

CDC's Quality Assurance and Harmonization Activities

Normalization of NBS Laboratory MS/MS Biomarker Results and the Development of a New Generation of Proficiency Testing Materials



Kostas Petritis, PhD

Chief, Biochemical Mass Spectrometry Laboratory
Newborn Screening and Molecular Biology Branch

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Services Provided by CDC's Newborn Screening Quality Assurance Program (NSQAP)

The only comprehensive NBS quality assurance program using dried blood spots

- Proficiency testing
- Quality control materials
- Linearity materials
- Filter paper evaluation
- Training and consultation
- NBS translation research



CDC's Newborn Screening Quality Assurance Program

By the Numbers

Annual# bloodspots: \approx 1,000,000

Liters of blood per year: \approx 100

Labs participated (2017): 660

Countries participated (2017): 84

Distribution frequency: Each quarter

Years of operation: 40 years

PT programs: 16

- AC, AA, BIOT, GALT, G6PD, HORM, IRT, CAH, CFDNA, Hb, HIV, LSD, TREC, TOXO, XALD, UDOT.

QC programs: 13

- 17OHP/TGAL, AAAC, GALT, IRT, T4, TSH, XALD, CAH, GAMT, MSUD-PKU, MMA-HCY, HIV, LSD

Number of biochemical analytes: 64

- Excludes Hb phenotypes, CF genotypes etc

**NORMALIZATION OF NBS LABORATORY
MS/MS BIOMARKER RESULTS**

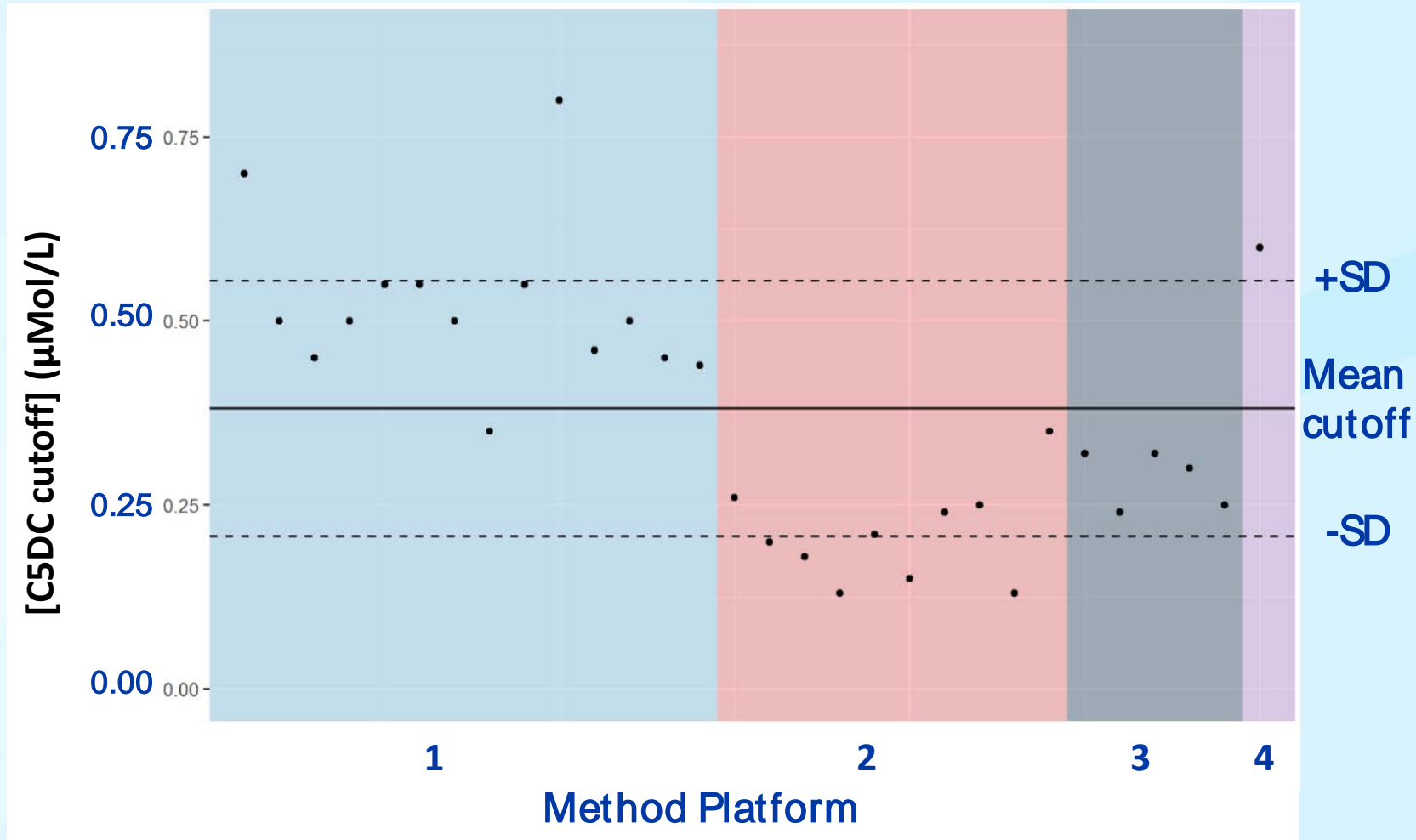
MS/MS Biomarker Measurements and Cutoffs Can Vary Significantly Among Different Labs

>70% (23/32) of RUSP bloodspot disorders can be screened by MS/MS

MS/MS analyte results and cutoff values vary due to:

Major Contributors	<ul style="list-style-type: none">• Extraction methodologies• Derivatized vs. non-derivatized• Few labs account for analyte recovery, most labs do not• Use of additional/different analytes per disorder or second-tier screening
Other Factors	<ul style="list-style-type: none">• Population tested• Instrumentation• Internal standards• Calibration techniques

