Pompe Disease 101

Clinical Aspects and Screening Methods

Part 2: Pompe Disease: Newborn Screening Methodology
February 26th, 2014

Pompe Disease 101: Agenda

- **Welcome and Introduction**
  Patricia R. Hunt
  Member, APHL Subcommittee
  Manager, Texas Newborn Metabolic Screening Group
- **Overview of Available Screening Methods for Pompe Disease**
  Dr. Dietrich Matern, MD, PhD
  Co-director, Mayo Clinic Biochemical Genetics Laboratory, Rochester, MN
- **The Missouri Experience**
  Patrick V. Hopkins
  Chair, APHL NBS Quality Assurance/Quality Control Subcommittee
  Chief, Newborn Screening Unit, Missouri State Public Health Laboratory

Pompe Disease 101: Agenda

- **The New York Experience**
  Dr. Joseph Orsini, PhD
  Research Scientist, New York State Department of Health-Wadsworth
  Newborn Screening Program Manager, Wadsworth Center
- **The Illinois Experience**
  Dr. George J. Dizikes, PhD, HCLD/CC(ABB)
  Chief of the Illinois Department of Public Health Newborn Screening Laboratory
- **Proficiency Testing Materials for Pompe Disease**
  Dr. Joanne V. Mei, PhD
  Dr. Hui Zhou, PhD
  Newborn Screening Quality Assurance Program
  Centers for Disease Control and Prevention

Pompe Disease 101: Agenda

- **Q&A and Closing Remarks**
  Patricia R. Hunt
- **Closing Remarks**
  Jelili Ojodu, MPH
  Director, Newborn Screening and Genetics
  Association of Public Health Laboratories

Newborn Screening for Pompe Disease in Missouri

Patrick V. Hopkins
Chief, Missouri NBS Laboratory

February 26th, 2014

Utilizing Digital Microfluidics

- Received a legislative mandate for LSD screening.
- Missouri annual birthrate is around 78,000 (91,000 samples/yr with repeat screens).
- Chose this method due to cost, space and time constraints.
- Currently conducting a 4-plex assay: Pompe, Gaucher, Fabry and MPS-I (NY testing Krabbe for us).
- Conducted validations, and pre-pilot in 2012.
- Started Full Population Pilot Screening January 11, 2013 after full IRB review and approval.

2 Work Stations
8 Digital Microfluidics Platforms

Open Platform

48 Well Sample Cartridge

Enzyme Reaction in DMF Method

SUBSTRATE + Enzyme ⟶ PRODUCT

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

Workflow for Testing

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.

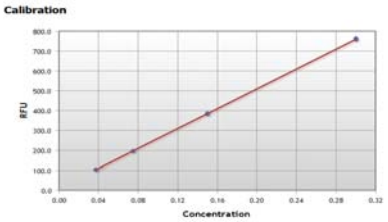
Machine run time

(2.5 h for 4-plex assay)

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

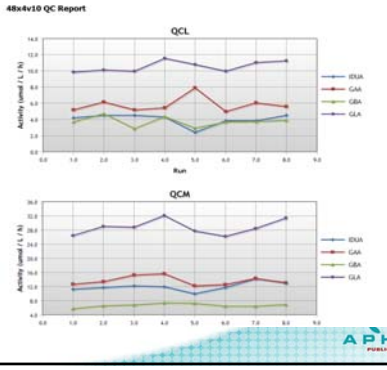
- 2 scientists currently working full time in LSD section
- 48 sample wells assayed per instrument
  - 10 controls (2 blanks, 4 calibrators, 2 low controls and 2 medium controls)
  - 38 patient samples
- Sample punch to enzymatic activity results in ~4 hours

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



Results Screen

Results

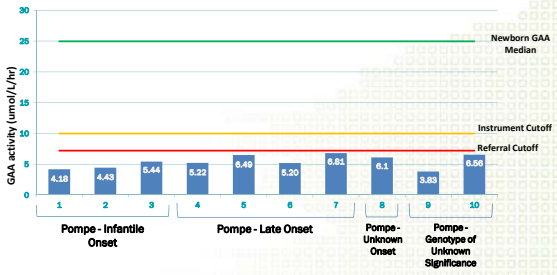
Sample	Location	IDUA (pmol / L / h)	GAA (pmol / L / h)	GBA (pmol / L / h)	GLA (pmol / L / h)
		For research use only. Not for use in diagnostic procedures.			
Sample Mean		22.77	23.92	20.80	24.74
CDCBF281	A02	3.51	1.60	2.39	3.87
CDCL282	A03	5.13	3.64	2.72	5.49
CDCM283	A04	24.16	22.73	16.18	37.34
CDCQ284	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	26.62	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

