Quality Assurance for Tandem Mass Spectrometry (MS/MS) Assays in Newborn Screening

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National Conversation: Tandem Mass Spectrometry in Newborn Screening

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National Center for Environmental Health
Division of Laboratory Sciences

MS/MS in Neonatal Screening

- **Today**
  - All US labs use MS/MS
  - Widespread adoption worldwide
  - QA materials available from CDC
  - Several manufacturers provide kits
  - Second-tier tests to improve specificity
    - CAH, MSUD, others
  - Additional compounds/disorders have been incorporated
    - SUAC, LSDs

MS/MS Use in Neonatal Screening System Activities Worldwide

- **North America**
  - 100% US labs
  - Canada, Mexico
- **Central America**
  - Costa Rica
- **South America**
  - Argentina, Brazil, Chile, Colombia, Uruguay
- **Europe**
  - Germany, Italy, Netherlands, Spain, United Kingdom
- **Middle East**
  - Israel, Lebanon, Qatar, Saudi Arabia
- **Asia-Pacific**
  - Australia, China, Japan, Philippines, South Korea, Taiwan, Thailand

Laboratory Considerations when Implementing MS/MS Testing

- **Maintenance**
  - PMs; daily checks
- **Reagents**
  - 3N HCl (DEER); hydrazine
- **Laboratory equipment**
  - Corrosion-resistant dryers; Fume hoods; vacuum exhaust
- **Training**
  - Hands-on; best practices guidelines
- **EQAS: CDC**

CDC'S QUALITY ASSURANCE ACTIVITIES FOR MS/MS

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

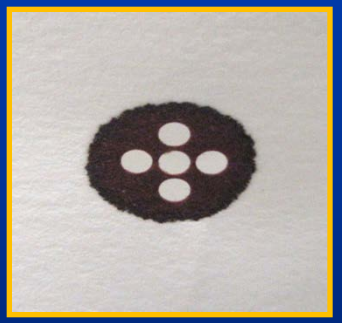
The only comprehensive quality assurance program using dried blood spots

- Proficiency testing
- Quality control materials
- Reference materials
- Training and consultation
- NBS translation research
- Filter paper evaluations

Preparation of whole blood pools

Certification of Blood Spots

Packaging and Shipment to Participating Labs



Filter paper evaluations

National Impact of CDC's Newborn Screening Quality Assurance Program (NSQAP)

- Sole provider of comprehensive quality assurance services for screening labs
  - Essential for evaluation of method performance
- Over 850,000 dried-blood spots (DBS) produced each year
  - 3 challenges of 5 blind-coded samples/year
  - UDOT
  - Proactive follow-up of false negative results
- 100% of US states covered by program
  - Allows for accreditation of screening labs
  - Provide summary reports and feedback for all participating labs

Program Timeline – MS/MS Analytes

2001	2002	2003	2007	2008	2009	2010	2011	2012	2013
Phe	Cit	C5	C0	C3DC	C5OH	Arg	C18:1	Ala	C18OH
Leu	C6	C5DC	C2	SUAC	C18	C4OH		C10:2	
Met	C10		C10:1			C5:1			
Tyr			C14:1			C12			
Val						C16OH			
C3									
C4									
C8									
C14									
C16									

100% Coverage of RUSP MS/MS Primary Biomarkers Achieved in 2012

MS/MS-Detectable Markers in CDC QA Materials

- Alanine
- Citrulline
- Phenylalanine
- Leucine
- Valine
- Methionine
- Arginine
- Tyrosine
- Succinylacetone (SUAC)
- Free carnitine (C0)
- Acetylcarnitine (C2)
- Propionylcarnitine (C3)
- Malonylcarnitine (C3DC)
- Isobutyrylcarnitine (C4)
- Hydroxyisobutyrylcarnitine (C4OH)
- Isovalerylcarnitine (C5)
- Glutarylcarnitine (C5DC)
- Tiglylcarnitine (C5:1)
- Hydroxyisovalerylcarnitine (C5OH)
- Hexanoylcarnitine (C6)
- Octanoylcarnitine (C8)
- Decanoylcarnitine (C10)
- Decenoylcarnitine (C10:1)
- Decadienoylcarnitine (C10:2)
- Dodecanoylcarnitine (C12)
- Myristoylcarnitine (C14)
- Tetradecenoylcarnitine (C14:1)
- Hydroxypalmitoylcarnitine (C16OH)
- Palmitoylcarnitine (C16)
- Stearoylcarnitine (C18)
- Oleoylcarnitine (C18:1)
- Hydroxystearoylcarnitine (C18OH)
- 17-OHP/Androstenedione/cortisol/11-deoxy/21-deoxy (2nd tier CAH)
- Isoleucine/allo-isoleucine (2nd tier)
- 24:0 LPC, 26:0-LPC
- MMA, EMA, MCA, HCY (2nd tier)
- Orotic acid