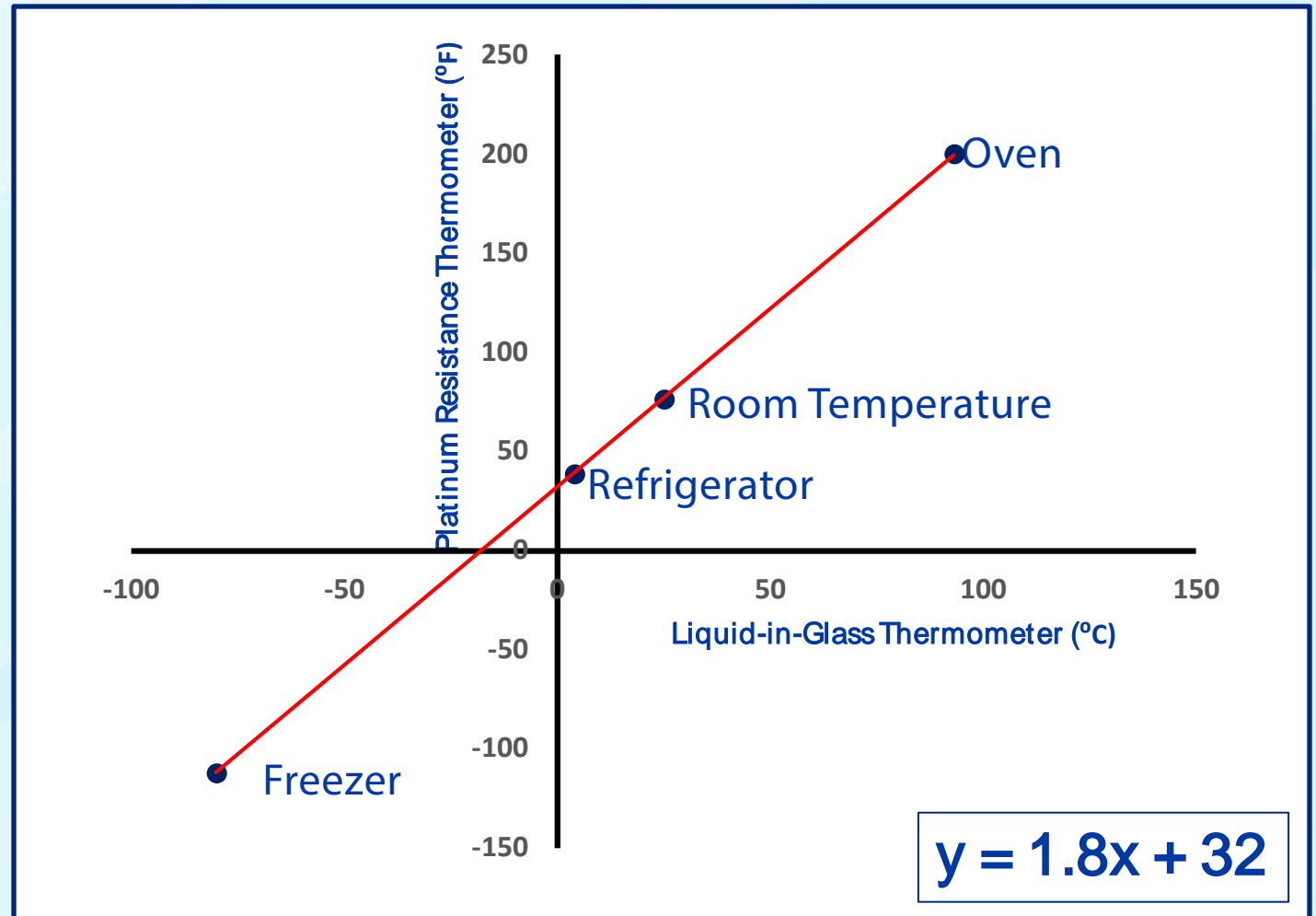
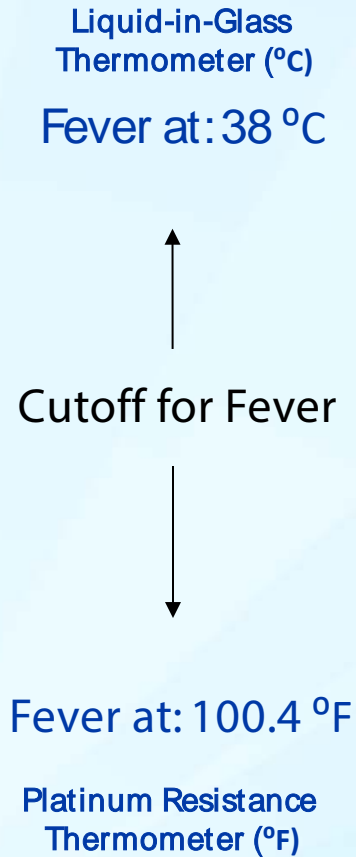
Method-specific Variability in C5DC Cutoffs in U.S. NBS Laboratories



C5DC: Glutaryl carnitine, CV: Coefficient of Variation, SD: Standard Deviation

How normalization works:

Simple analogy: Normalization of thermometer results



How normalization works:

Use of CDC Quality Control (QC) bloodspot materials to normalize mass spectrometry results

Same idea as previous slide but instead of:

- Thermometers ... we use Mass Spectrometers
- Four different temperatures ... we use 4 different concentrations of each biomarker in QC Samples