10 Confirmed Pompe Genotypes in First Year



First Year's Findings

- We are very pleased with the DMF methodology.
- 33 Positive Pompe were referred in Year 1.
  - 10 Pompe genotypes
  - 6 Pompe Psuedo-deficiencies
  - 6 Pompe Carriers
  - 8 False Positives
  - 3 Pending
- Positive Predictive Value = 30%
- False Positive Rate = 0.026 %
- Detection rates so far:
  - Infantile Onset = 1:26,000
  - Late Onset = 1:19,500

Other Important 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. May need more repeat screens.
- Multiplexing with other enzyme assays greatly helps asses quality of sample and risk for referral.
- Some seasonal variation is observed with enzyme activities, similar to GALT assay in that more carriers and psuedo-deficiencies can be detected during higher heat and humidity months.

Detected on Second Day of Pilot

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

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

Acknowledgements

- 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.

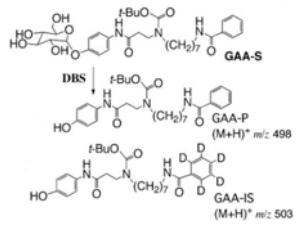
Newborn Screening for Pompe Disease in New York State

Joseph J. Orsini, PhD
February 26th, 2014

Varied Method Population Studies: NY

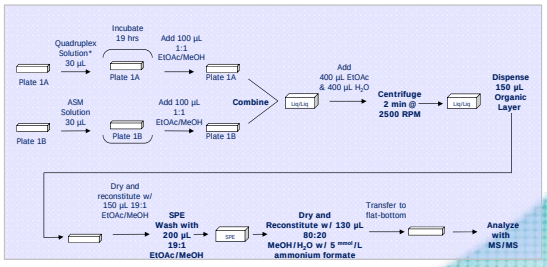
1. NY is using a multiplexed tandem mass spectrometry assay (based on Michael Gelb assay).
2. Why MS/MS? Only method that was available at the time we started screening for Krabbe disease.
3. We modified the Gelb assay so can screen for 6 LSD's using two 3-mm dried blood spot punches. Can screen for Pompe, Krabbe, Fabry, Niemann-Pick A/B, and MPS-1/X-ALD (Dr. Dieter Matern from Mayo added these).
4. Currently performing a consented Pilot study with Dr. Melissa Wasserstein to screen for Pompe, Fabry, Gaucher, and Niemann Pick A/B.
5. Methods have been validated/approved using NYS Clinical Laboratory Evaluation Program guidelines.
   - requires inter/intra day precision, accuracy, linearity, possible interference studies, as well as reporting methods.

Basic Principal of Method



Li/Gelb/Scott et al. 2004

NYS modified 5-plex method



*Quadruplex solution, time consuming to make. Easy if just doing single assay. Each Substrate/Internal standard pair added separately.

New York State Assay

Punch 3-mm specimen, add assay solution reagent and incubate

⇩ 19 hours

Quench reaction (50/50 MeOH/EtAc), perform Liquid / liquid extraction (EtAc/H20), remove organic phase

⇩

Dry plates (10 min)

⇩

Reconstitute extract in 19:1 EtOAc/MeOH, perform SPE (unnecessary for Pompe only)

⇩

Dry plates (30 min)

⇩

Re-dissolve in MS suitable solvent (80/20 MeOH/H20)

⇩

Analyze samples, 1.5 minutes per sample

⇩

Calculate activity/sample, daily mean activity, *% of daily mean act/sample*

Population Studies/Statistics

	Activity		
Stats	4+1 Pop Study	4+1 Pilot	ALD/Triplex
Mean	16.7	15.2	15.4
Median	15.9	14.3	14.5
Std Dev	6.5	6.0	6.3
Min	1.5	1.6	1.0
Max	109.13	70.50	65.80
N	5403	5720	3306
< 20% PMA	6	6	4
< 15% PMA	3	3*	3