N=46

NBS MS/MS Methods/Kits

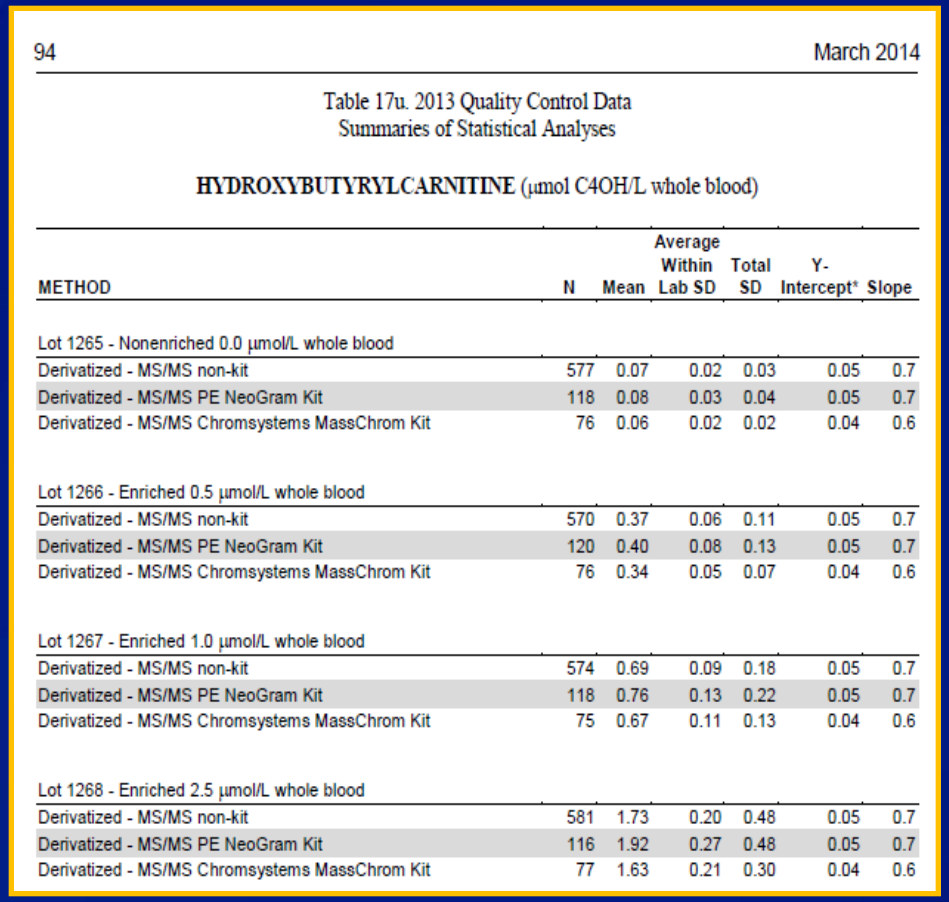
- **Derivatized**
  - PerkinElmer NeoGram
  - Chromsystems MassChrom
  - Laboratory-developed tests
- **Non-derivatized**
  - PerkinElmer NeoBase
  - Chromsystems MassChrom
  - Siemens MS-Neo
  - Laboratory-developed tests

The choice is yours!*

*Maybe

QC DBS Materials

- QC data 2x per year
- 4 concentration levels
  - Certification: 20 runs in duplicate
- Data sorted by method
- Mean, SD, slopes
- Reports on web site



94 March 2014

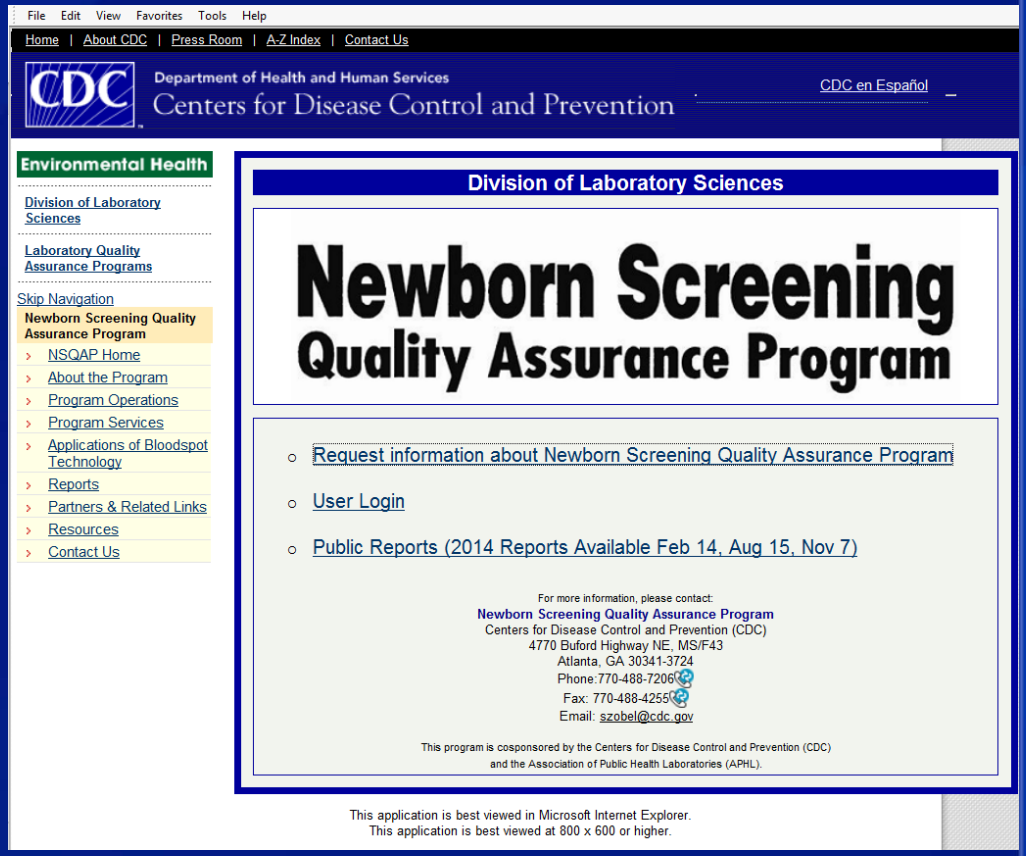
Table 17u. 2013 Quality Control Data
Summaries of Statistical Analyses

