Assuring the Quality of MS/MS Dried Blood Spot Newborn Screening Testing

Víctor R. De Jesús, PhD

Team Lead, Biochemical Mass Spectrometry Laboratory
Newborn Screening and Molecular Biology Branch

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National Center for Environmental Health
Division of Laboratory Sciences

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
  - 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

Available MS/MS-Detectable Markers

- Alanine
- Citrulline
- Phenylalanine
- Leucine
- Valine
- Methionine
- Arginine
- Tyrosine
- Succinylacetone (tyrosinemia type I)
- Free carnitine (C0)
- Acetylcarnitine (C2)
- Propionylcarnitine (C3)
- Malonylcarnitine (C3DC)
- Isobutyrylcarnitine (C4)
- 3-Hydroxyisobutyrylcarnitine (C4OH)
- Isovalerylcarnitine (C5)
- Glutarylcarnitine (C5DC)
- Tiglylcarnitine (C5:1)
- 3-Hydroxyisovalerylcarnitine (C5OH)
- Hexanoylcarnitine (C6)
- Octanoylcarnitine (C8)
- Decanoylcarnitine (C10)
- Decenoylcarnitine (C10:1)
- Decadienoylcarnitine (C10:2)
- Dodecanoylcarnitine (C12)
- Myristoylcarnitine (C14)
- Tetradecenoylcarnitine (C14:1)
- 3-Hydroxypalmitoylcarnitine (C16OH)
- Palmitoylcarnitine (C16)
- Stearoylcarnitine (C18)
- Oleoylcarnitine (C18:1)
- Androstenedione/cortisol/11-deoxy/21-deoxy
- ABG, ASM, GAA, GALC, GLA, IDUA

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Comparison of amino acids and acylcarnitines assay methods used in newborn screening assays by tandem mass spectrometry

Víctor R. De Jesús [a,*], Donald H. Chace [b], Timothy H. Lim [a], Joanne V. Mei [a], W. Harry Hannon [a]

[a] Newborn Screening Quality Assurance Program, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Mail Stop F-19, Atlanta, GA 30341, United States
[b] Pediatrix Analytical, The Center for Research and Education, Pediatrix Medical Group, Inc., 1301 Concord Terrace, Sunrise, FL 33323, United States

- Minor differences found for most analytes
- Arg, Cit can differ also depending on method
- C3DC is most different
- NSQAP Data Examples

4. Discussion

Overall differences between amino acid and acylcarnitine quantitative values resulting from derivatized and underivatized techniques were <15% for the majority of the amino acids and acylcarnitines detected by both methods. However, the most striking difference between the MS/MS analysis of acylcarnitines by butyl esterification techniques as compared to underivatized forms is for dicarboxylic species such as C5DC. These differences can be explained, in part, based on the results from a previous study of C3DC [1]. Laboratories report concentrations based on ion ratios of the unlabeled dicarboxylic acylcarnitine to an internal standard. That internal standard is

De Jesús VR, Chace DH, Lim TH, Mei JV, Hannon WH. Comparison of Amino Acids and Acylcarnitines Assay Methods Used in Newborn Screening Assays by Tandem Mass Spectrometry. Clinica Chimica Acta 2010; 411: 684-689.

Figure 15. Bias Plot of Phenylalanine Values by Method
Quarter 3, Specimen 3
Expected Value (EV) 295.25 µmol/L whole blood

