The Effects of Low Birth Weight on the Newborn Screening Activities of Enzymes Associated with Lysosomal Storage Disorders

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Lysosomal Storage Disorders Screening Assay in Illinois Newborn Screening Program

- **Illinois State Newborn Screening Program started a high-throughput multiplex assay of evaluating five enzymes involved with Mucopolysaccharidosis1, Fabry, Pompe, Nieman-Pick A/B, and Gaucher disease on June 1, 2015.**
- **We adopted UPLC-Tandem Mass Spectrometry technology to analyze 17 hour incubation enzymes products with minimal post analytical steps. From Nov 3, 2014 pilot to Feb 15, 2016, total 142,902 samples have been tested.**

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Multiplex LC-MS/MS Assay

- Modification of method developed at the University of Washington for six LSDs: Pompe, Gaucher, Fabry, Niemann-Pick A/B, Mucopolysaccharidosis type I (MPS I).

  Single DBS punch

  Single buffer

  In-line chromatographic purification (no LLE and solid-phase extraction)

  17-hour incubation.

- UPLC column separates products, substrates, ISTDs and removes salt, detergent, & phospholipids by directing the flow to the waste.

- 2.5 minute injection cycle, 500 injections/instrument/day, column guard change after 2500 injections; column change after >10,000 injections.

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6-Plex Assay

Final Composition of Assay Cocktail & Assay Conditions*

Ammonium formate **Sodium cholate** **Acarbose** **N-Acetyl-α-galactosamine**	**0.1 M, pH 4.4** **10 g/L** **0.08 M** **50 mM**
IDUA Substrate (S), Internal Standard (IS) GLA S, IS GAA S, IS ASM S, IS (d7-C6 Ceramide) GALC S, IS (d7-C8 Ceramide) ABG S, IS (d7-C12 Ceramide)	500 µM, 3.5 µM 600 µM, 1.2 µM 200 µM, 2.0 µM 150 µM, 2.5 µM 450 µM, 2.5 µM 300 µM, 2.5 µM
3 h/17 h incubation at 37 ^{0}C	

- Reaction was quenched with 200 µL acetonitrile (ACN) and centrifuged for 5 min at 1000 x g.
- 100 µL top layer was transferred to a glass-lined plate, and 100 µL MS-grade water was added to each well.

*Spacil Z, Tatipaka H, Barcenas M, Scott CR, Turecek F, Gelb MH. Clin Chem. 2013 Mar;59(3):502-11

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Acquity TQD Instrument

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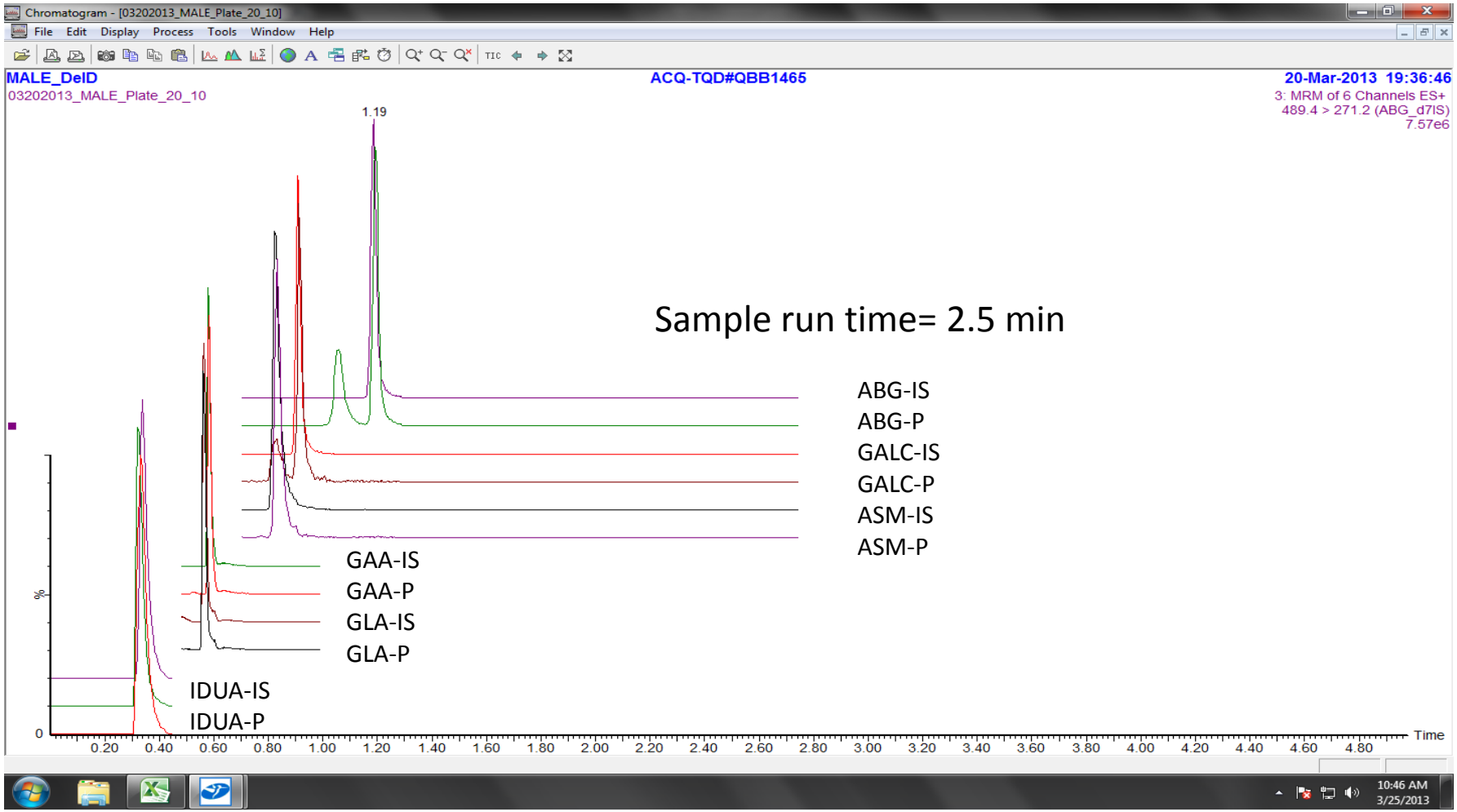
UPLC Chromatogram