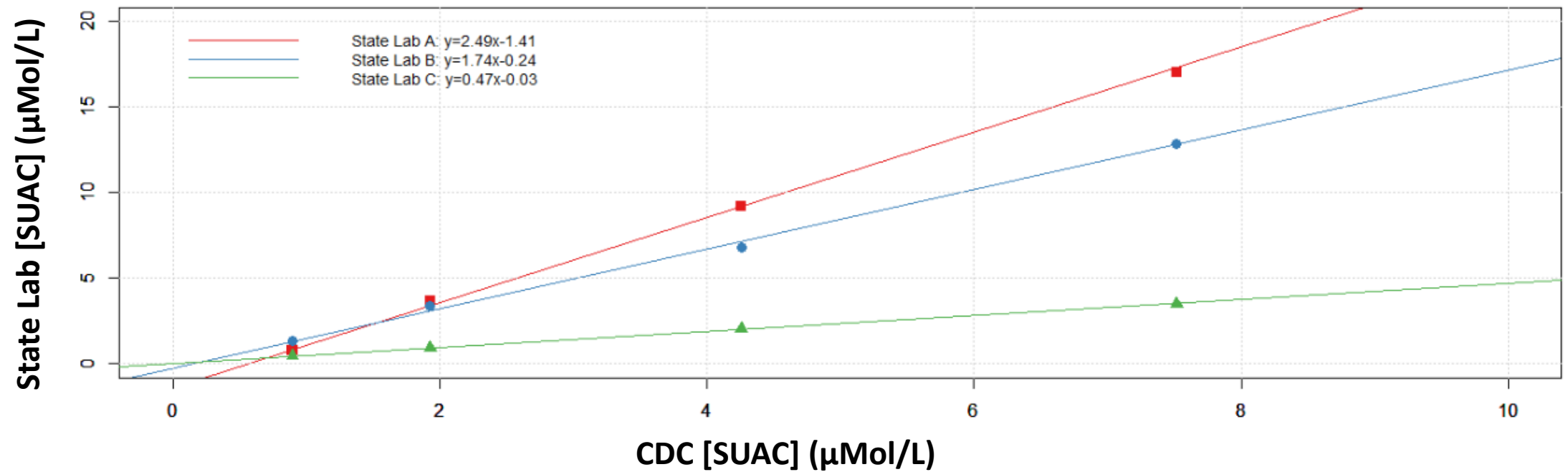
QC Mass Spectrometry Materials for Amino Acids and Acylcarnitines (AAAC)

- 29 biomarkers
- 4 concentration levels
- 5 duplicate MS/MS inter-day runs of each level
- Data reported back to CDC

NBS laboratories could use the QC materials to answer the following questions:

- What is the variability of each instrument within the same day?
- What is the variability of each instrument between days?
- How similar are the results between instruments?

Addressing Succinylacetone (SUAC) Lab-to-Lab Variability by Normalizing Results



- Use QCs to normalize
- Use PTs to validate the normalization worked

Expectation:

- NBS labs receive the same PT specimens
- PT analytical results should be the same

Methods:

- FIA-MS/MS results
- PT specimens are analyzed only once
- QC and PT results from USQ3 2016 event

Addressing SUAC Lab-to-Lab Variability by Normalizing Results

SUAC PT Normalization		
Lab	Raw Value	Normalized Value
State Lab A	62.7	25.7
State Lab B	44.3	25.6
State Lab C	10.7	22.9
CDC	27.3	27.3
CV	62%	7%

Times Difference between
Lab A and Lab C

Normalization
Before → After
5.9 → 1.12

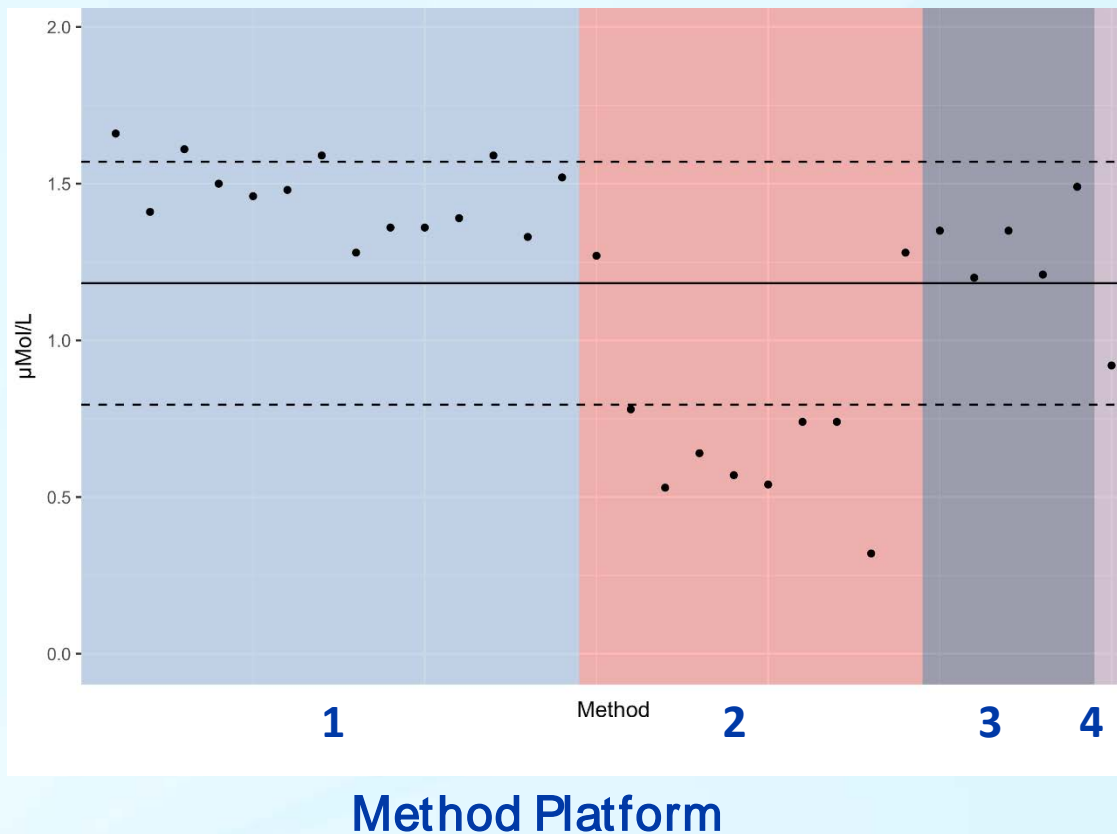
SUAC Cut-off Normalization		
Lab	Raw Value	Normalized Value
State Lab A	5.4	2.7
State Lab B	3.0	1.9
State Lab C	1.0	2.1
CDC	2.2	2.2
CV	64%	15%