* 3 referrals/psuedo-deficiency gene

Positive Controls	Activities			Percent of population mean		
GAA(+)9167636-adult	0.81			4.8		
GAA(+)P14 04-002	0.5			3.0		
GAA(+)66948	0.21			1.3		
GAA(+)MO-12		0.18			1.2	
GAA(+)MO-13		0.27			1.8	
GAA(+)MO-21		0.18			1.2	
GAA(+)120121583_7		0.19	0.23		1.3	1.5
GAA(+)120382519_7		0.38	0.2		2.5	1.3

NYS General Population and Known Positive controls: Comparison to other newborn screening program results

	Disease	Population	Population Mean (umol/L/h)	Std Deviation	Min	Max	N	Median	% of mean*
NYS (Zhang)	Pompe	newborns	16.5	7.40	0.03	144	6750	15.30	3.8**
		+ controls	0.30	0.23	0.01	0.62	8	0.29	
Austria Dajnoki	Pompe	newborns	14.6	7.20	N/A	N/A	11279	13.50	13.6
		Diseased				1.99	29	N/A	
Taiwan Chien	Pompe	newborns	16.5	N/A	1.78	40.76	132538	N/A	13.9
		Diseased				2.3	4		

Many Things to Consider

1. Pompe only, or other LSDs in future, what disorders may be recommended next (MPS-I, Fabry, ALD ?)
2. Budget/Space
3. Kits or homebrew
4. Staff capabilities
5. Krabbe NP-A/B: currently only can be done by MS/MS
6. Not so obvious considerations:
   a. Planning to run ALD?
   b. Do you use derivatized or underivatized kits for AA/AC
   c. Succinyl acetone for Tyrosinemia type 1

Conclusions

1. Validations for the MS/MS methods have gone smoothly
2. If had to do this again, would do mostly the same. MS/MS is still the only available method to screen for Krabbe disease.
3. Multiplexed enzyme assays with currently provide materials is complicated as each Substrate/Internal Standard pair is provided for each disease. Makes validations complicated due to "moving target" of diseases that may be screened.

Acknowledgements

- Monica Martin, Amanda Showers (New York State Newborn Screening/Pilot LSD Testing).
- Dr. Melissa Wasserstein, Nicole Kelly and the Mt. Sinai School of Medicine team.
- Dr. Robert Vogt, Dr. Hui Zhou, and the CDC LSD quality assurance support group.
- Dr. Dietrich Matern and Coleman Turgeon (Rochester, MN, Mayo).
- Dr. Micheal Gelb and the University of Washington support team.

Screening for Pompe Disease and other Lysosomal Storage Disorders (LSDs) – Illinois Experience

George J. Dizikes, Ph.D.
Section Chief, Newborn Screening Laboratory
Illinois Department of Public Health (IDPH)

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Acknowledgments

IDPH
- Khaja Basheeruddin, Ph.D. – Unit Supervisor
- Rong Shao, M.D. – Laboratory Research Scientist
- Fran Balster – Clinical Laboratory Technologist
- Pearlie Gardley – Clinical Laboratory Technologist
- Tamara Simulick – Clinical Laboratory Technologist

Others
- Barbara K. Burton, M.D. – Lurie Children's Hospital
- Michael H. Gelb, Ph.D. – University of Washington
- Joseph J. Orsini, Jr., Ph.D. – Wadsworth Center
- CDC Newborn Screening and Molecular Biology Branch
- PerkinElmer Corporation

Pompe Testing Timeline

- 2007– Legislative mandate for five LSDs, including Pompe.
- 2010 – Pilot screening for Pompe and two other LSDs using microfluidic platform.
  - o 8,012 DBS screened
  - o Two had abnormal GAA activities, confirmed negative by second-tier tests

Pompe Testing Timeline (cont'd)

- 2011 – Legislative mandate expanded to seven LSDs, with the following provisions before screening:
  - A method either cleared by the US Food and Drug Administration (FDA) or validated under the Clinical Laboratory Improvement Amendments (CLIA)
  - Availability of quality control and proficiency testing materials
  - Appropriate equipment for high-volume screening
  - Adequate funding

Pompe Testing Timeline (cont'd)

- 2011 – Decision made to switch from microfluidic platform to tandem mass spectrometry (MS/MS).
  - Microfluidic platform did not have substrates for all LSDs.
  - Microfluidic platform lacked throughput for Illinois' volume (~170,000 newborns per year).
  - Recent developments with multiplex MS/MS promised adequate testing throughput for more disorders and with less staff.
- 2014 – Statewide testing expected to begin July 1.