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Five Lysosomal Enzymes Normal and Abnormal Ranges based on % of Batch Median

	Normal Range	1st Cut-off	Borderline	2nd Cut-off (positive)
IDUA (Mucopolysaccharidosis1)	> 31%	=<35%	> 28 and =< 31	=< 28%
GLA (Fabry)	> 18%	=<20%	> 13 and =< 18	=< 13%
GAA (Pompe)	> 22%	=<24%	> 18 and =< 22	=< 18%
ASM (Nieman-Pick A/B)	> 15%	=<20%	> 11 and =< 15	=< 11%
ABG (Gaucher)	> 20%	=<25%	> 17 and =< 20	=< 17%

Preterm/low birth weight infants exhibit unexpectedly elevated enzyme levels

- Newborn screening for some Lysosomal Storage Disorders (LSDs) has shown that preterm/low birth weight infants exhibit unexpectedly elevated enzyme levels. This phenomenon may affect proper evaluation of their disease status. Data from 100,777 neonates were reviewed. Infants with very low birth weight (VLBW <1000 g) comprised 0.65% of the study cohort; low birth weight (LBW 1000-2000 g) comprised 2.59%.

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The effects of birth weight on lysosomal enzymes activities

Median Activities: Percent of Batch median

	<1000 g	1001-1500 g	1501-2000 g	2001-2500 g
(n)	658	807	1804	6392
GLA	432	307	172	117
ABG	112	90	84	89
GAA	116	93	84	89
IDUA	113	108	92	92
ASM	102	85	80	87

