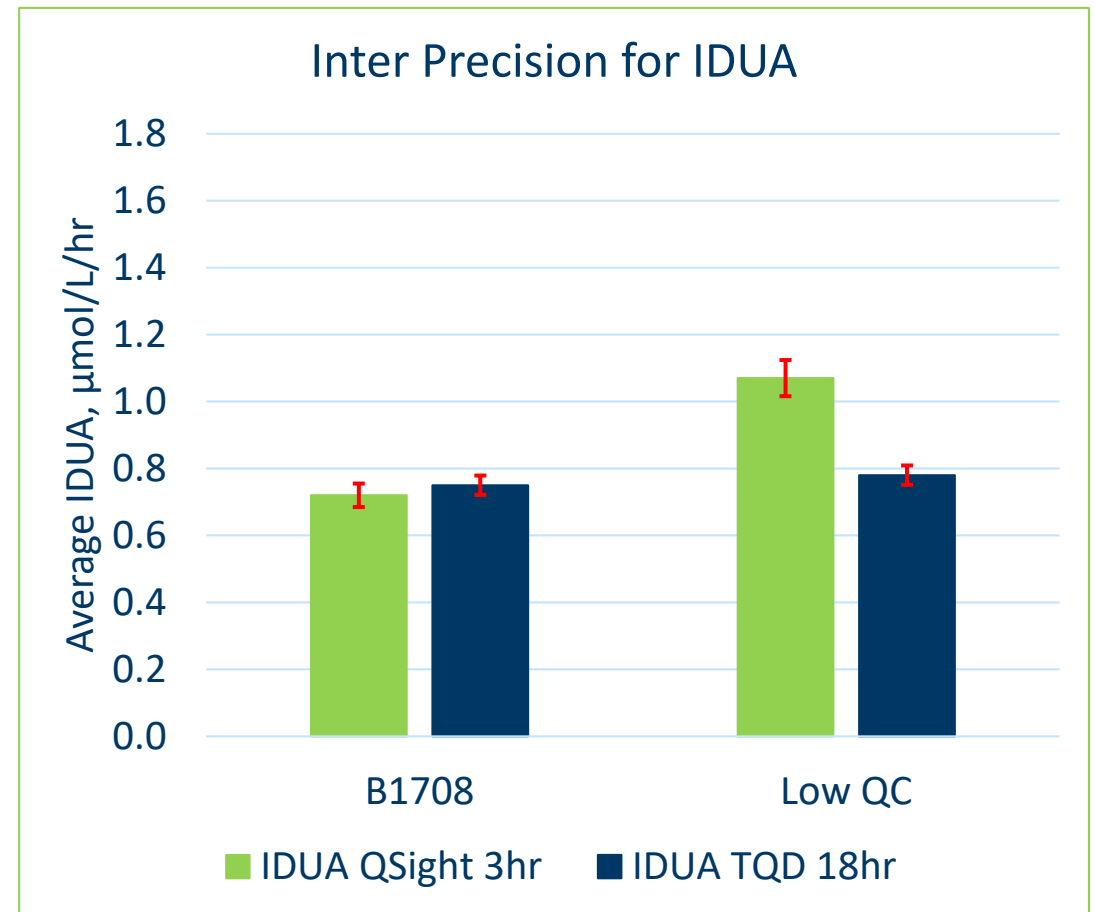
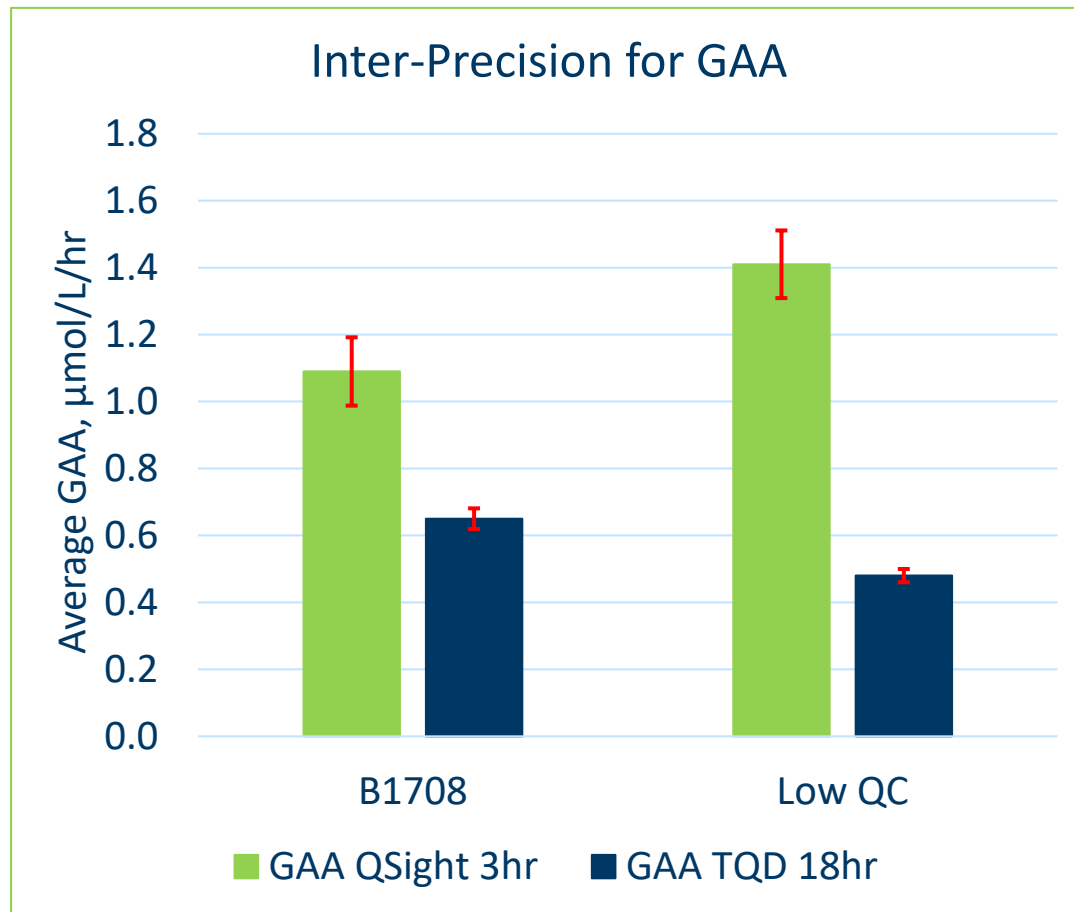
- Accuracy was tested using 10 specimens with reduced IDUA activity
- 3 previously graded PT panels from CDC's NSQAP were also tested
- 100% concordance between the 3hr on QSight and the 18hr on TQD
- 2 confirmed MPS I (Hurler) cases were correctly identified
 - IDUA activity $<0.3 \mu\text{mol/L/hr}$ and $< 3\%$ daily median
- Accuracy testing did indicate cutoffs should be raised from 13 to 20% daily median in order to reflex the same number of specimens for 2nd tier biochemical testing



