Supplemental Data

Assay solution recipes and storage conditions:

Quadruplex Buffer (0.2 M sodium phosphate + 0.10 M citrate, pH 4.4):
6.9 g sodium phosphate monobasic monohydrate
7.35 g sodium citrate tribasic dihydrate
Dissolve in ~200 mL deionized water
Adjust pH to 4.40 (± 0.05) using 6N HCL
Fill to 250 mL with deionized water
Store @ 2-8°C for up to 6 months

ASM Buffer (0.840 M sodium acetate + 0.604 mM zinc chloride, pH 5.7):
17.14 g sodium acetate
Dissolve in ~200 mL deionized water
1.34 mL glacial acetic acid
1.51 mL 0.1 mol/L zinc chloride
Adjust pH to 5.7 using sodium hydroxide solution
Fill to 250 mL with deionized water
Store @ 2-8°C for up to 6 months

GAA Inhibitor (0.8 mM acarbose in water):
12.9 mg acarbose
Dissolve in ~20 mL
Fill to 25 mL with deionized water
Prepare on day of use, shelf life has not yet been determined.

Detergent (96 g/L sodium taurocholate in water):
9.6 g sodium taurocholate
Dissolve in 100 mL deionized water
Store @ ≤ -20°C for up to 1 year

Quadruplex Solution (ABG, GAA, GALC, GLA):
Using 1 vial each of ABG, GAA,GALC and GLA S/IS
Transfer contents of ABG, and GAA vials into GALC vial using MeOH
Remove MeOH by evaporation
Transfer contents of GLA vial into GALC/ABG/GAA vial using 1.8 mL detergent
Vortex until solids are completely dissolved. Heat in water bath if necessary.
Add 0.3 mL GAA inhibitor
Add 15.9 mL Quadruplex Buffer
Vortex to homogenize.
Store @ ≤ -20°C for up to 1 month

ASM Solution:
Using 1 vial of ASM S/IS
Add 0.15 mL detergent
Add 17.85 mL ASM Buffer
Vortex to homogenize
Store @ ≤ -20°C for up to 1 month

Quadruplex and ASM buffer were stored in glass Wheaton bottles.

Detergent and final assay solutions were stored in polypropylene screw cap tubes.

List of Reagents and Materials:

Sodium Phosphate Monobasic Monohydrate (Sigma-Aldrich #S9638, USA)
Sodium Citrate Tribasic Dihydrate (Sigma-Aldrich #S4641, USA)
Hydrochloric Acid (Mallinckrodt Chemicals #H613-45, NJ, USA)
Sodium Acetate (Sigma-Aldrich #241245, USA)
Zinc Chloride (Sigma #39059, USA)
Sodium Hydroxide (Krackeler Scientific #11-3722-04, NY, USA)
Taurocholic Acid Sodium Salt Hydrate (Sigma #T4009, USA)
Acarbose (Toronto Research Chemicals # A123500, Canada)
Methanol (JT Baker, NJ, USA)
Ethyl Acetate (JT Baker, NJ, USA)
Water (Barnstead Filtration System, Nanopure Diamond Filter # OC-ZL502X366, Iowa, USA)
Silica Gel (Sigma-Aldrich #227196, 230-400 mesh, 60A, Merck, Grade 9385, USA)
Ammonium Formate (Sigma-Aldrich #516961, USA)

Wallac DBS puncher (Perkin Elmer, MA, USA)
V-Bottom Plates (Costar V-Bottom #3363, NY, USA)
Plate Storage Mats (Costar, NY, USA)
Centrifuge (Eppendorf, Centrifuge 5810)
Plate Shaker (Barnstead Titer Plate Shaker #4625, Iowa, USA)
Deep-well Plates (Costar, Square V-Bottom #3961, NY, USA)
Filter Plates (E & K Scientific #2202, CA, USA)
Plate Dryer (Caliper Life Sciences, MA, USA)
Flat-Bottom Plates (Costar, Flat Bottom #3364, NY, USA)
Tip Boxes (Axygen, 250 uL pre-sterilized tips, #FX-250-LRS, CA, USA)
Aluminum Foil (Krackeler Scientific #L610282, NY, USA)
12 Channel Pipette (Biohit Proline 25-250 µL)
Biomek® NX Laboratory Workstation (Beckman Coulter, USA)
Cytomat Hotel (Kendro, Germany)

ESI-MS/MS instrument settings:

Analyte	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
GALC-IS	m/z 454.30 → m/z 264.25	15	20
GALC-P	m/z 426.30 → m/z 264.25	15	20
GALC-S	m/z 588.40 → m/z 264.25	15	20
GAA-IS	m/z 503.25 → m/z 403.28	15	20
GAA-P	m/z 498.25 → m/z 398.25	15	20
GAA-S	m/z 660.30 → m/z 560.25	15	20
GLA-IS	m/z 489.25 → m/z 389.25	25	20
GLA-P	m/z 484.15 → m/z 384.25	25	20
GLA-S	m/z 646.25 → m/z 546.25	25	20
ABG-IS	m/z 510.45 → m/z 264.28	15	20
ABG-P	m/z 482.40 → m/z 264.28	15	20
ABG-S	m/z 644.45 → m/z 264.28	15	20
ASM-IS	m/z 370.25 → m/z 264.25	15	20
ASM-P	m/z 398.25 → m/z 264.25	15	20
ASM-S	m/z 561.70 → m/z 398.30	15	20

Transitions were conducted with a dwell time of 0.15 s and delay of 0.03 s.