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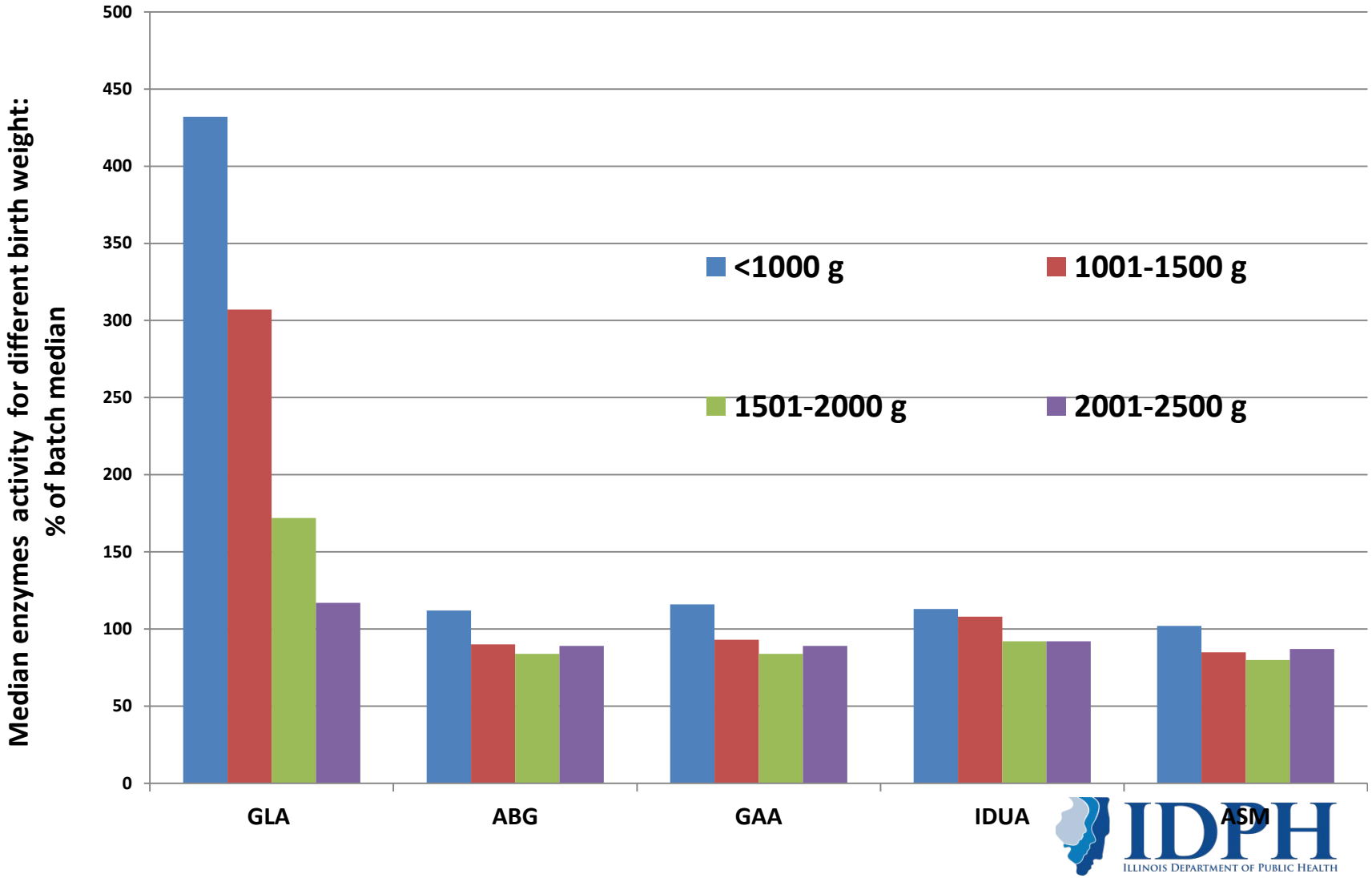
The effects of birth weight on lysosomal enzymes activities



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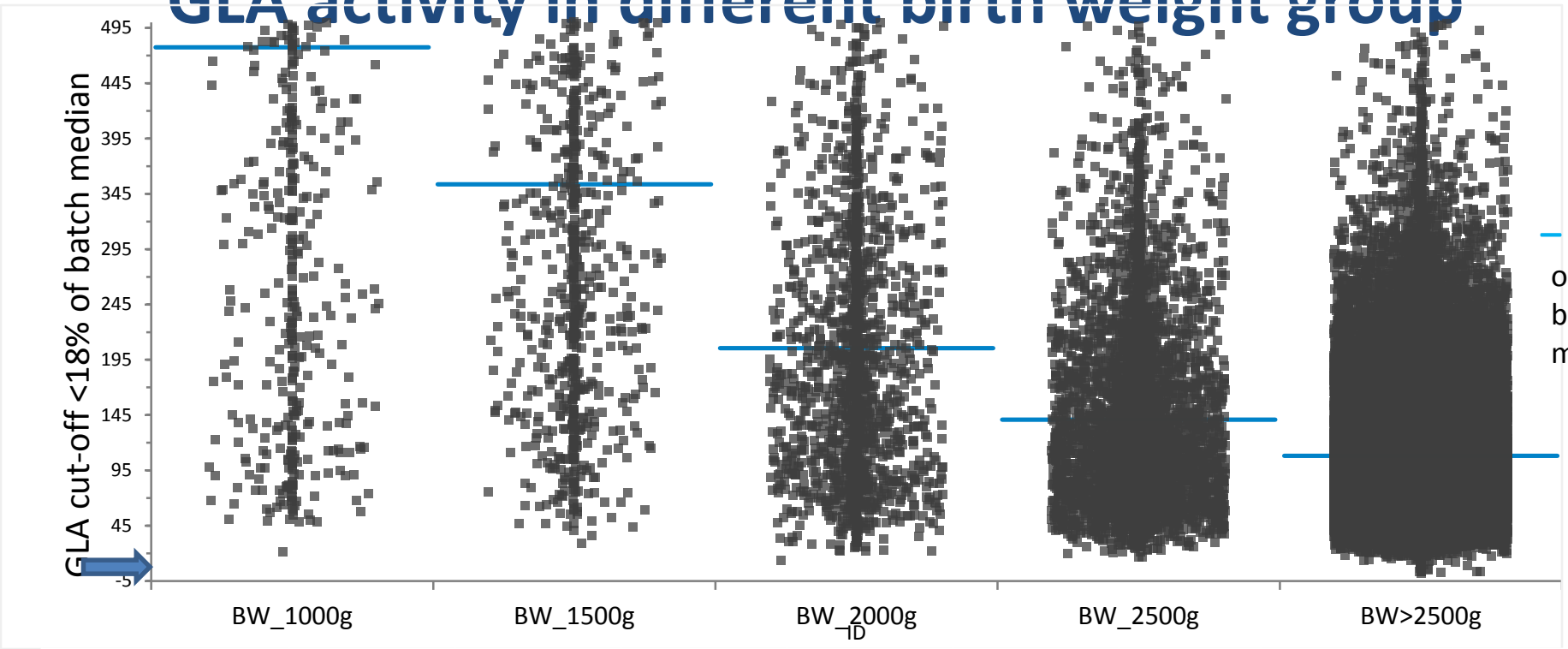
GLA activity in different birth weight group