Normalization
Before → After
5.4 → 1.28

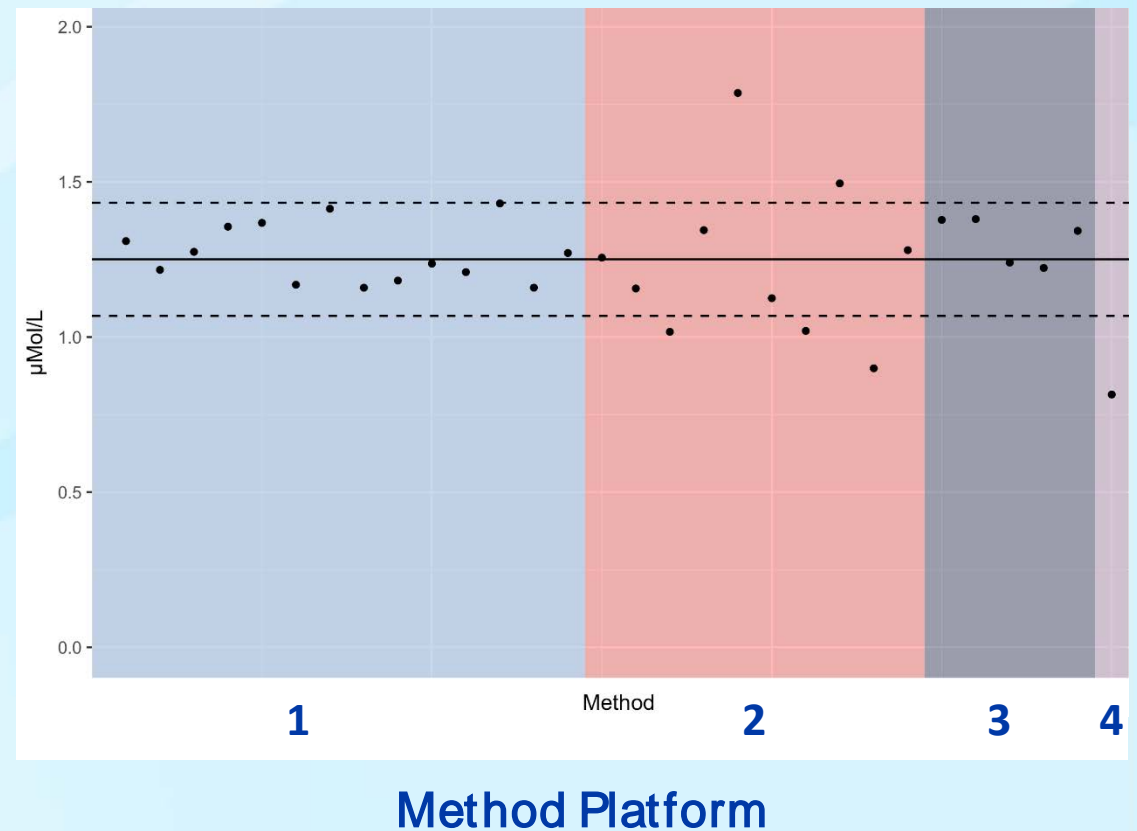
Does Normalization Work?

Orthogonal validation using the PT results

Glutaryl carnitine (C5DC) PT results
Without normalization ... CV: 32.8%



Glutaryl carnitine (C5DC) PT results
After normalization ... CV: 14.6%



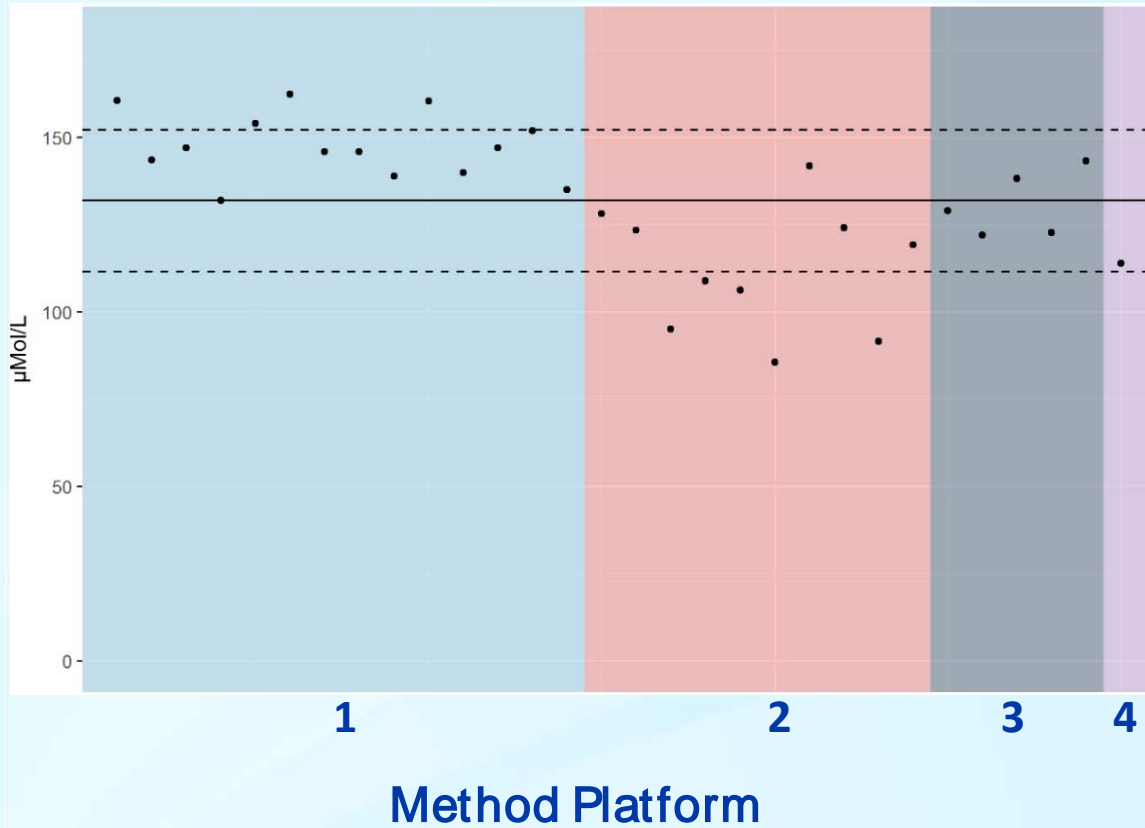
CV: Coefficient of Variation, PT: Proficiency Test

Does Normalization Work?