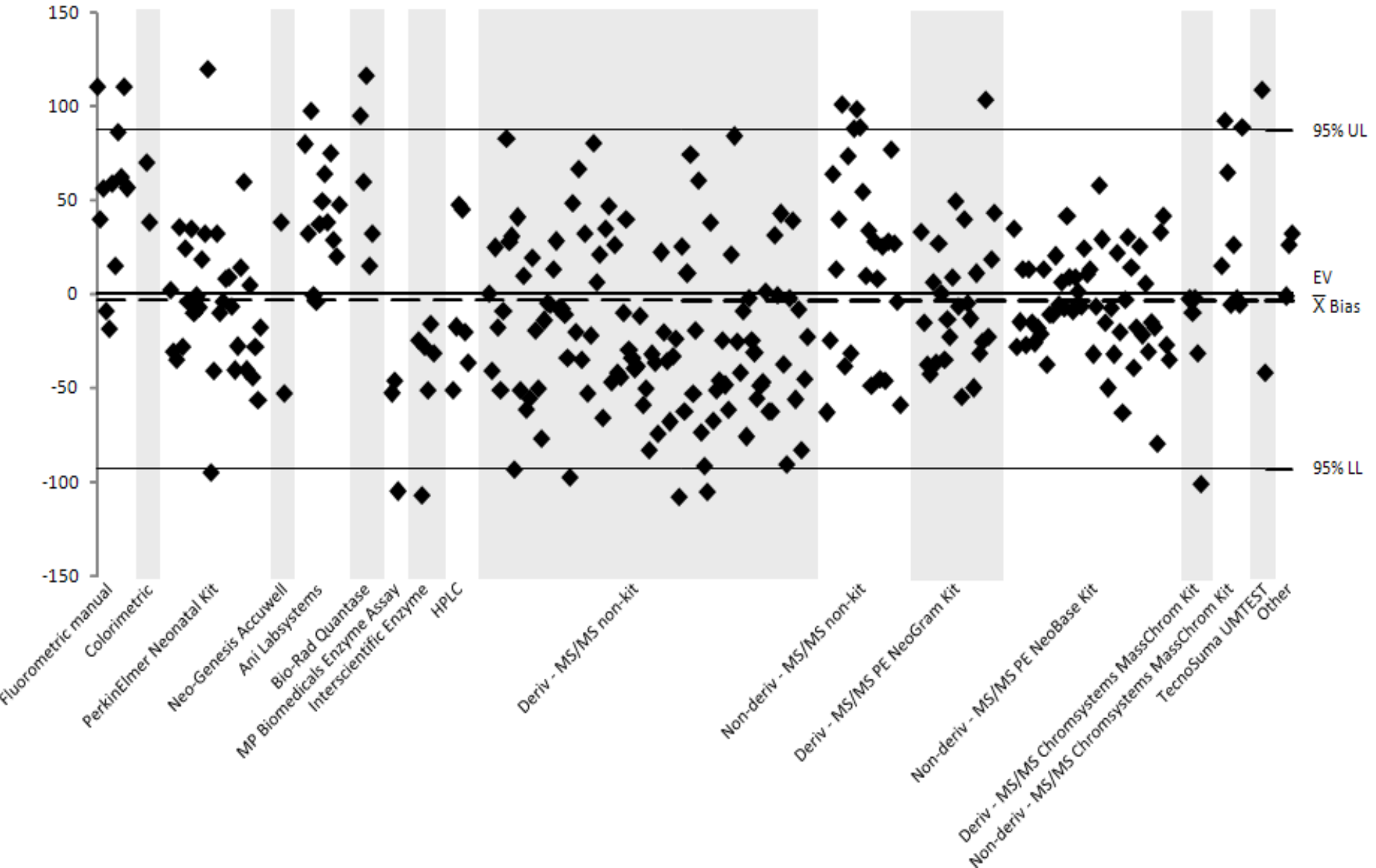


Figure 23. Bias Plot of Free Carnitine (C0(L)) Values by Method
Quarter 1, Specimen 1
Expected Value (EV) 5.66 µmol/L whole blood