N	100777							
GLA Activities BW	Minimum	1st Quartile	Median	95% CI		3rd Quartile	Maximum	Inter-quartile
BW up to 1000g	22	216	458	411	to 488	664	2361	448
BW 1001-1500g	29	183	312	293	to 337	478	1192	295
BW 1501-2000g	14	105	172	167	to 180	274	1032	168
BW 2001-2500g	16	81	118	116	to 120	179	1642	98
BW>2500g	2	73	98	98	to 98	131	1172	57

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GLA median activity in different collection time and birth weight (unit: % of batch median)

	Time of collection	<1000 g	1001-1500 g	1501-2000 g	2001-2500 g	>2500 g
(n)	<1 week	658	807	1804	6392	103583
(n)	1-2 weeks	557	1394	2076	2059	3939
(n)	2-3 weeks	169	210	408	387	660
(n)	3-4 weeks	257	333	370	153	229
Analyte	Time of collection	<1000 g	1001-1500 g	1501-2000 g	2001-2500 g	>2500 g
	<1 week	432	307	172	117	98
GLA	1-2 weeks	210	199	121	100	87
	2-3 weeks	232	195	120	105	89
	3-4 weeks	219	195	127	114	79

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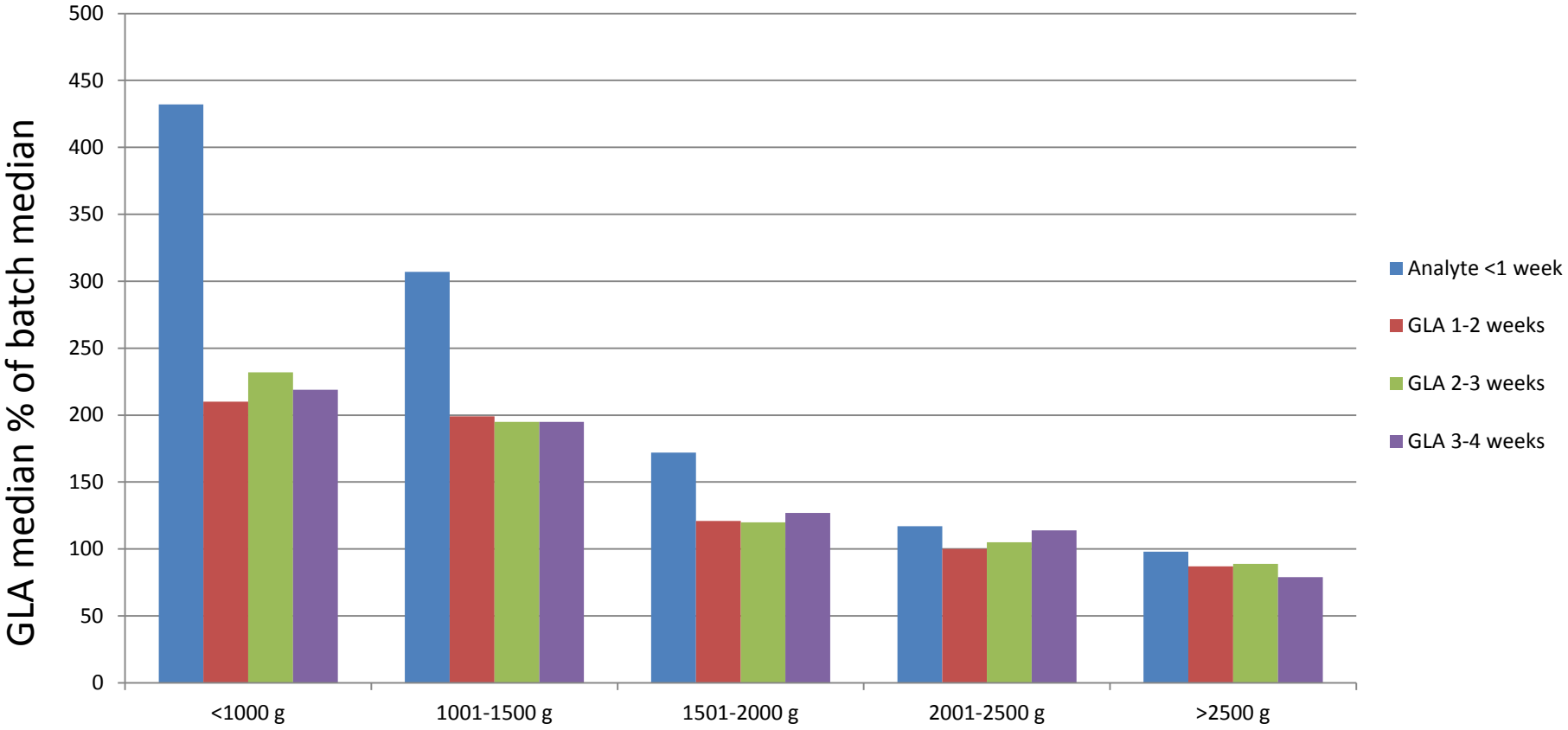
GLA median activity in different collection time and birth weight (unit: % of batch median)