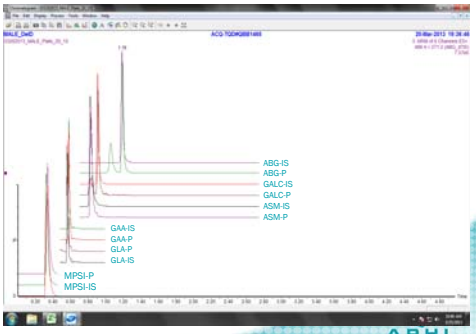
Multiplex LC-MS/MS Assay

- Modification of method developed at the University of Washington for six LSDs: Pompe, Krabbe, Gaucher, Fabry, Niemann-Pick (A/B), Mucopolysaccharidosis type I (MPS I).
  - Single DBS punch
  - Single buffer
  - In-line chromatographic purification (no solid-phase extraction)
- Three-hour incubation (maintains work flow).
- UPLC column separates product/ISTD pairs and removes salt, detergent, & phospholipids by valving.
- 2.5 minute injection cycle, 500 injections/instrument/day, >10,000 injections/PM.

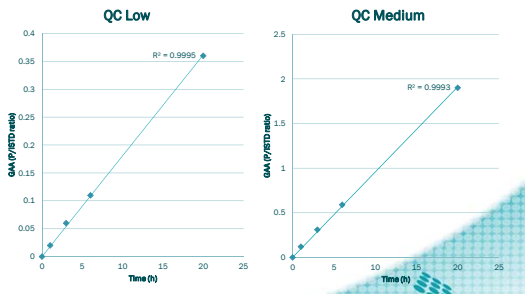
Duffey, et al., *Clin Chem* (56), 2010. Spacil, et al., *Analyt Chem* (83), 2011.

Acquity TQD Instrument

UPLC Chromatogram



Linearity of Assay



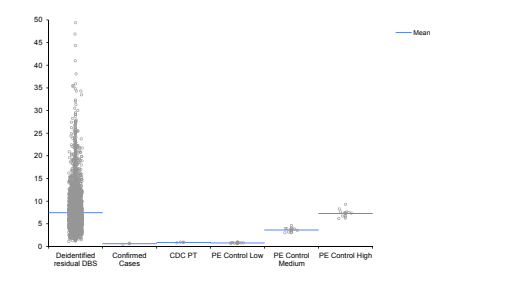
Method Validation

- Evaluate different levels of Quality Control samples (Low, Medium, and High).
- Perform precision studies.
- Perform accuracy studies.
- Participate in the CDC pilot Proficiency Testing (PT) program for Pompe (and Krabbe).
- Obtain DBSs from confirmed cases.

Method Validation (cont'd)

- Test de-identified specimens from male, female, low birth weight, and 7+ day-old babies.
- Study the effects of detergents and DBS storage conditions on LSD enzyme activities.
- Determine cut-off values.
- Exchange specimens with a qualified testing laboratory to establish comparability of results.

Assay Results for de-ID DBSs, Confirmed Pompe cases, PTs, and Quality Controls



Statistical Analysis of Assay Results for DBSs

	n	Mean	95% CI		SE	SD
De-identified residual DBS	10003	7.45	7.37	7.52	0.04	3.64
Confirmed Cases	3	0.58	0.31	0.85	0.06	0.11
CDC PT	4	0.88	0.76	1.00	0.04	0.08
Control Low	16	0.78	0.73	0.83	0.02	0.09
Control Medium	16	3.63	3.40	3.87	0.11	0.44
Control High	15	7.28	6.84	7.73	0.21	0.80

	n	Min	Median	95% CI		Max
De-identified residual DBS	10003	1.12	6.70	6.64	6.76	49.3
Confirmed Cases	3	0.48	0.57	N/A		0.69
CDC PT	4	0.77	0.90	N/A		0.94
Control Low	16	0.65	0.77	0.71	0.87	0.99
Control Medium	16	3.01	3.62	3.19	3.90	4.63
Control High	15	6.16	7.34	6.72	7.60	9.30