Orthogonal validation using the PT results

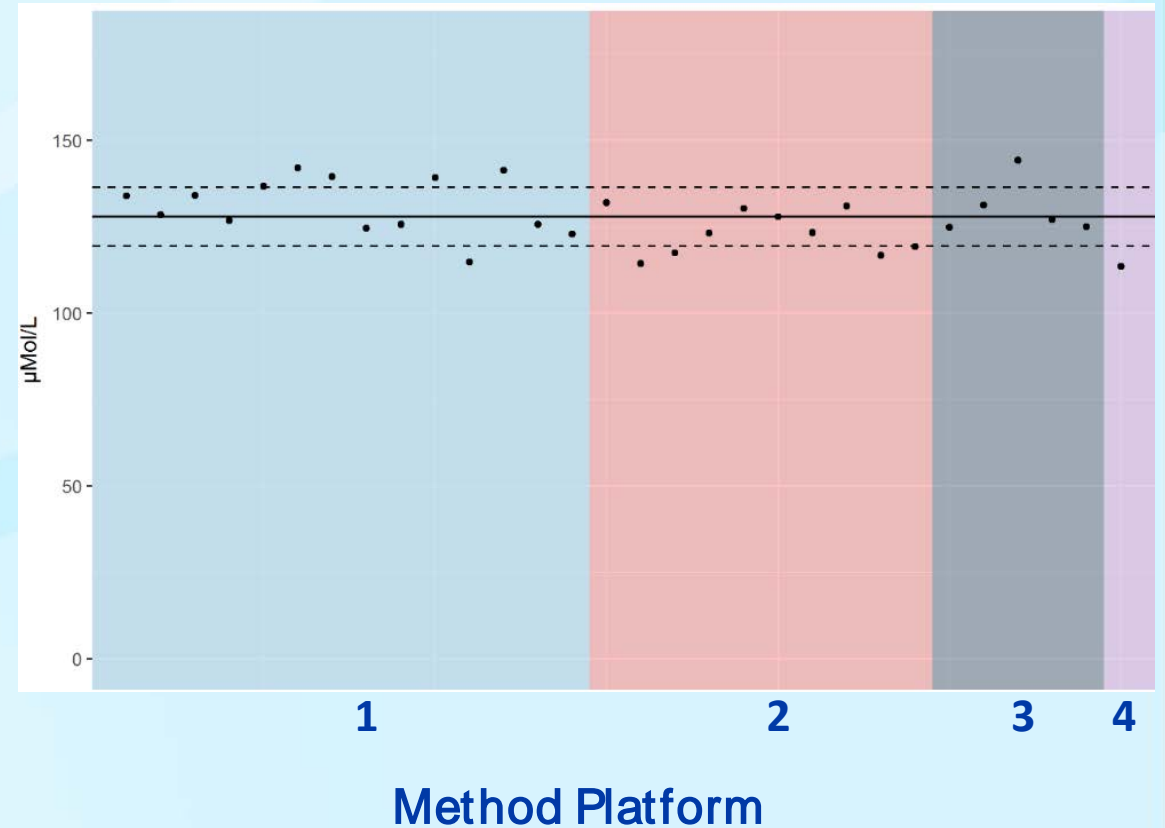
Citrulline (Cit) PT results

Without normalization ... CV: 15.4%



Citrulline (Cit) PT results

After normalization ... CV: 6.6%



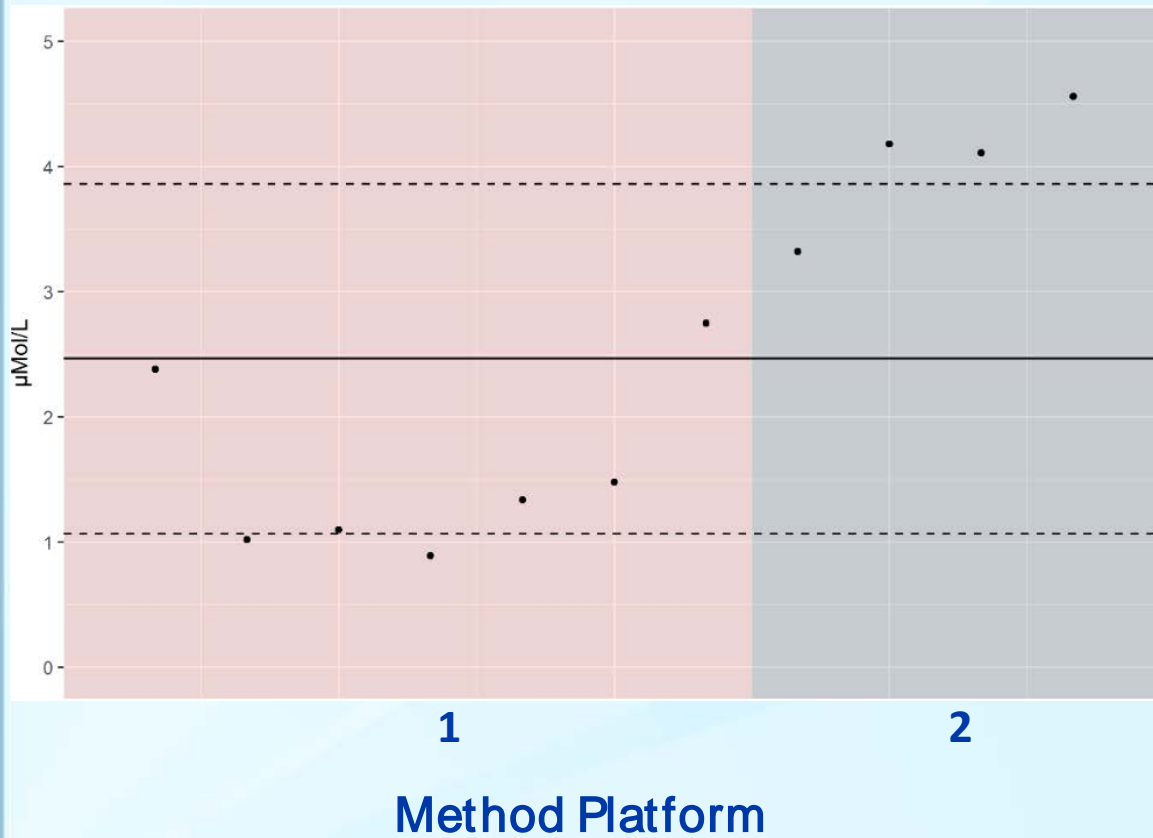
CV: Coefficient of Variation, PT: Proficiency Test

Does Normalization Work?