Capillary voltage (V)	3000
Cone (V)	15 (25 GLA)
Extractor (V)	2
RF Lens (V)	0.1
Source Temperature (°C)	90
Desolvation Temperature (°C)	150
Cone Gas Flow (L/hr)	600
Desolvation Gas Flow (L/hr)	45
LM 1 Resolution	15
HM 1 Resolution	15
Ion Energy 1	0.2
MSMS mode Entrance	2
Collision	20
MSMS mode Exit	1
LM 2 Resolution	14
HM 2 Resolution	14
Ion Energy 2	1
Multiplier (V)	750
Collision Cell Pressure (mbar)	6.98 e-4
Collision Gas	Argon

A Waters Micromass® Quattro *micro* ™ API tandem mass spectrometer was used in positive ion mode and data was aquired and anlayzed using MassLynx 4.0 software. Above are the settings used for data aquisition on the MS/MS.

Step	Zhang, et al., 2008 method	Modified & Automated Method	4plex + 1 Method
Punch	2 V-bottoms	2 V-bottoms	2 V-bottoms
Extract	4 V-bottoms	4 V-bottoms	n/a
	70 µL extract buffer	70 µL extract buffer	n/a
Add Assay Solution	5 Solutions	5 Solutions	2 Solutions
Incubate	20 - 24 hr	19 hr	19 hr
Quench	100 µL/plate	100 µL/plate	100 µL/plate
Combine	1 deepwell	1 deepwell	1 deepwell
Liq/Liq	400 µL / 400 µL	400 µL / 400 µL	400 µL / 400 µL
Remove Organic	300 µL	200 µL	150 µL
Dry	-	-	-
Reconstitute	300 µL	200 µL	150 µL
SPE	1600 µL	800 µL	200 µL
Dry	-	-	-
Recon/transfer	200 µL	100 µL	100 µL

Table 1. Summary of modifications to the previously published assay by Zhang et al., 2008.

Analyte	CDC Mean Activity (95% Confidence Limits).		
	Low QC	Med QC	High QC
GALC	0.25 (0.12-0.38)	1.59 (1.11-2.08)	3.47 (3.14 - 3.80)
GAA	1.13 (0.90-1.37)	7.67 (6.29-9.05)	15.76 (12.13-19.40)
GLA	0.56 (0.31-0.80)	3.87 (3.11-4.64)	8.31 (6.90 - 9.72)
ABG	0.99 (0.52-1.46)	5.83 (4.42-7.23)	12.59 (10.37-14.81)
ASM	0.28 (0.00-0.60)	1.30 (0.92-1.67)	2.54 (2.02 - 3.07)
IDUA	0.45 (0.00-1.05)	5.31 (3.84-6.79)	11.46 (9.15 -13.76)

Table 2: LSD QC analytical information as received from the Centers for Disease Control and Prevention. Values were obtained using the previously published MS/MS method by Zhang, et al. 2008 [6 and 14]. Units are expressed as µmol/L/hr.

Blank	Uncorrected Blank Activity (µmol/L/hr)				
	GALC	GAA	GLA	ABG	ASM
Average	0.03	0.06	0.12	0.13	0.10
Std Dev	0.01	0.06	0.07	0.05	0.05
CV	34.61	88.43	59.70	37.40	47.00
N	174	174	174	174	174
Min	0.00	0.03	0.06	0.06	0.03
Max	0.09	0.65	0.87	0.5	0.43

Table 3A

Low CDC	Activity (CDC Certified Value)				
	GALC	GAA	GLA	ABG	ASM
Average	0.21 (0.25)	1.13 (1.13)	1.51 (0.56)	0.92 (0.99)	0.74 (0.28)
Std Dev	0.09	0.17	0.19	0.23	0.38
CV	42.71	15.22	12.48	25.11	51.40
N	118	118	118	118	118
Min	0.09	0.72	1.22	0.5	0.44
Max	1.01	2.07	2.63	1.68	4.16

Table 3B

Med CDC	Activity (CDC Certified Value)				
	GALC	GAA	GLA	ABG	ASM
Average	1.31 (1.59)	6.29 (7.67)	9.53 (3.87)	3.90 (5.83)	4.32 (1.30)
Std Dev	0.27	0.98	1.11	1.22	0.87
CV	20.68	15.56	11.67	31.40	20.04
N	118	118	118	118	118
Min	0.90	3.83	7.72	1.31	2.24
Max	2.28	8.83	12.48	7.11	7.54

Table 3C

High CDC	Activity (CDC Certified Value)				
	GALC	GAA	GLA	ABG	ASM
Average	2.30 (3.47)	12.22 (15.76)	18.81 (8.31)	7.46 (12.59)	8.94 (2.54)
Std Dev	0.25	1.26	1.73	1.71	1.51
CV	10.77	10.34	9.19	22.98	16.92
N	118	118	118	118	118
Min	1.70	8.92	15.07	3.97	5.42
Max	3.16	16.42	23.16	12.02	13.23

Table 3D

Tables 3A-3D: A summary of QC results broken down by specimen type. Note that in Table 3A values show uncorrected (not blank subtracted) activity (µmol/L/hr) while in Tables 3B-D values are expressed as blank subtracted activities. CDC certified values were incorporated from Table 3 for ease of comparison.

DBS extraction/distribution experiment details: One plate of specimens consisting of 87 de-identified newborns and three of each control specimen (blanks, low, high LSD QC) was punched in triplicate. One plate (Plate 3, Table 4 of publication) had DBS extract distributed to individual assay plates in alphabetical order (ABG, GAA, GLA) while the other two plates (Plates 1 and 2, Table 4) used reverse alphabetical order (GLA, GAA, ABG) to distribute extract. Sample processing then proceeded in accordance with our modified version of the previously published method from Zhang and coworkers.