HYDROXYBUTYRYLCARNITINE (µmol C4OH/L whole blood)

METHOD	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 1265 - Nonenriched 0.0 µmol/L whole blood						
Derivatized - MS/MS non-kit	577	0.07	0.02	0.03	0.05	0.7
Derivatized - MS/MS PE NeoGram Kit	118	0.08	0.03	0.04	0.05	0.7
Derivatized - MS/MS Chromsystems MassChrom Kit	76	0.06	0.02	0.02	0.04	0.6
Lot 1266 - Enriched 0.5 µmol/L whole blood						
Derivatized - MS/MS non-kit	570	0.37	0.06	0.11	0.05	0.7
Derivatized - MS/MS PE NeoGram Kit	120	0.40	0.08	0.13	0.05	0.7
Derivatized - MS/MS Chromsystems MassChrom Kit	76	0.34	0.05	0.07	0.04	0.6
Lot 1267 - Enriched 1.0 µmol/L whole blood						
Derivatized - MS/MS non-kit	574	0.69	0.09	0.18	0.05	0.7
Derivatized - MS/MS PE NeoGram Kit	118	0.76	0.13	0.22	0.05	0.7
Derivatized - MS/MS Chromsystems MassChrom Kit	75	0.67	0.11	0.13	0.04	0.6
Lot 1268 - Enriched 2.5 µmol/L whole blood						
Derivatized - MS/MS non-kit	581	1.73	0.20	0.48	0.05	0.7
Derivatized - MS/MS PE NeoGram Kit	116	1.92	0.27	0.48	0.05	0.7
Derivatized - MS/MS Chromsystems MassChrom Kit	77	1.63	0.21	0.30	0.04	0.6

Proficiency Testing (PT)

- 3X per year
- One-month reporting period
- Dedicated web page
  - Methods, quantitative values, cutoffs, classifications
- Reports issued to participants **one week** after data reporting deadline