Orthogonal validation using the PT results

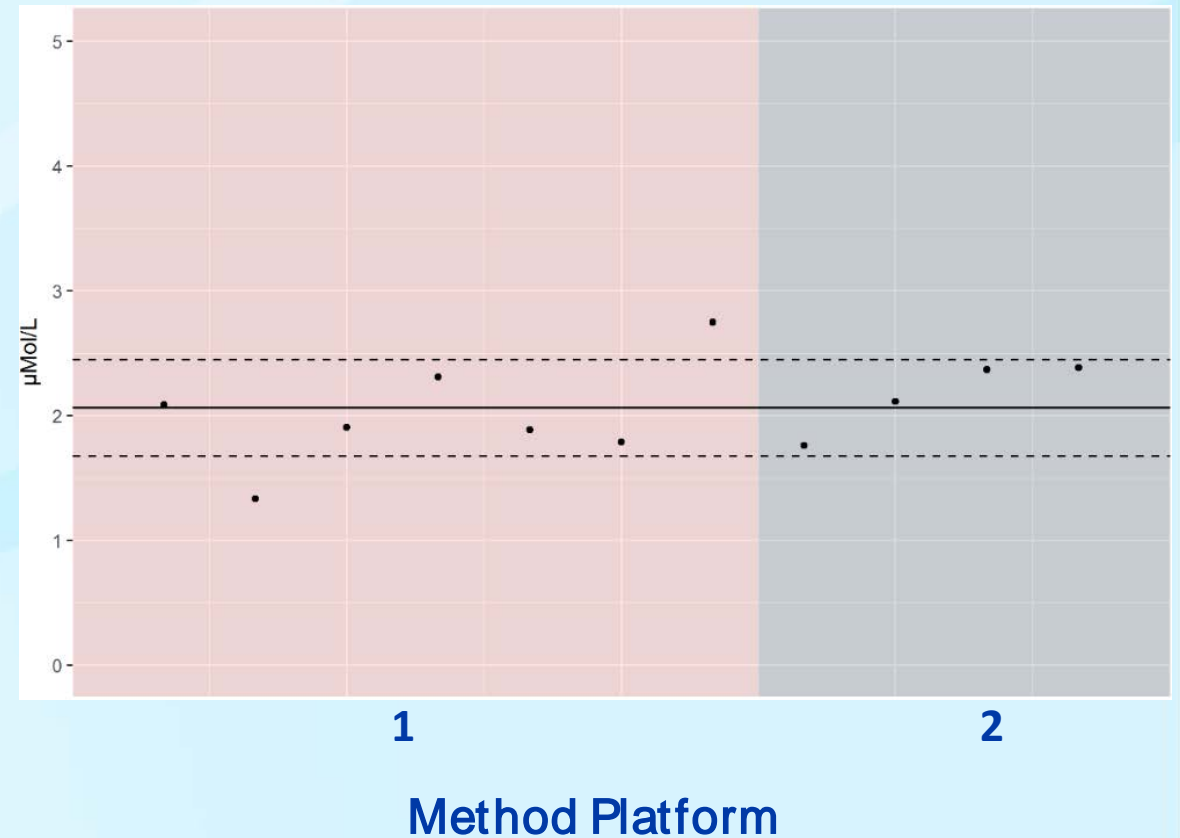
Malonylcarnitine (C3DC) PT results

Without normalization ... CV: 56.7%



Malonylcarnitine (C3DC) PT results

After normalization ... CV: 18.7%



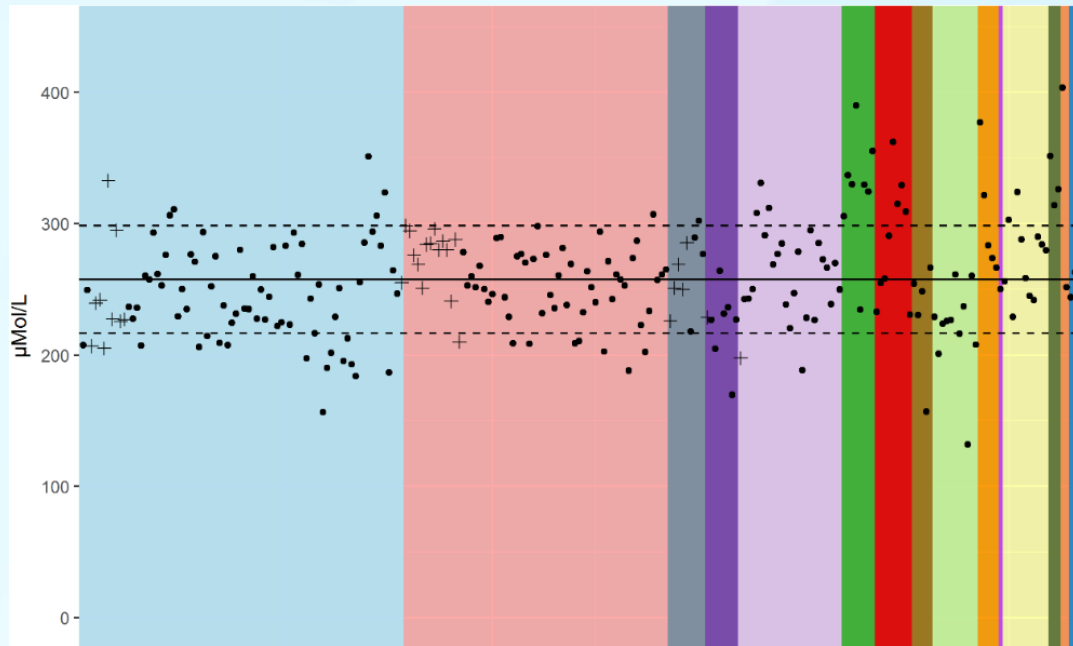
CV: Coefficient of Variation, PT: Proficiency Test

Does Normalization Work?