Lessons Learned

- Many different individuals with a wide range of skills need to work together to successfully develop a complex, high-throughput analytical assay.
- The process will take longer than initially anticipated; regular interactions and good communications are vital.
- MS/MS platform permits expanded test menu and multiplexing with a single injection.

Lessons Learned (cont'd)

- There are many challenges in adapting a research procedure to a high-throughput newborn screening assay (e.g., analytical, personnel, physical plant, and IT). FDA-cleared tests are vastly preferable.
- If at all possible for mandated testing, have legislation or administrative rules written to permit adequate preparation and milestones (e.g., method validation, QC and PT availability, acquisition of high volume equipment, & funding).

Thank you

George J. Dizikes, Ph.D. george.dizikes@illinois.gov
312-793-4745

Proficiency Testing Materials for Pompe Disease

Hui Zhou, PhD
Newborn Screening Translation Research Initiative
Joanne Mei, PhD
Team Lead, Newborn Screening Quality Assurance Program

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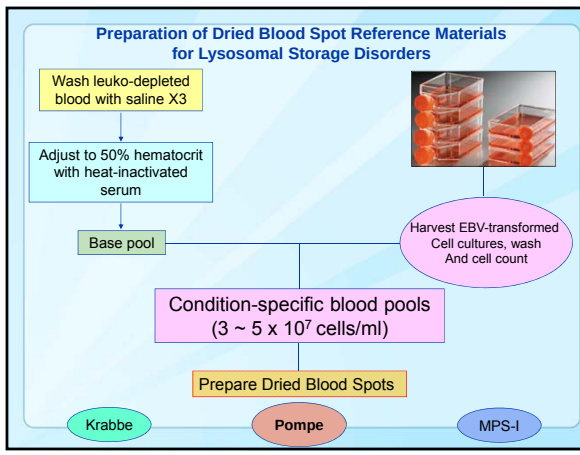
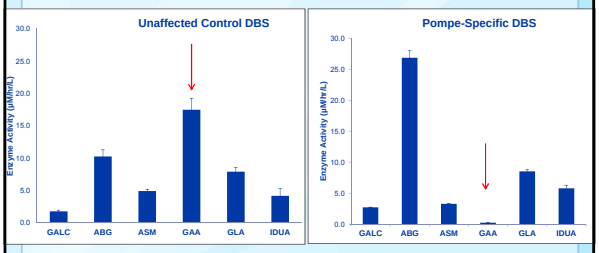
National Center for Environmental Health
Division of Laboratory Sciences

Preparation of Dried Blood Spot Reference Materials for Lysosomal Storage Disorders



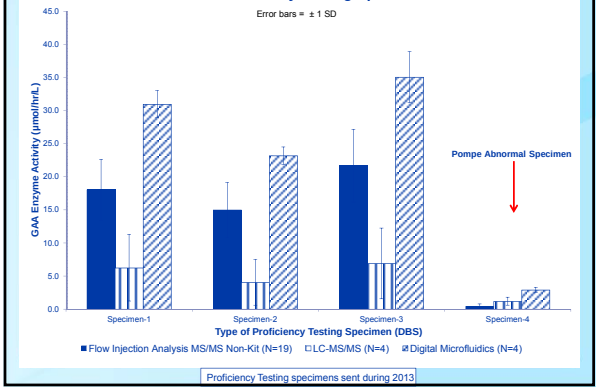
Enzyme Activity in DBS Prepared from EBV-Transformed Cell Cultures



NSQAP Pilot Proficiency Testing For LSD

- Available for Pompe and Krabbe disease
- 4 times per year for US programs
- Information collected:
  - Analytical data
  - Clinical assessments
  - Methods
  - Cutoffs
- One-month data turnaround
- Summary reports issued two weeks after deadline