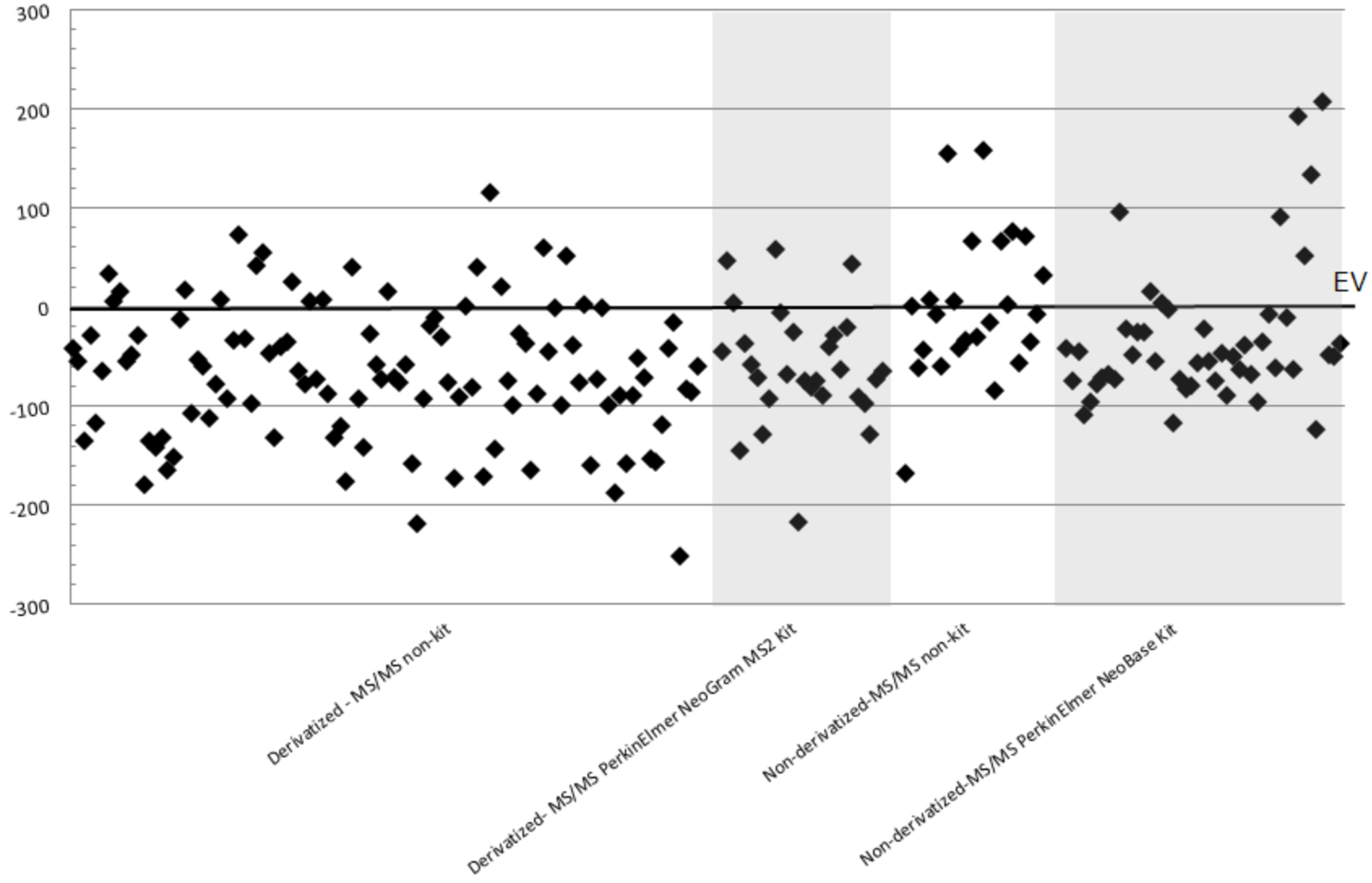


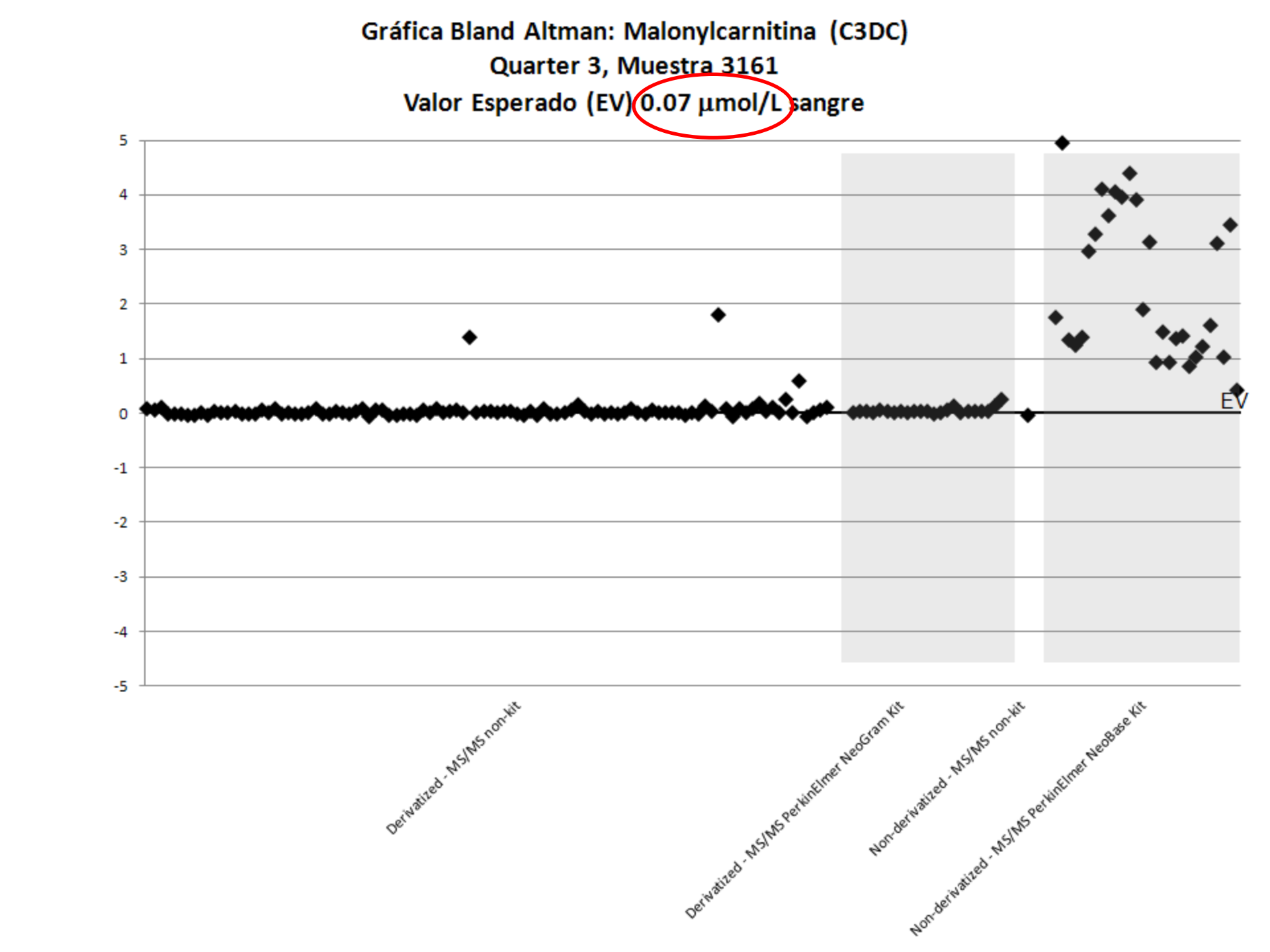
https://wwwn.cdc.gov/nsqap/Public/default.aspx