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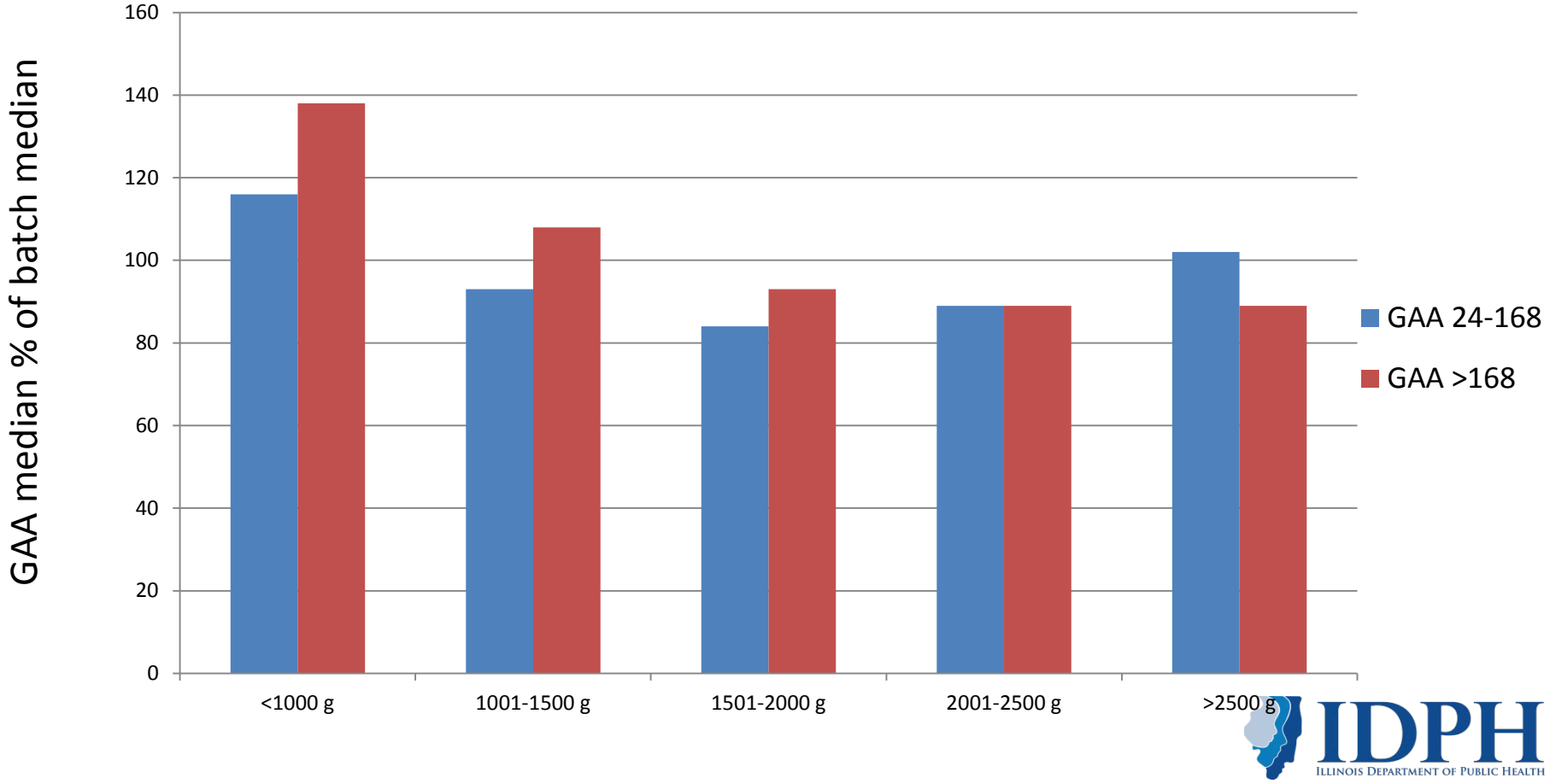
GAA median activity in different collection time (hour) and birth weight (unit: % of batch median)