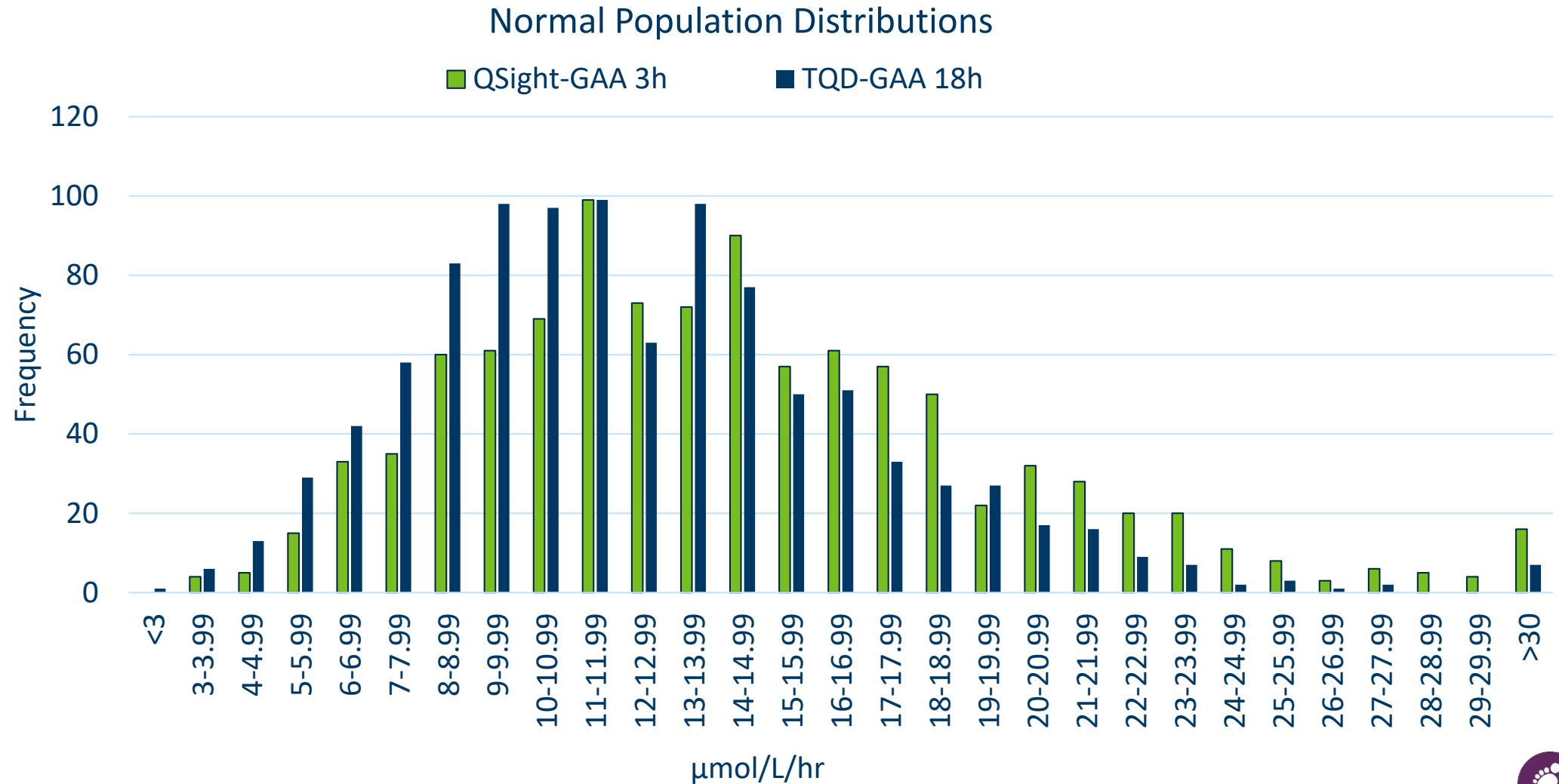
Intra-Precision



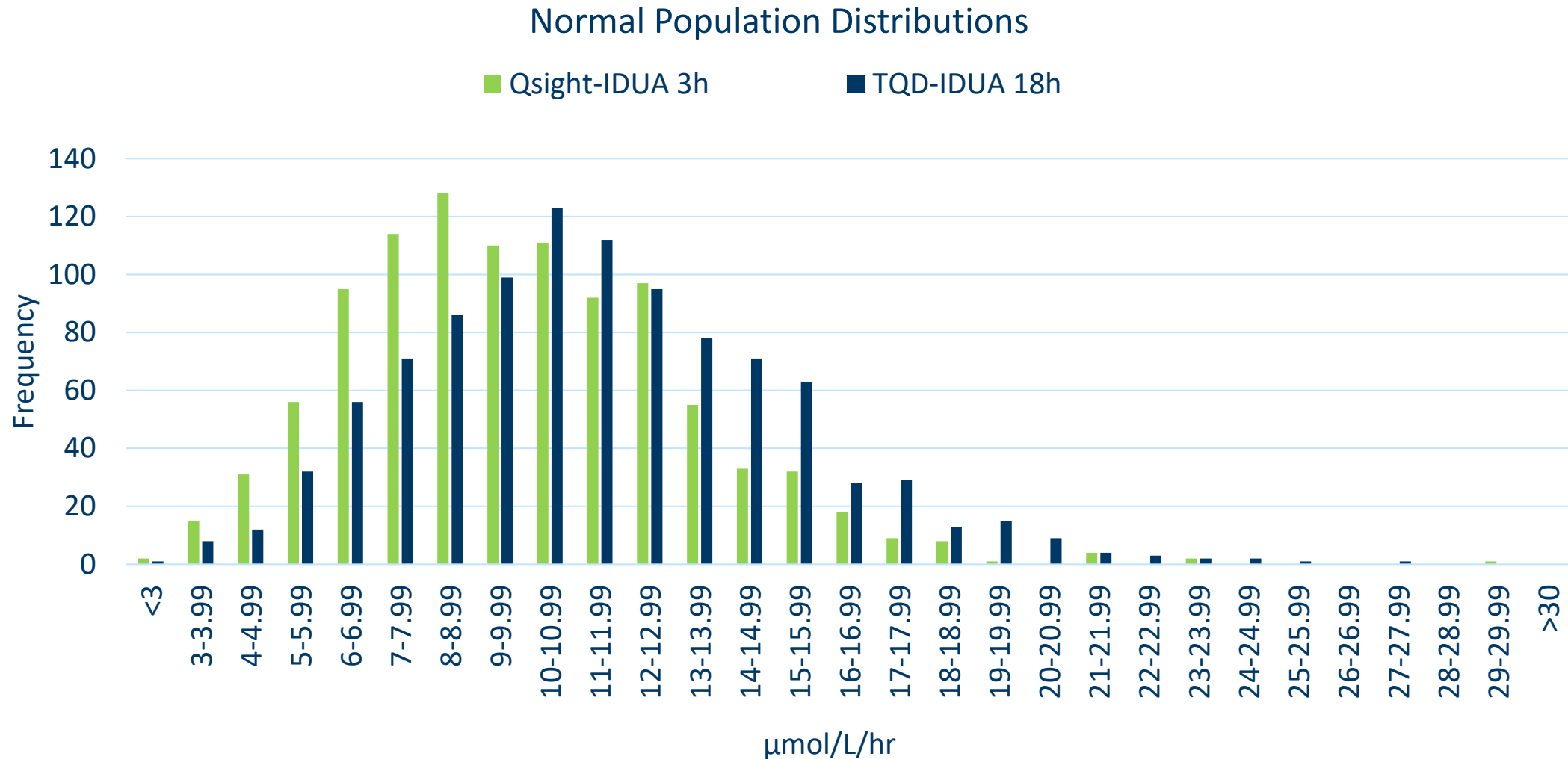