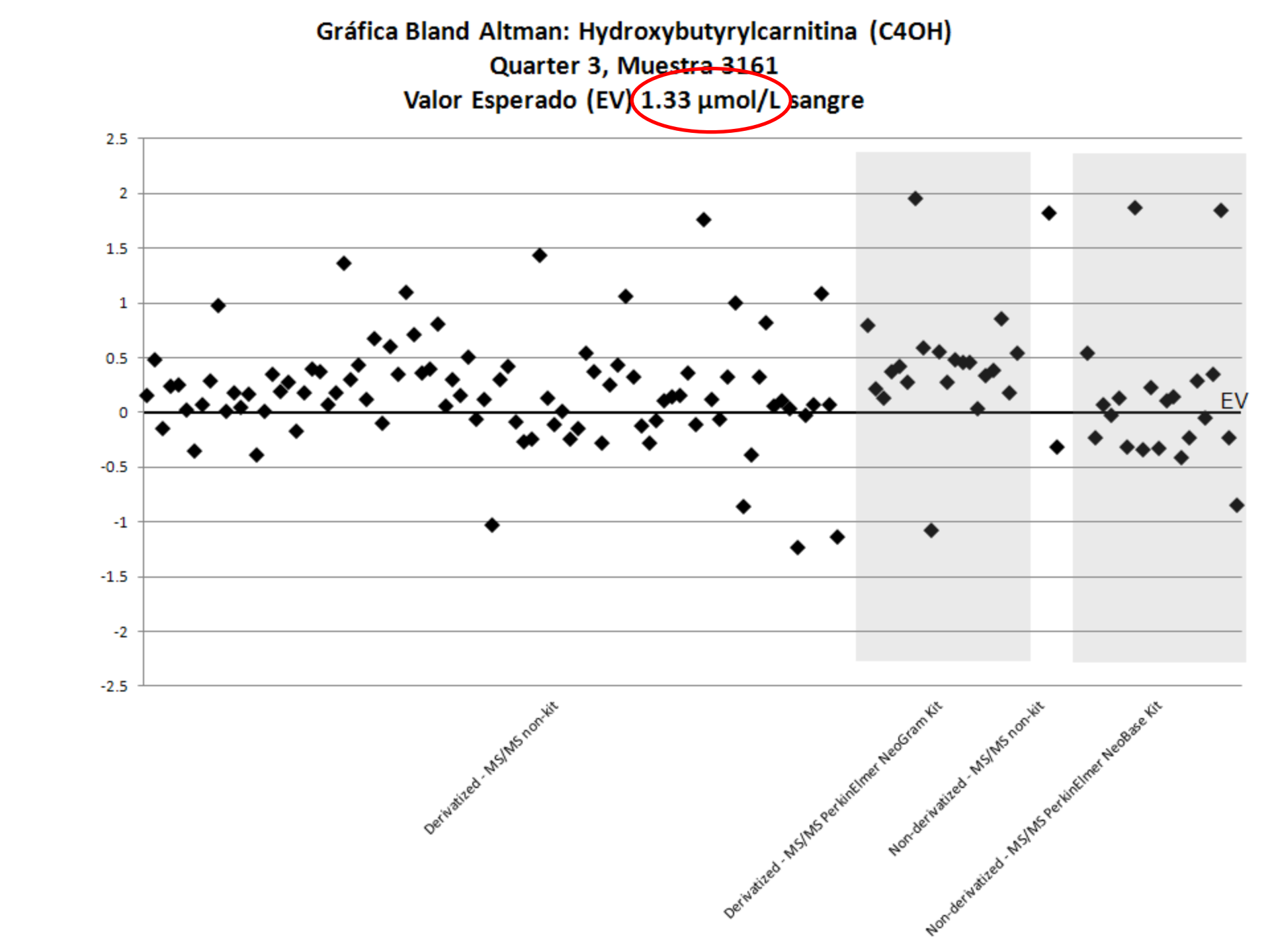
Importance of Quality Assurance *Proficiency Testing*

- Laboratory performance assessed at one point in time
- Required for regulatory purposes and lab certification
  - Labs should participate in a certain number of challenges each year
  - If formal PT programs are not available, labs should establish alternative PT
- Proficiency must be demonstrated to inspectors to maintain certification

Gráfica Bland Altman: Fenilalanina (Phe)
Quarter 1, Muestra 1154
Valor Esperado (EV) = 503 µmol/L sangre