Orthogonal validation using the PT results

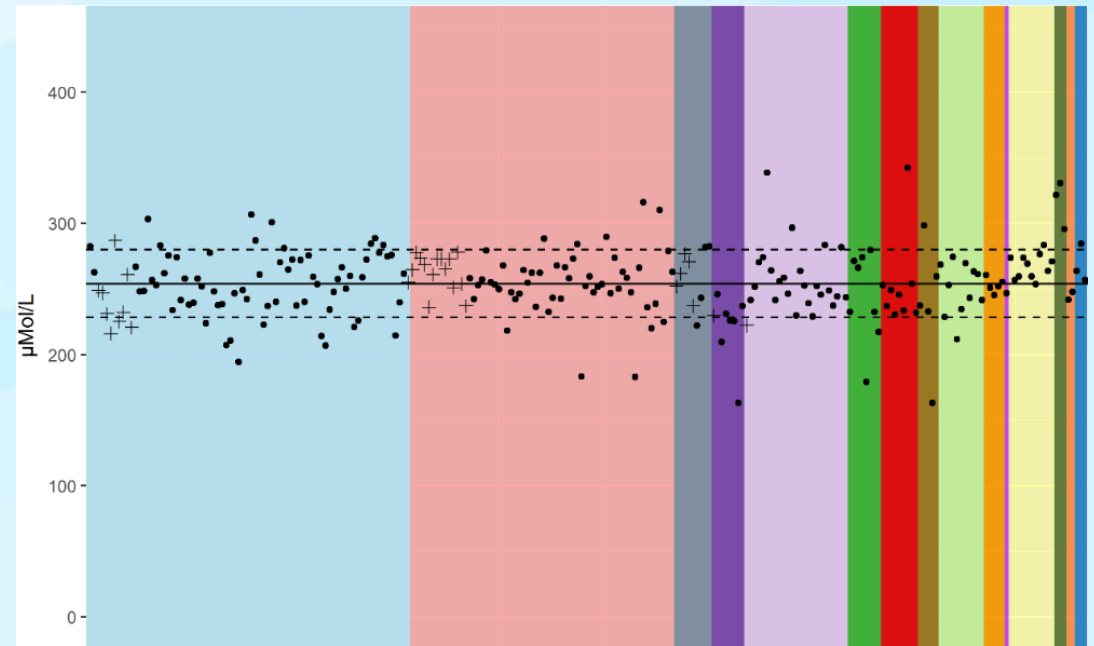
US and International NBS labs
15 different methods (most non-MS/MS)
+: US Labs, ●: International Labs

Phenylalanine PT results
Without normalization ... CV: 20.8%



Method Platform

Phenylalanine PT results
After normalization ... CV: 10.1%



Method Platform

CV: Coefficient of Variation, PT: Proficiency Test

NBS labs LC-MS/MS biomarker CV results before and after normalization

Biomarker	US Labs		US + International labs	
	Raw	Normalized	Raw	Normalized
C3DC	56.7%	18.7%	54.5%	24.3%
SUAC	50.8%	24.5%	148.4%	31.7%
Arg	34.7%	18.2%	37.3%	22.1%
C5DC	32.8%	14.6%	39.5%	19.5%
C16OH	23.1%	14.9%	70.5%	21.4%
Val	19.9%	15.0%	22.9%	12.6%
C5OH	19.8%	16.2%	36.0%	14.8%
C0(L)	19.0%	15.9%	20.5%	13.7%
C5	18.8%	13.2%	17.9%	12.0%
C10	17.7%	12.3%	24.1%	12.9%
Cit	15.4%	6.6%	22.6%	13.0%
C18	15.3%	11.6%	20.2%	16.8%

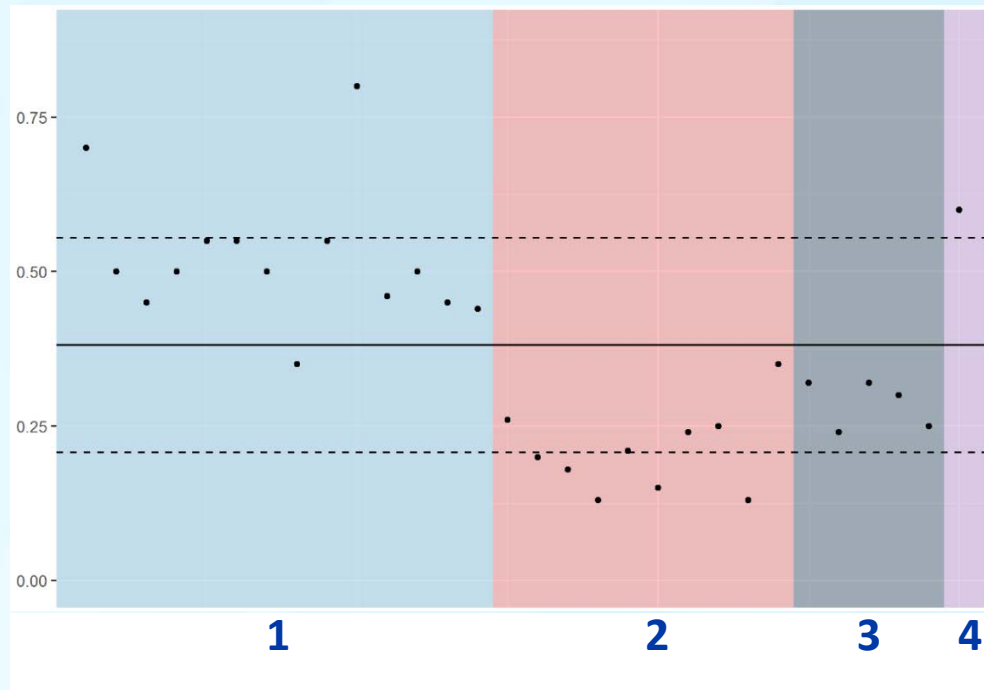
Biomarker	US Labs		US + International labs	
	Raw	Normalized	Raw	Normalized
Met	14.8%	9.5%	22.9%	12.4%
C4	14.3%	10.0%	18.0%	12.0%
C18OH	13.3%	11.7%	49.4%	21.9%
Phe	13.1%	7.9%	20.8%	10.1%
Tyr	12.7%	9.0%	19.0%	15.7%
C8	12.2%	11.9%	19.7%	15.1%
C3	12.1%	9.7%	20.4%	16.6%
C6	10.9%	10.7%	20.9%	15.5%
Leu	10.8%	8.2%	18.2%	10.1%
C14	8.5%	8.2%	19.1%	15.2%
C16	7.2%	7.1%	14.9%	13.3%