Inter-Precision



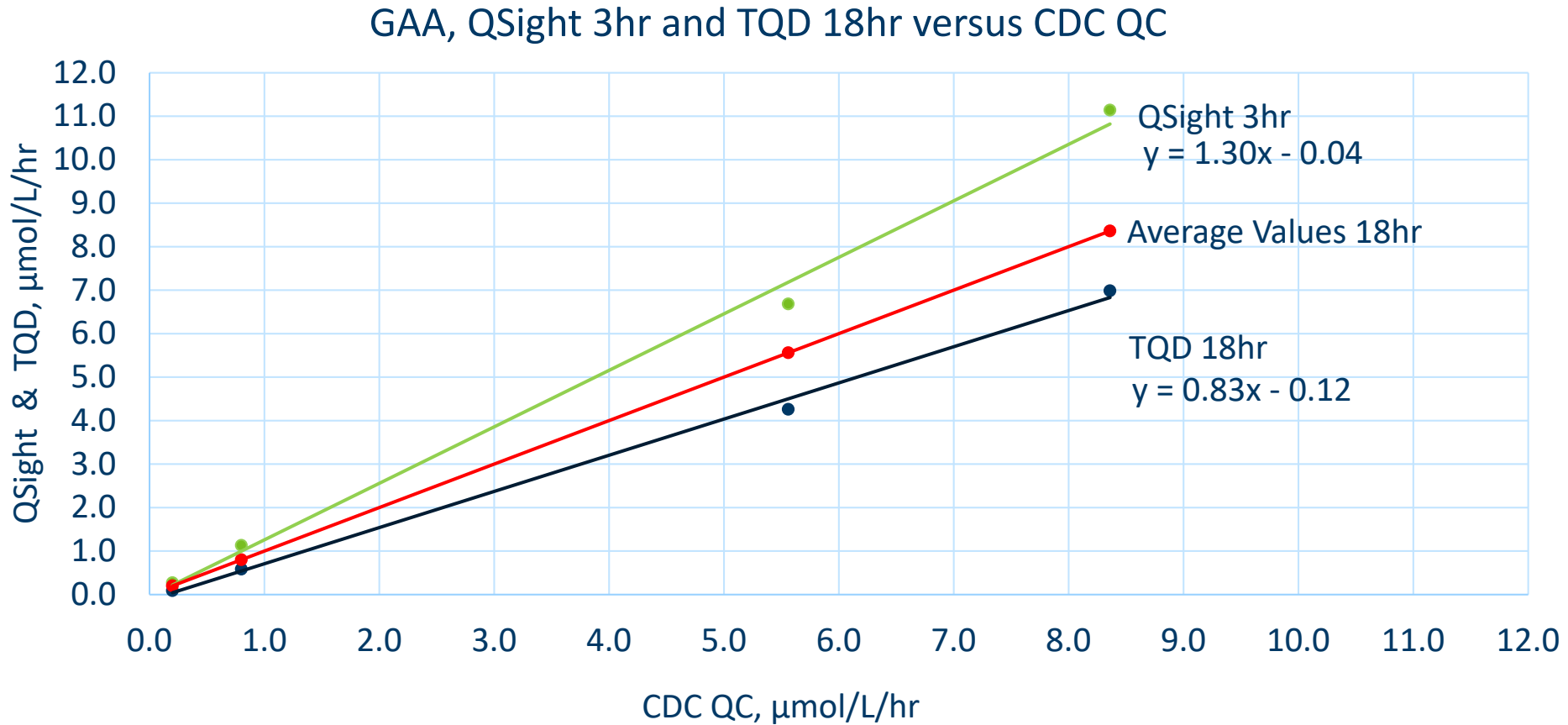
Reference Range for GAA



Reference Range for IDUA