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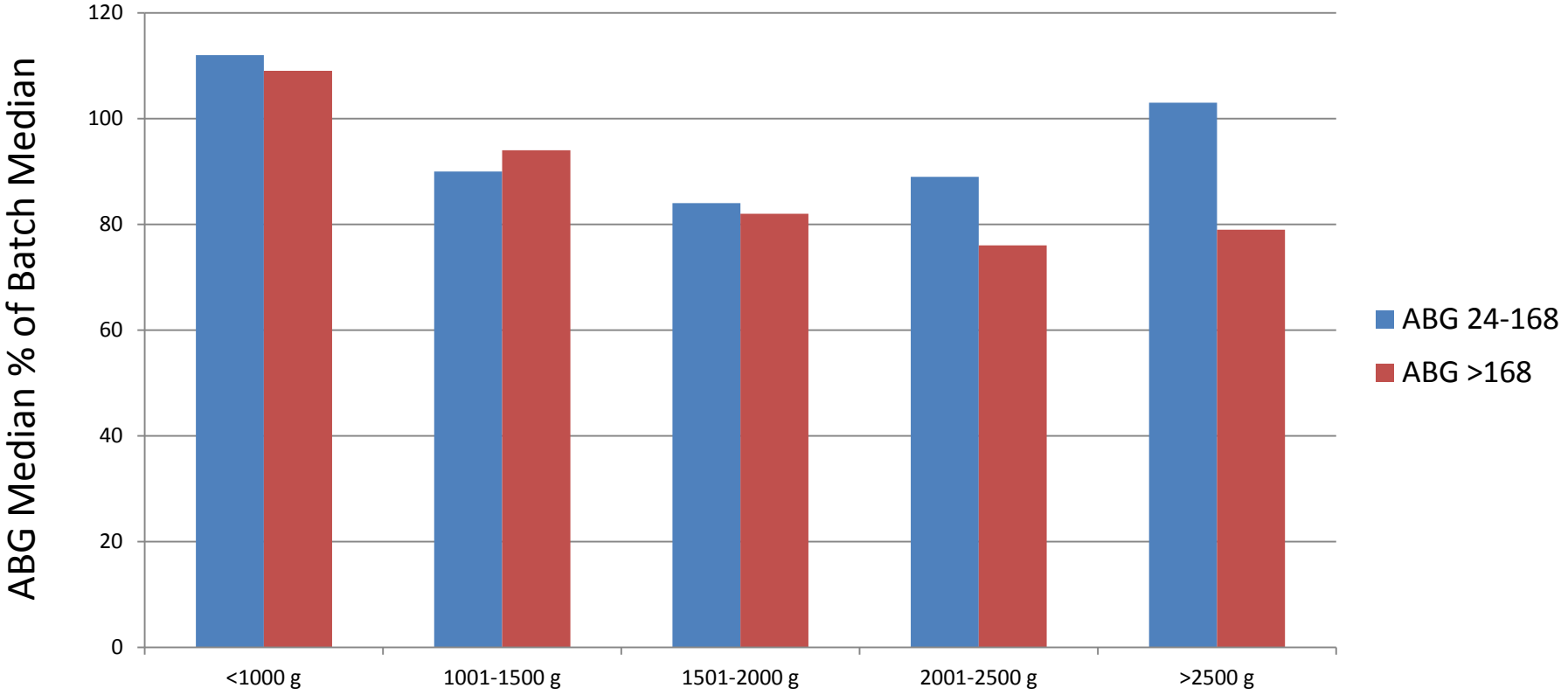
ABG median activity in different collection time (hour) and birth weight (unit: % of batch median)