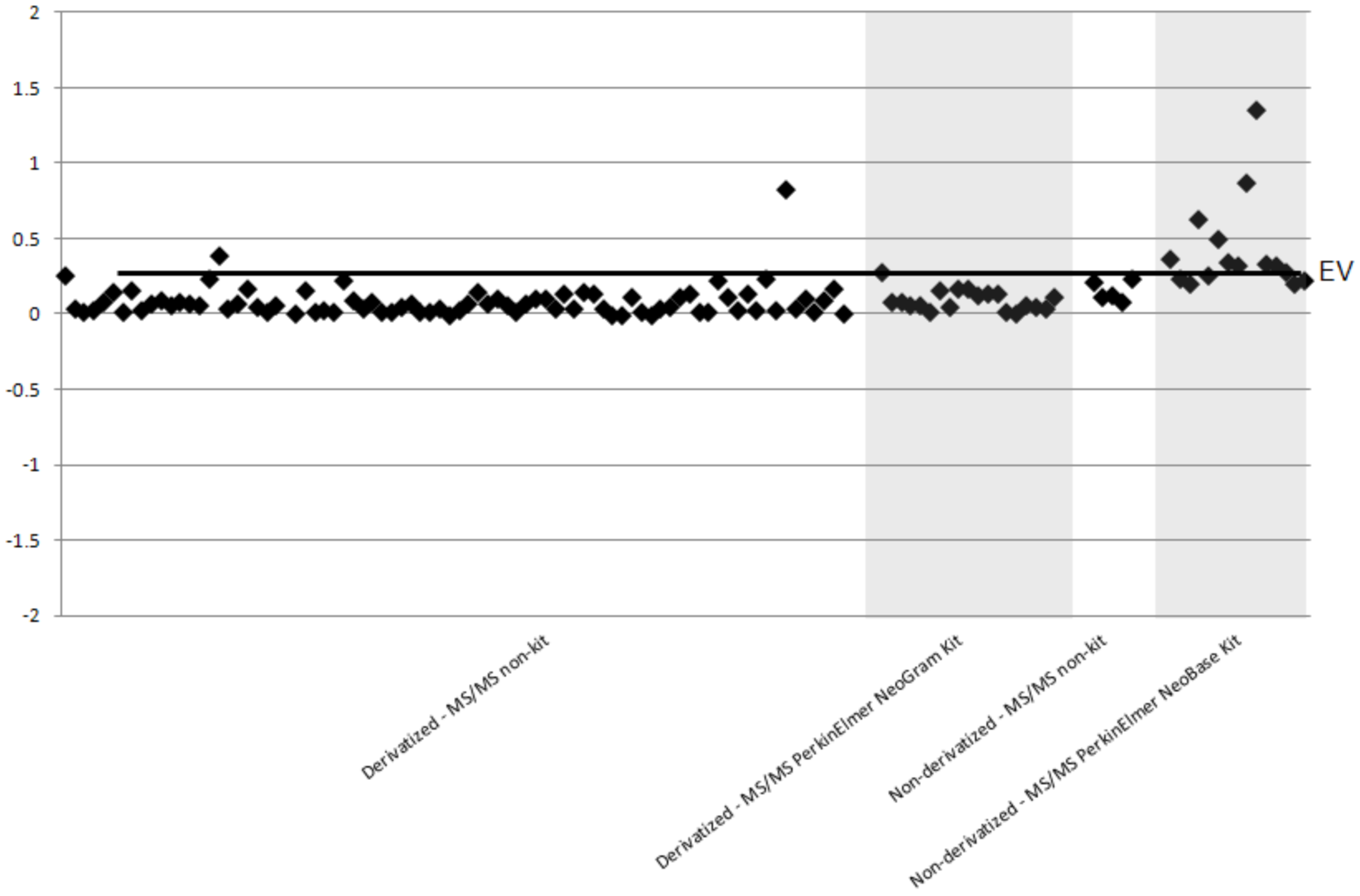

Gráfica Bland Altman: Malonylcarnitina (C3DC)
Quarter 3, Muestra 3161
Valor Esperado (EV) 0.07 µmol/L sangre
5
4
3
2
1
0
-1
-2
-3
-4
-5
EV
Derivatized - MS/MS non-kit
Derivatized - MS/MS PerkinElmer NeoGram Kit
Non-derivatized - MS/MS non-kit
Non-derivatized - MS/MS PerkinElmer NeoBase Kit


Gráfica Bland Altman: Hydroxybutyrylcarnitina (C4OH)
Quarter 3, Muestra 3161
Valor Esperado (EV) 1.33 µmol/L sangre
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
EV
Derivatized - MS/MS non-kit
Derivatized - MS/MS PerkinElmer NeoGram Kit
Non-derivatized - MS/MS non-kit
Non-derivatized - MS/MS PerkinElmer NeoBase Kit

Gráfica Bland Altman: Hydroxybutyrylcarnitina (C4OH)