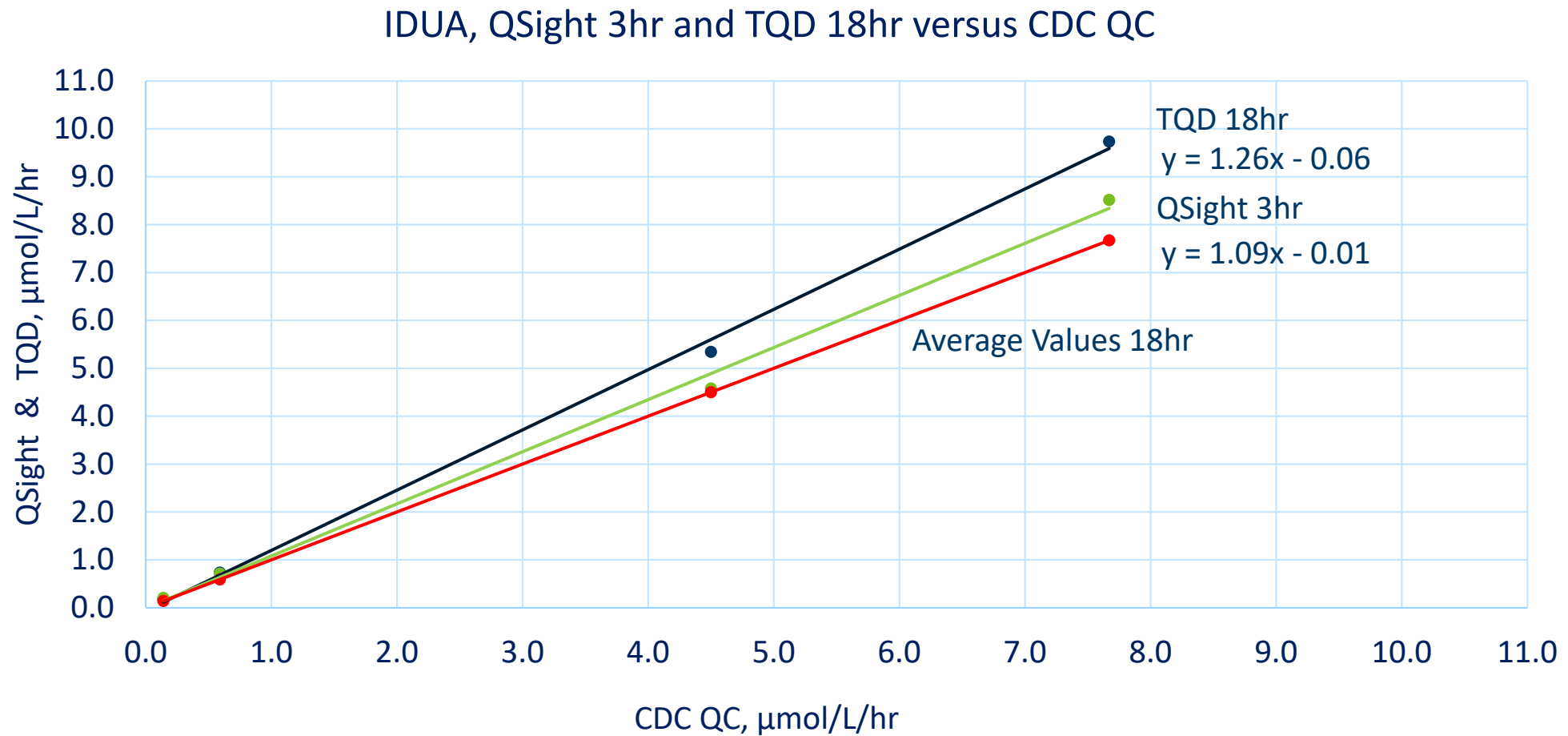
GAA – QSight 3 hr & TQD 18 hr vs. CDC 18 hr