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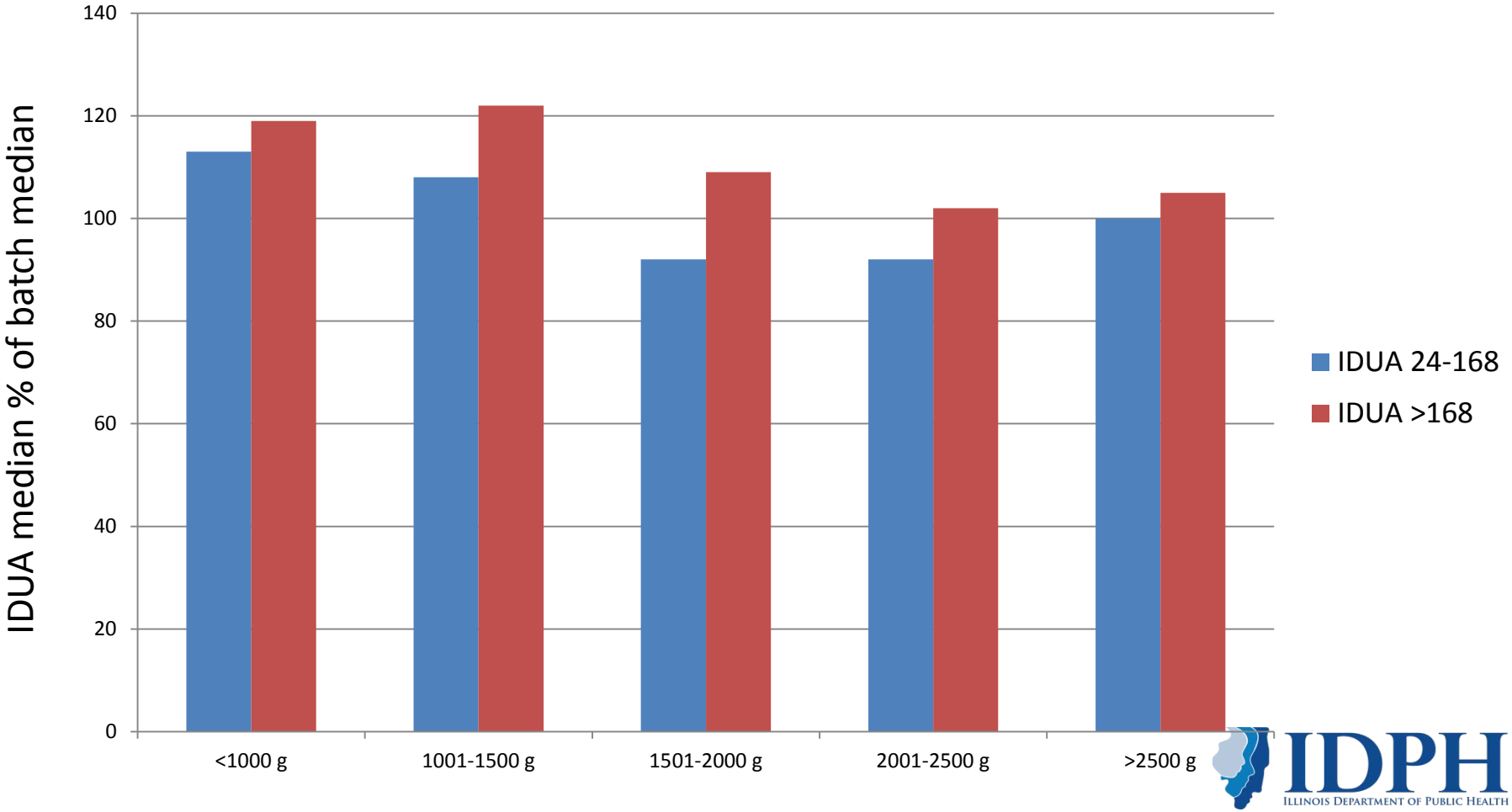
IDUA median activity in different collection time (hour) and birth weight (unit: % of batch median)