Quarter 1, Muestra 1163

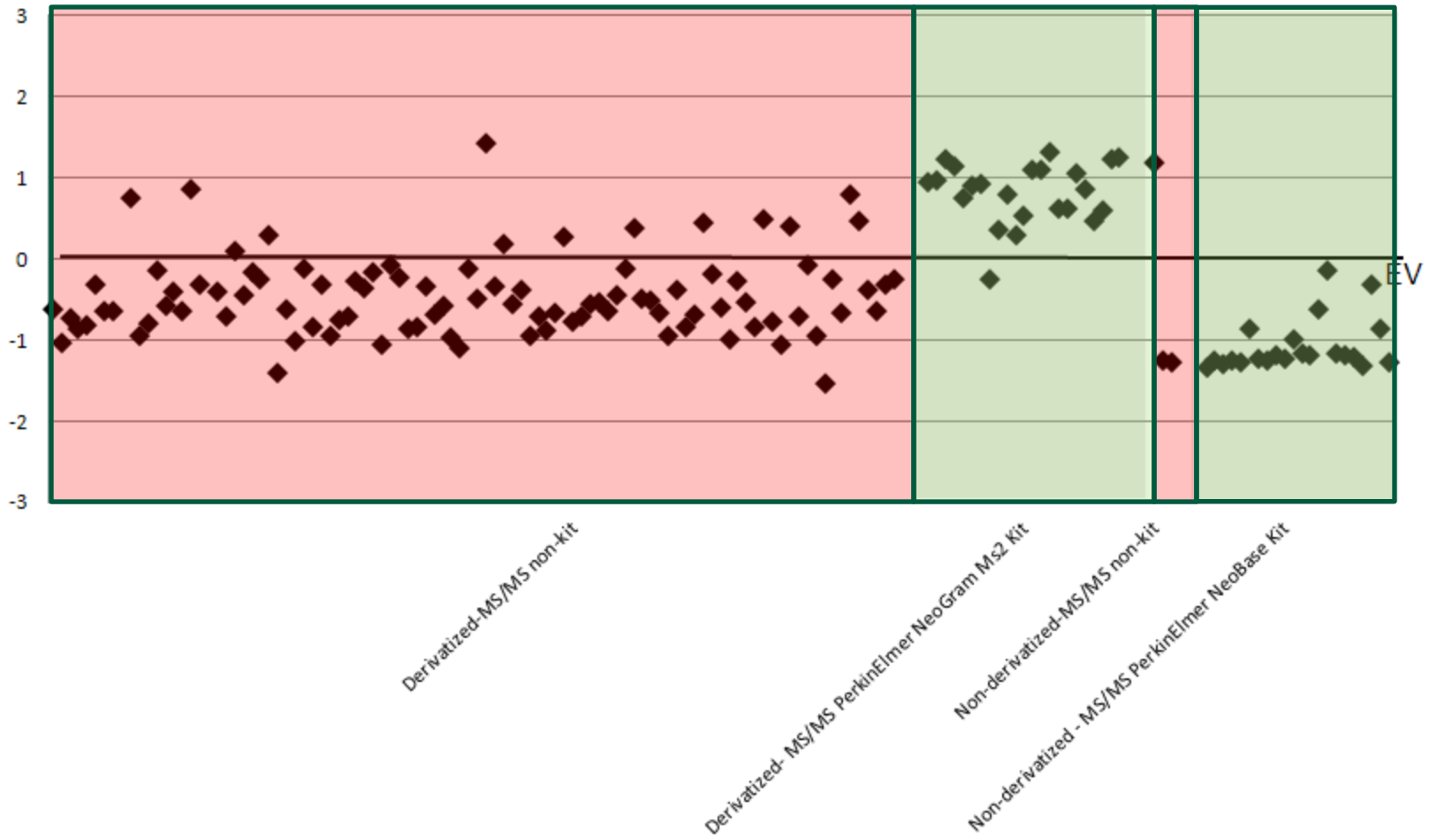
Valor Esperado (EV) = 0.07 µmol/L sangre



Gráfica Bland Altman: Malonylcarnitina (C3DC)

Quarter 1, Muestra 1163

Valor Esperado(EV) = 1.57 µmol/L sangre



Other MS/MS Programs at CDC

- CURRENT:
  - Second tier CAH
  - Second tier MSUD
  - X-ALD
  - Hemoglobin peptides
- UNDER DEVELOPMENT:
  - Second tier MMA
  - Linearity materials (CLSI EP6-A)
  - ORN, GLY for AAQC
  - GAMT
  - ADA-SCID

MS/MS TRAINING WORKSHOP

Newborn Screening by Tandem Mass Spectrometry: A Hands-On Course in Understanding Laboratory Issues and Interpreting Test Results

- 5-day workshop
- 10 participants per course
- Lectures:
  - Biochemical pathways
  - Interferences
  - Stable isotopes
- Lab exercises:
  - MS Tuning
  - Der, Non-der
  - 2nd tier CAH, X-ALD, Plasma AC

The reviews are in…

- I enjoyed very much learning from the hands-on lab activities. An opportunity to familiarize myself with various MS/MS instruments and to learn basic instrument PM/tuning ***which I can practically use***.