QSight and TQD results are regressed on the average values reported by all labs using FIA-MS/MS



IDUA – QSight 3 hr & TQD 18 hr vs. CDC 18 hr



QSight and TQD results are regressed on the average values reported by all labs using FIA-MS/MS



Performance Metrics for Pompe

- From 8/1/17 to 2/28/19 we screened 104,221 infants (110,750 specimens)
- 42 specimens sent to Mayo for 2nd tier testing, 6 infants were positive and referred for DX testing while 3rd tier molecular testing was completed. Results so far are:
 - 2 are confirmed with IOPD and both are on enzyme replacement therapy
 - 1 likely has late-onset Pompe
 - 2 are likely pseudodeficiencies
 - 1 is a homozygous VUS that may be non-pathogenic
- Pompe birth incidence in Minnesota thus far is 1:34,740
- Only around 14% of specimens remain positive after 2nd tier testing



Performance Metrics for MPS I

- From 8/1/17 to 2/28/19 we screened 104,221 infants (110,750 specimens)
- 56 specimens sent to Mayo for 2nd tier testing, 3 infants were positive and referred for DX testing while 3rd tier molecular testing was completed. Results so far are:
 - 3 are confirmed positive MPS I, all likely Severe
 - 1 is a false positive with 2 VUS, but urine GAGs were normal
- MPS I birth incidence in Minnesota thus far is 1:34,740
- Only around 5% of specimens remain positive after 2nd tier testing



Pros – QSight and 3 hour incubation

- The QSight really does have less maintenance than TQD
 - Daily-prime pumps and rinse syringe, check pressures and spray, load method and run
 - Monthly-change needle/capillary, auto-calibration with PPG, possible DV adjustment
- QSight injection to injection time is much faster than TQD
 - 2 hours to run 1 plate on QSight compared to 3 hours on TQD
- The 3 hour incubation decreases turn-around-time by one day
- The MS/MS WorkStation configured for just Pompe/MPS 1 and a 3hr incubation is really slick



QSight Simplicity software vs. MS/MS WorkStation

Processing QSight LSD Results	Simplicity with Excel, Time in Minutes	MS/MS WorkStation, Time in Minutes
Review chromatography and QC	12	3
Create LIMS upload file	9	3
Enter data and report	27	13
Total number of steps in process	140 steps	58 steps
Total time for a 6 plate batch	48	19



Cons – QSight and 3 hour incubation

- QSight and Simplicity software are new products and there is definitely a learning curve for everyone
- The ISTD blanks are higher with a 3 hour incubation on QSight which results in a higher background subtraction for the results
- There is a little more variability with a 3 hour incubation on QSight for GAA
- These changes are acceptable to us because we have a second tier biochemical test
 - This may not be acceptable to other labs that don't have a 2nd tier or if they are screening for all 6 LSD enzymes



Bonus! xALD on QSight

- Simplicity for QSight is only FIA, not HPLC, therefore you cannot separate and quantify individual peaks. However this works fine for the xALD method (LC-MS/MS in negative ion mode)
- Very few pressure issues on QSight system-possibly due to seal wash and better QC on pump components, column disconnected daily to purge QSight needle(capillary) at 1ml/min to clean it, if pressure is high-change needle
- Source area gets very dirty, Crisco-like coating, but still performs as of 5 months
- Simplicity software is not the mature product that MassLynx is, you will have to use Excel to process your data and create your own LIMS compatible file-if this is your primary screen



Acknowledgments

Washington State University

Michael Gelb

PerkinElmer

Joe Trometer, Jesse Cornejo, Min Weng, James DiPerna, Janet Albrecht

Minnesota Department of Health

Holly Winslow, Alisha Wruck, Nga Gross, Colin Lord, Jill Simonetti, Amy Gaviglio, Mark McCann



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Thank you!



Accuracy for GAA

	QSight 3h		TQD 3h		TQD 18h		Final Dx
Specimen ID	GAA	%DM	GAA	%DM	GAA	%DM	Diagnosis
2016027090	0.081	0.6	0.2	1.8	0.3	3	IOPD
2016021263	0.58	4.3	0.44	4	0.53	5	IOPD
20180671093	2.12	16	1.67	15	1.30	11	WNL
20180171109	1.58	12	1.18	11	1.29	11	WNL
20180291027	2.41	19	2.17	20	1.49	12	WNL
20180181097	1.81	14	1.24	11	1.36	12	WNL
20180391135	2.05	15	1.51	13	1.47	14	WNL
20180521072	2.23	17	1.68	15	1.48	14	WNL
20180201127	2.16	16	1.54	14	1.80	15	WNL



Accuracy for IDUA

Specimen ID	QSight 3h		TQD 3h		TQD 18h		Final Dx
	IDUA	%DM	IDUA	%DM	IDUA	%DM	Diagnosis
20181371152	0.21	2	0.28	3	0.09	1	MPS I
20181211064	0.17	2	0.17	2	0.07	1	MPS I
20173451004	0.8	8	0.92	7	1.16	8	WNL
20173331230	0.66	7	0.95	8	1.29	9	WNL
20172131254	0.68	7	1.03	9	1.24	10	WNL
20180171079	0.96	9	1.1	9	1.56	10	WNL
20173031354	0.89	11	1.04	10	1.52	11	WNL
20173101315	1.3	15	1.49	15	1.75	12	WNL
20172861195	0.83	10	1.02	10	1.56	12	WNL
20180591045	2.07	22	2.34	22	1.78	12	WNL
20181361104	1.33	13	1.54	13	1.29	12	WNL
20180671093	1.72	19	1.96	18	2.38	16	WNL