Note: The represents data from CDC's NSQAP Proficiency Testing Program in the 3rd Quarter of 2016

Normalization of MS/MS results allows the normalization of cutoffs

Glutaryl carnitine (C5DC) US labs cutoffs

Without normalization ... CV: 45.55%

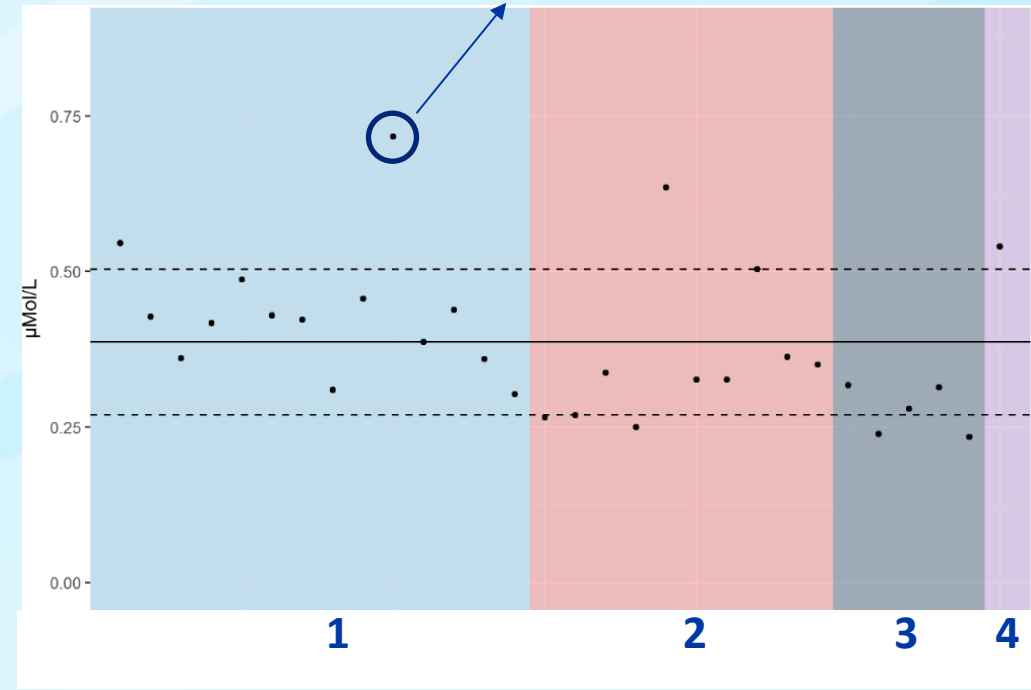


Method Platform

Glutaryl carnitine (C5DC) US labs cutoffs

After normalization ... CV: 30.22%

NBS labs with high biomarker normalized cutoffs could reevaluate them



Method Platform

**DEVELOPMENT OF A NEW GENERATION
OF PROFICIENCY TESTING MATERIALS**

High Accuracy Multi-Analyte Dried Blood Spot Enrichment Method

Breakthrough: Enrichment within 5% of desired concentration

+

Ability to normalize MS/MS data

+

Confirmed cases MS/MS data from NBS labs with quarter + year info



Proficiency testing materials that are “biochemical carbon copies” of babies that were diagnosed with the disorder

New Generation of PT materials

When: July Shipment (Q3-2018 PT event)

What: Proficiency testing materials that are “biochemical carbon copies” of babies that were diagnosed with the disorder for the analytes and ratios of interest

Which ones: Amino acid, Fatty Acid Oxidation and Organic Acid Disorders

Where: From MS/MS data submitted to CDC from US state labs that contained quarter and year of specimen collection information

How: Report as usual, working on updating NSQAP website

Interpretive algorithms: Q3 2018 PTs should work with any workflow, including reflex to biochemical second-tier screening

Looking forward to feedback from NBS labs!

Future Directions

- ❑ CDC will continue to improve normalization and visualization of the results and will expand the number of analytes in QC materials
- ❑ High accuracy multi-analyte bloodspot enrichment will allow the creation of borderline materials for educational purposes
- ❑ CDC will be creating reference materials for MS/MS kits to use for difference applications, including:
 - Changes of instrumentation, method, kit lots
 - Abnormal and borderline specimens to assess cut-offs
 - Linearity materials for method performance
 - Provide assistance for method development, validation
- ❑ CDC is redesigning the data reporting website to improve QC and PT data submission and to accommodate expanded programs

Conclusions

- ❑ Based on preliminary results: It seems possible to normalize MS/MS analyte results by using the CDC's QC materials
- ❑ The CV of all PT analytes improved after normalization
- ❑ CDC will be reporting de-identified normalized cutoffs to NBS laboratories to help them compare their cutoffs to their peers
- ❑ CDC has begun the development of new PT and borderline materials that more closely mimic the pattern and concentrations of biochemical analytes as screened in babies diagnosed with the disease
- ❑ CDC is developing a repository of artificial blood spot specimens to be used as kits for verification/validation or program evaluation. Samples will be distributed upon request

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Many thanks to all NBS
laboratories that submitted
de-identified confirmed cases
data to CDC

Thank you for your attention!



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

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