Performance of Methods that Detect GAA Enzyme Deficiency in DBS Proficiency Testing Specimens



Proficiency Testing specimens sent during 2013

Analytical Summary Data for GAA Activity in Proficiency Testing DBS

Expected Results (GAA Activity µmol/hr/L)	Normal-1		Normal-2		Normal-3		Pompe Abnormal		Cutoff		
	19.25		17.24		24.83		0.23				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	Min	Max
Flow Injection Analysis MS/MS Non-Kit (N=19)	18.04	4.59	14.98	4.14	21.65	5.49	0.40	0.39	2.81	1.50	4.00
LC-MS/MS (N=4)	18.64	9.51	9.56	7.66	13.57	11.43	0.40	0.01	1.62	1.00	2.90
Digital Microfluidics (N=4)	30.98	2.02	23.18	1.31	35.05	3.84	2.91	0.37	7.25	7.00	8.00

Proficiency Testing for Pompe (GAA) in DBS Results for 2013*

Condition	No. Labs Reporting	Positive Specimens Assayed (N)	False Negative Errors (%)	Negative Specimens Assayed (N)	False Positive Errors (%)
Pompe (GAA)	8	27	0	108	0

**Data compiled from 4 quarters, 5 specimens per proficiency testing panel.*

Summary

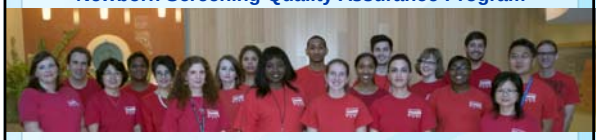
- CDC LSD Reference Materials can be used for assay development and validation for all methods in current use
- EBV-transformed lymphoblast cells derived from LSD patients provide a sustainable resource for condition-specific reference materials
- NSQAP's Pilot PT Program for Pompe (GAA)
  - No false positive results reported
  - No false negative results reported

CDC Contacts
DBS Reference Materials for LSDs

- DBS reference materials, certain reagents, training
  - Dr. Hui Zhou (HZhou2@cdc.gov) or 770.488.4861
- Proficiency testing materials (US only)
  - Dr. Hui Zhou (HZhou2@cdc.gov or 770.488.4861)
  - Dr. Joanne Mei (JMei@cdc.gov or 770.488.7945)
- Proficiency testing instructions and data reporting
  - Ms. Irene Williams (IWilliams1@cdc.gov or 770.488.7024)

Thank You!

Newborn Screening Quality Assurance Program



For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division Name in this space

Pompe Disease 101
Clinical Aspects and Screening Methods

Jelili Ojodu, MPH
Director, Newborn Screening and Genetics

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES

Presenters

Part 1

- Alex R. Kemper, MD, MPH, MS
- Olaf A. Bodamer MD, PhD, FACMG, FAAP
- Neena Champaigne, MD

Part 2

- Dietrich Matern, MD, PhD, FACMG
- Patrick V. Hopkins, BS
- Joseph J. Orsini, PhD
- George J. Dizikes, PhD, HCLD(ABB)
- Joanne Mei, PhD
- Hui Zhou, PhD

APHL NBS QA/QC Subcommittee

- Patrick Hopkins (MO)
- Fizza Gulamali-Majid, PhD (MD)
- Arthur Hagar, PhD (GA)
- Patricia Hunt (TX)
- Santosh Shaunak (WA)
- Neena Champaigne, MD (SC)
- Joseph Orsini, PhD (NY)
- Eleanor Stanley, MT (ASCP) (MI)
- Joanne Mei, PhD (CDC)
- Victor DeJesus, PhD (CDC)
- Ruhiyyih Degeberg, MPH (APHL)

Special Thanks

- Irene Williams, MMSc, MT(ASCP)
  Health Scientist
  Newborn Screening Quality Assurance Program
  Newborn Screening and Molecular Biology Branch
  Centers for Disease Control and Prevention
- Ruhiyyih Degeberg, MPH
  Specialist, Newborn Screening and Genetics Program
  Association of Public Health Laboratories

APHL 2014 Newborn Screening and Genetic Testing Symposium

- October 27-30, 2014
- Anaheim, California
- Theme: *Newborn Screening: Reassessing Business as Usual*