MS/MS WorkStation versus Simplicity

MS/MS Workstation	Simplicity and Excel
Drag drop Panthera W/L and plcodes to Specimen Gate folder	Copy paste W/L onto an old Simplicity worklist (can't make a template)
Drag drop created W/L to Simplicity folder	Run on Qsight
Load W/L and run on QSight	Review TICs one at a time in Simplicity
Export data to network folder then drag drop to Result Viewer	Export multi sheet Excel in text format to network folder
Calculate, Evaluate TICs, IStD intensities, QC, Medians in Result Viewer	Find the data and copy/paste into a template
Accept data and .csv automatically created ready for upload to Natus	Excel template does background subtraction, %DM, flagging, and makes .csv for upload into Natus
Similar to GSP (and NeoBase)	Find the QC and plot it in Excel, review QC
Time to Completion	Similar to nothing and quite painful! Prone to copy/paste errors
	Time to completion



Intra Precision

Avg, %CV	QS GAA 3h	TQD GAA 3h	TQD GAA 18h	QS IDUA 3h	TQD IDUA 3h	TQD IDUA 18h
Low QC Average	0.58	0.41	0.48	0.53	0.63	0.78
Low QC %CV	19.6	12.5	14.6	14.9	9.4	14.1
Mid QC Average	5.4	4.5	4.6	4.4	5.1	6.4
Mid QC %CV	10.2	10.3	6.5	9.5	9.9	5.4
High QC Average	9.3	7.9	8.5	9.1	10.7	12.7
Mid QC %CV	5.9	5.9	11.3	6.8	6.3	6.4



Inter Precision

Avg, %CV	QS GAA 3h	TQD GAA 3h	TQD GAA 18h	QS IDUA 3h	TQD IDUA 3h	TQD IDUA 18h
Low QC Average	1.1	0.87	0.65	0.72	0.90	0.75
Low QC %CV	21.3	10.9	16.9	11.1	8.6	14.7
Mid QC Average	1.4	1.1	0.48	1.1	1.3	0.78
Mid QC %CV	17.9	10.9	14.6	12.5	11.6	14.1
High QC Average	20.6	17.5	14.8	16.9	20.3	22.8
Mid QC %CV	10.8	9.0	8.2	11.3	11.7	11.7