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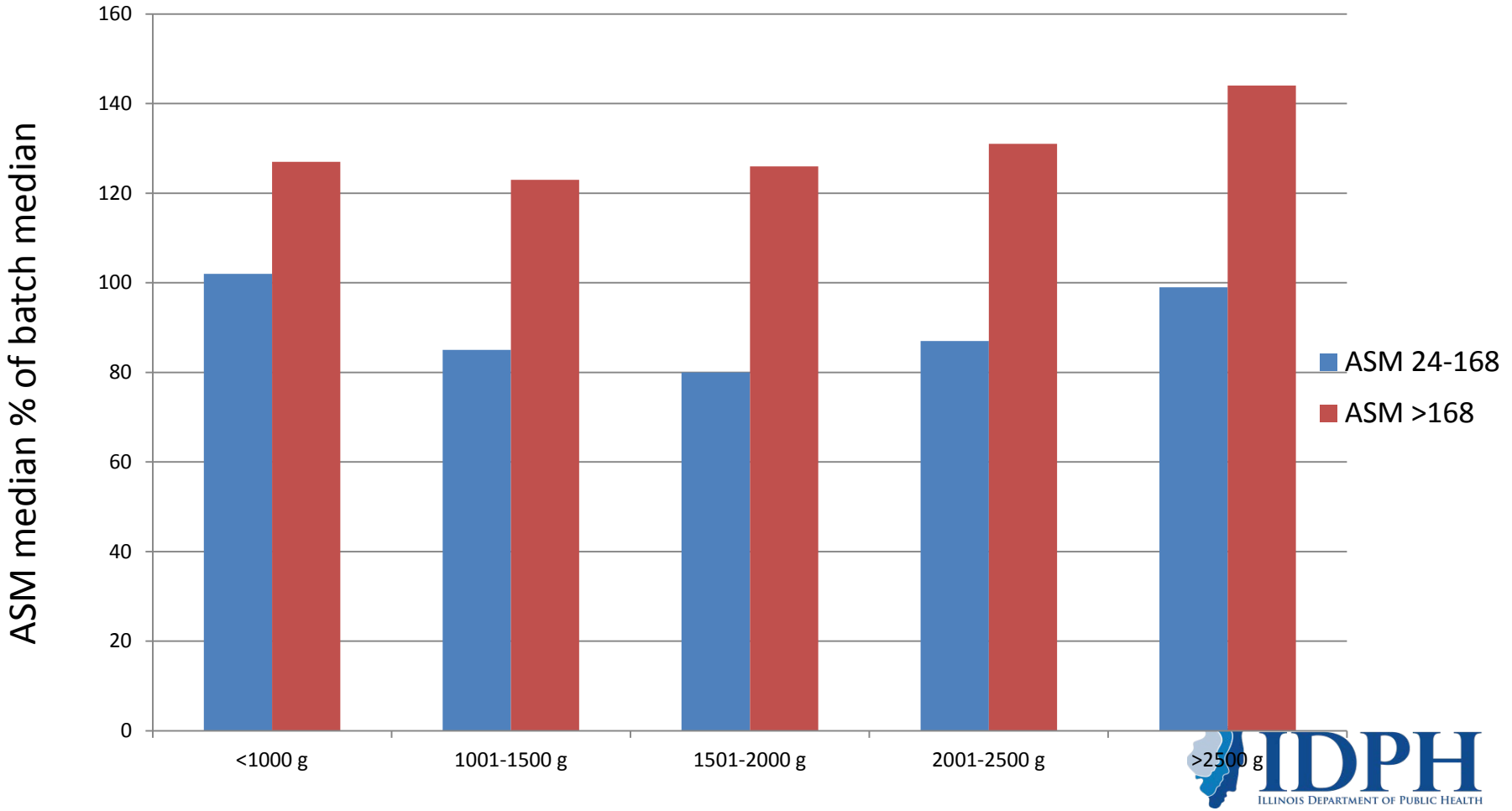
ASM median activity in Different collection time (hour) and birth weight (unit: % of batch median)



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Illinois Department of Public Health NewBorn Screening Program Abnormal Fabry Cases as of 11/03/14 through 1/15/2016

Pilot:	**Nov 3, 2014-May 31, 2015**		**15,154**				
Statewide:	**Jun 1, 2015-Jan 15, 2016**		**113,722**				
	Total		**128,876**				
Fabry	**Gestational Age**	**NICU**	**NonNICU**	**Total**	**Classical**	**Late Onset**	**PhenoUND**
	< 32weeks	**0**	**0**	**0**			
	32-36 weeks	**1**	**0**	**1**			
Borderline	**>37 weeks**	**7**	**25**	**32**		**3**	
	< 32weeks	**0**	**0**	**0**			
	32-36 weeks	**0**	**2**	**2**			
Positive	**>37 weeks**	**4**	**15**	**19**		**1**	
	Total	**12**	**42**	**54**	**0**	**4**	**1**

CONCLUSIONS

- GLA results increased with decreasing birth weight and were significantly increased in infants with VLBW compared with infants who weighed >2500 g.
- GLA (α- galactosidase) activity levels are disproportionately increased in VLBW infants. GLA levels fall after 1 weeks postnatal age and may only then reflect true activities of the enzyme. The data suggest that a request for resubmission of specimens from birth weight <2000 g should be applied to this subgroup.

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Acknowledgments

IDPH lab

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IDPH short-term follow up

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* Knoxville Regional Laboratory Director, Tennessee Department of Health

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THANK YOU

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