- The CAH & ALD tests were also beneficial as our NBS lab is looking into piloting it. The lab is super clean and organized and the staff were friendly. Over all it was ***one of the best learning experiences*** I have had……

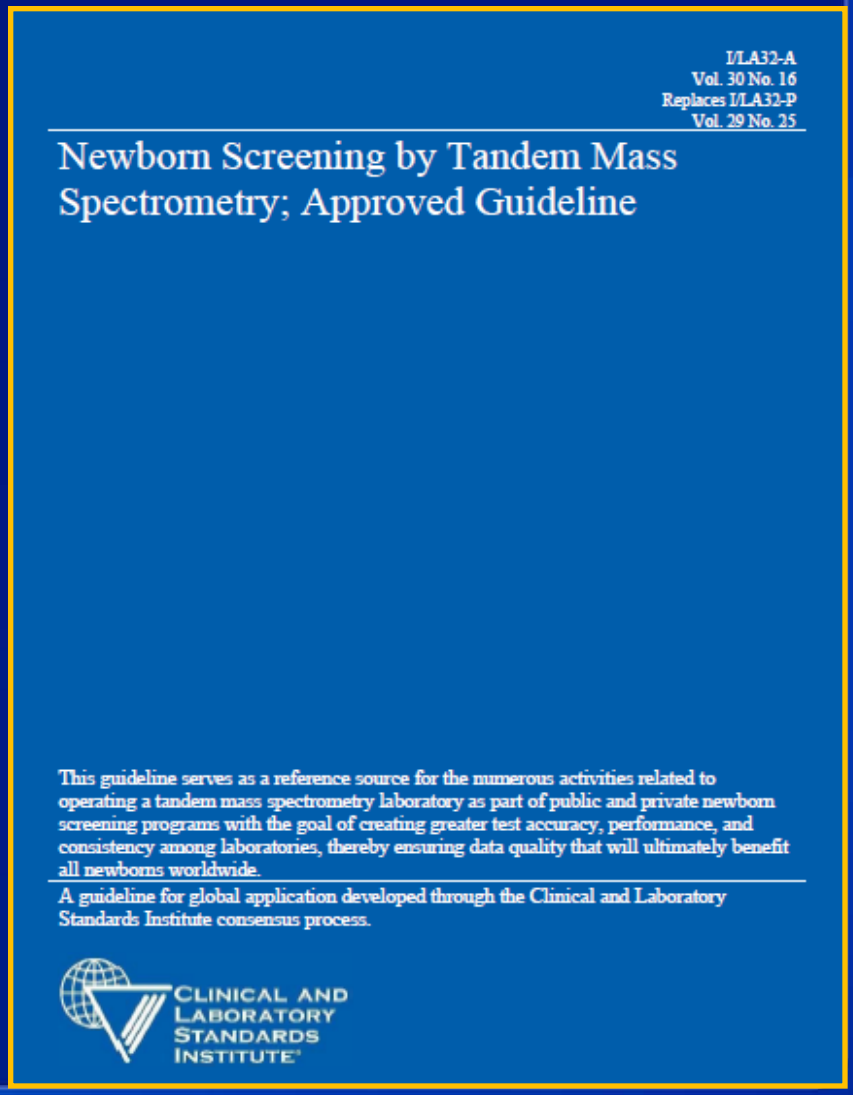
- The optimization of the common analytes did convince me that my method used at home ***could be significantly improved*** an example being front end fragmentation of citrulline.

*2014 Course evaluation results

ADDITIONAL RESOURCES: CLSI NBS04-A2 GUIDELINE UPDATE

CLSI I/LA32-A (NBS04)

- **WG members:**
  - Víctor R. De Jesús (Chair)
  - Mark A. Morrissey (Secretary)
  - Donald H. Chace, PhD, MSFS, FACB
  - Uttam Garg, PhD, DABCC
  - Christopher A. Haynes, PhD
  - Patricia R. Hunt
  - David C. Kasper, PhD
  - Mark Kuracina, MBA, BSc
  - Giancarlo la Marca, Pharm Sc
  - Raquel Yahyaoui Macías, MD, PhD
  - Adrienne Manning
  - Mary A. Seeterlin, PhD
  - Dianne Webster, PhD, FHGSA
  - Ronald J. Whitley, PhD, DABCC, FACB
  - William W. Wood, PhD



CLSI I/LA32-A (NBS04)

- **Updated content**
  - MS/MS basics and nomenclature
  - Enhanced guidelines for MS/MS data interpretation
  - Informative markers / ratios
  - Non-derivatized assay considerations
  - Improved documentation of interferences and other MS/MS-detectable disorders

- **Project timeline**
  - April 2014 – inaugural meeting
  - September 2014 – face-to-face meeting
  - December 2014 – final draft submitted to CLSI
  - 2015 – public comments, voting and publication

Summary

- Newborn screening by tandem mass spectrometry is a successful public health program
  - 42/57 RUSP disorders are MS/MS-detectable
- Multiple considerations to the selection of appropriate MS/MS method
- Many challenges remain for MS/MS screening
  - Establish proper steps to eliminate false positives and negatives
  - Second-tier testing?
  - Cutoffs and interpretation
  - Harmonization of analytical data
  - Quality indicators

NSQAP Web Site: http://www.cdc.gov/labstandards/nsqap.html

Summary

- Participation in EQAS helps maintain MS/MS assay proficiency
- NSQAP is a comprehensive resource for laboratory services
  - PT and QC materials invaluable for MS/MS laboratories
- Training opportunities available at CDC
  - Next course: April 2015
- Continued quality improvement is our mission

Acknowledgments

- Biochemical Mass Spectrometry Laboratory, NSMBB

- Newborn Screening Quality Assurance Program
  - Joanne V. Mei, Chief
  - Kelsey Sheard
  - Sarah Klass
- NSQAP Participating Laboratories

- APHL

Thank you!

For more information:

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