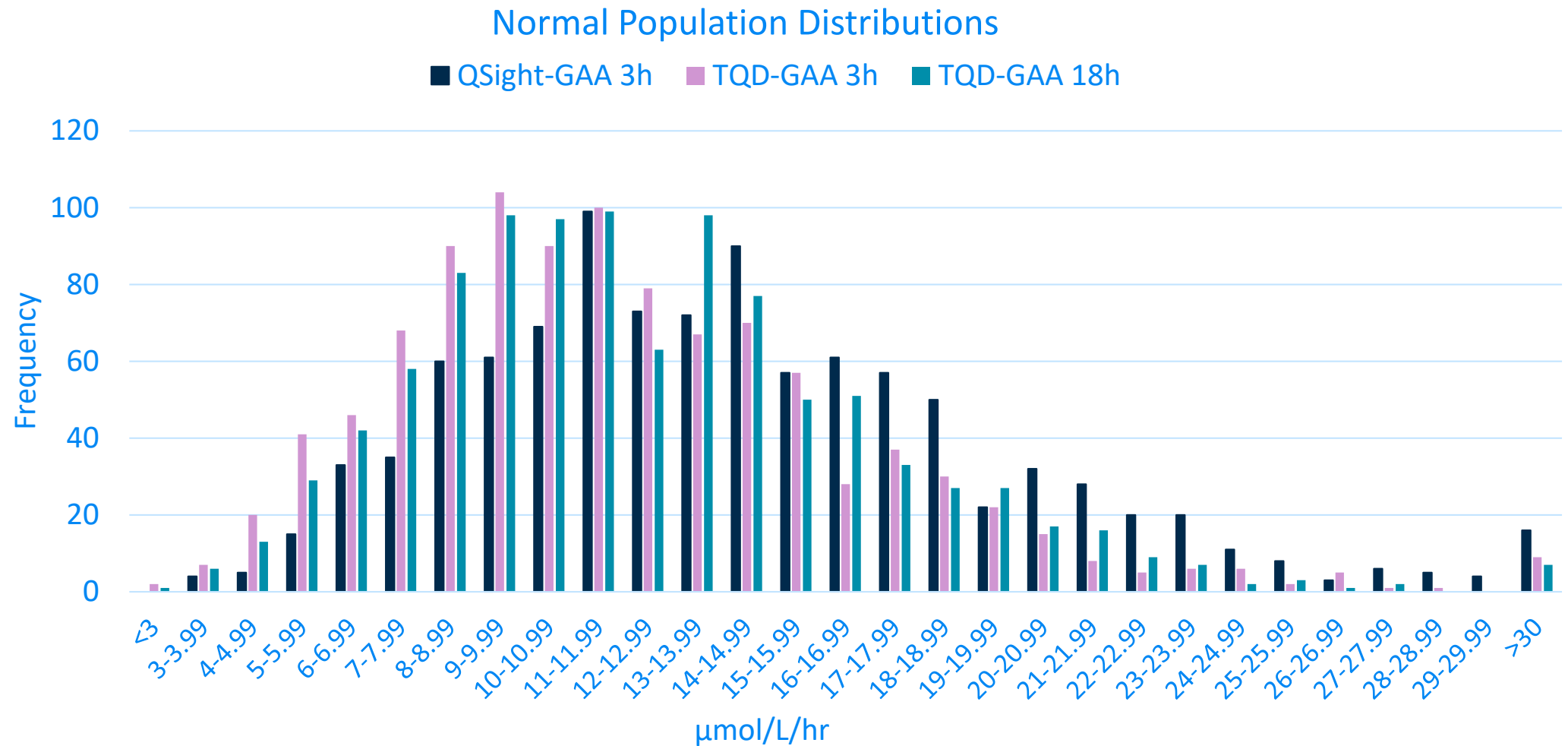
Sensitivity

	QS GAA 3h	TQD GAA 3h	TQD GAA 18h	QS IDUA 3h	TQD IDUA 3h	TQD IDUA 18h
Limit of Blank Filter Paper Average	0.029	0.036	0.019	0.013	0.02	0
Limit of Detection	0.15	0.095	0.044	0.09	0.092	0.02
*Average Internal Standard	0.37	0.18	0.048	0.25	0.22	0.119
*Standard Deviation Internal Standard	0.07	0.026	0.009	0.1	0.032	0.006

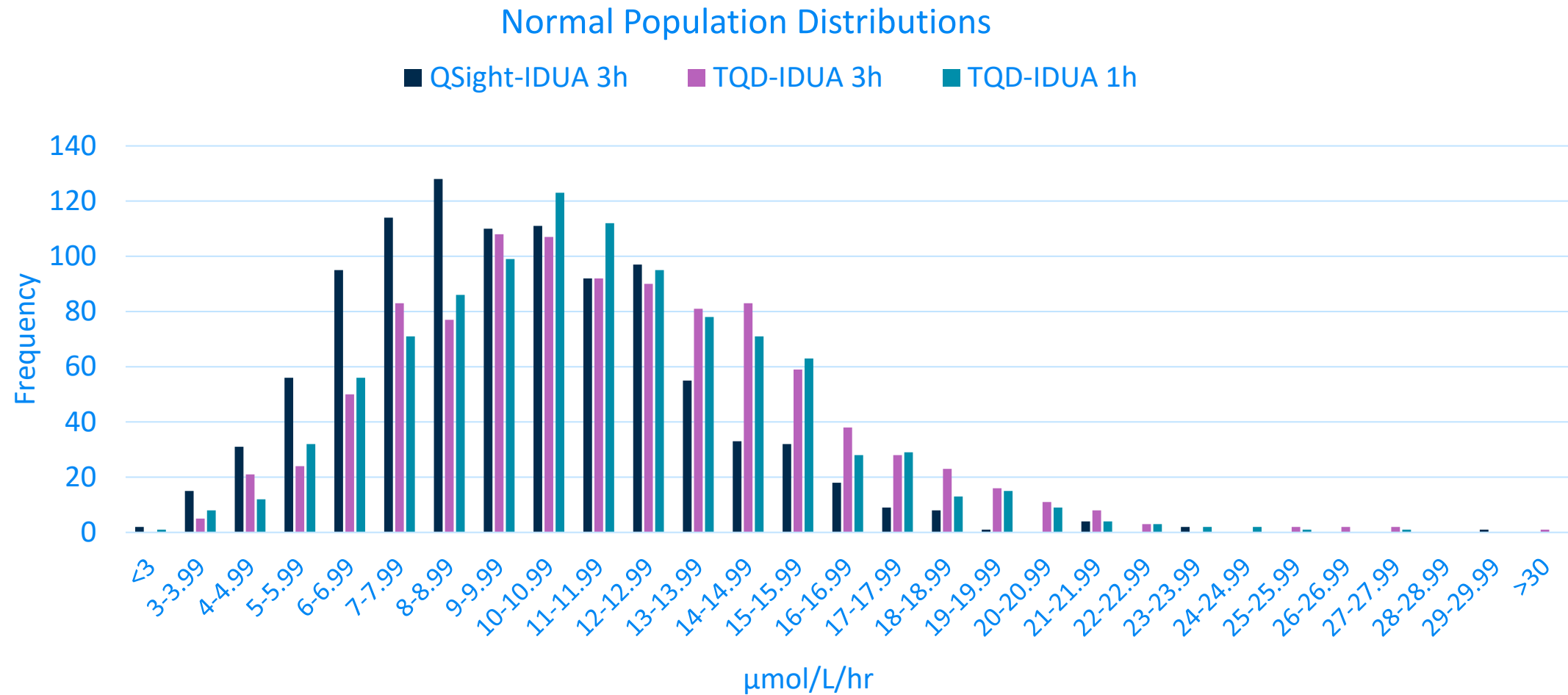
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 $LoD = LoB + 1.645 * Stdev$ of CDC's Base/Low Control



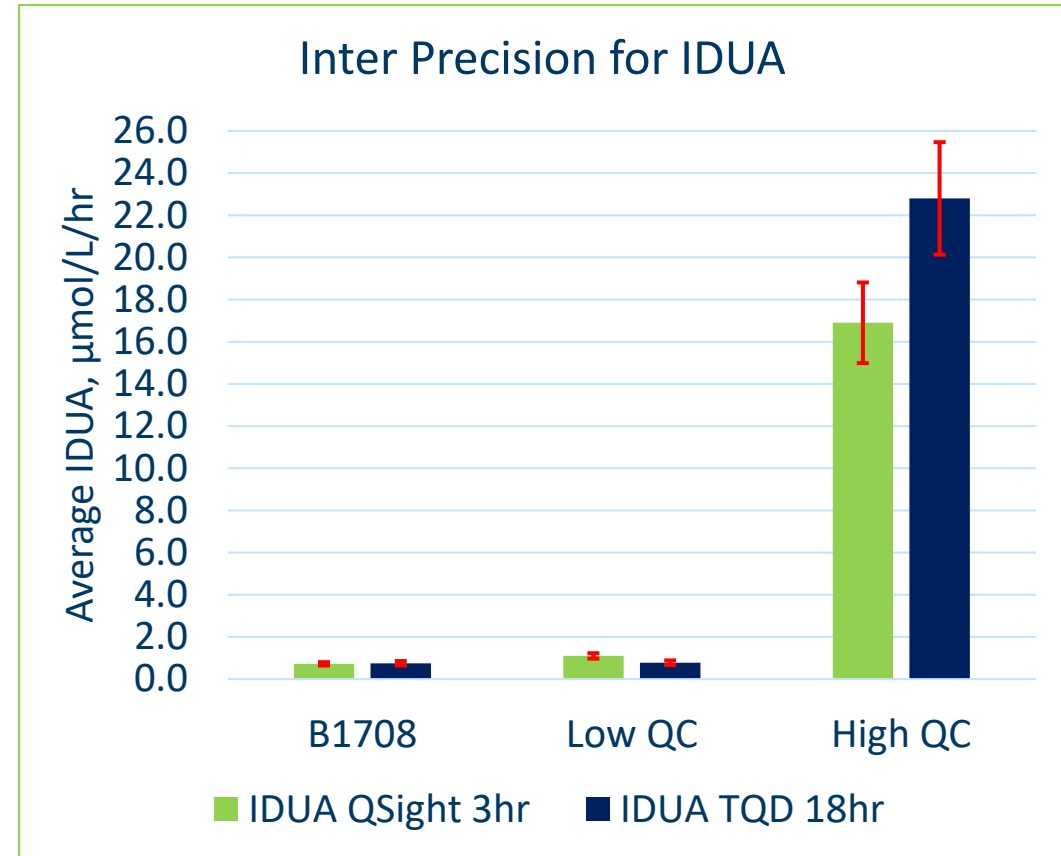
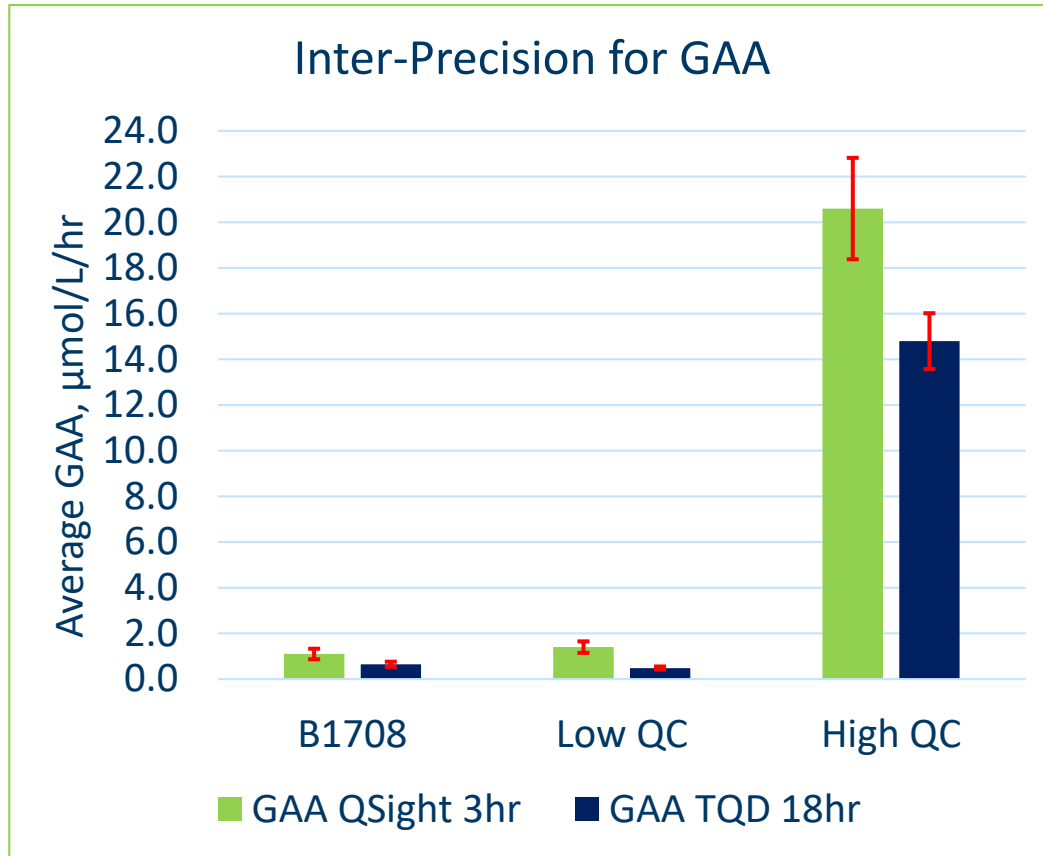
Reference Range for GAA



Reference Range for IDUA



Inter Precision



Reportable Range and Cutoffs

GAA Reportable Range

0.33 – 20.8 $\mu\text{mol/L/hr}$ for QSight 3 hr

0.04 – 14.18 $\mu\text{mol/L/h}$ for TQD 18 hr

IDUA Reportable Range

0.21 – 17.6 $\mu\text{mol/L/hr}$ for QSight 3 hr

0.02 – 21.7 $\mu\text{mol/L/h}$ for TQD 18 hr

GAA and IDUA Cutoffs are both 20% of the daily median



Inter-Precision