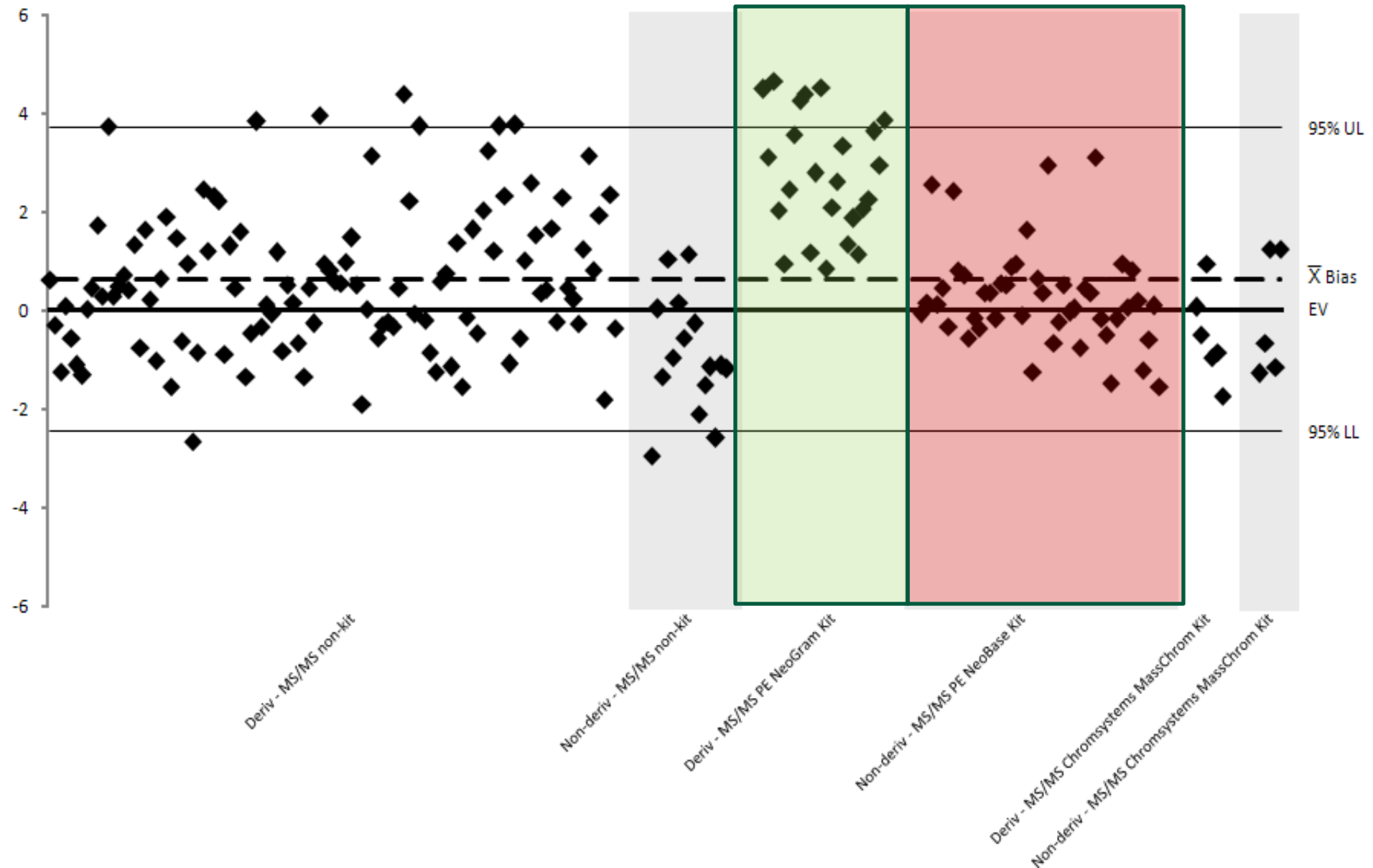
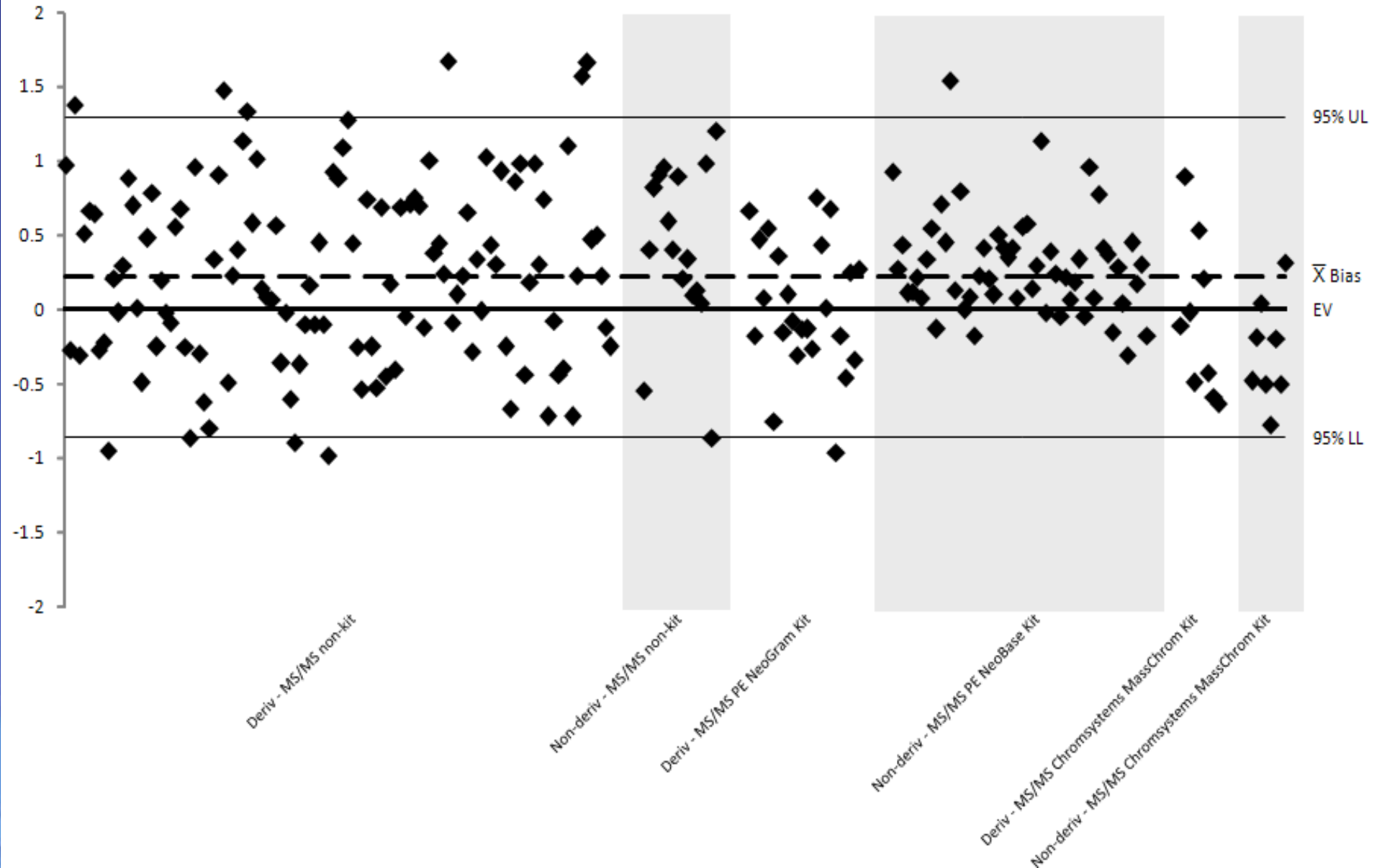
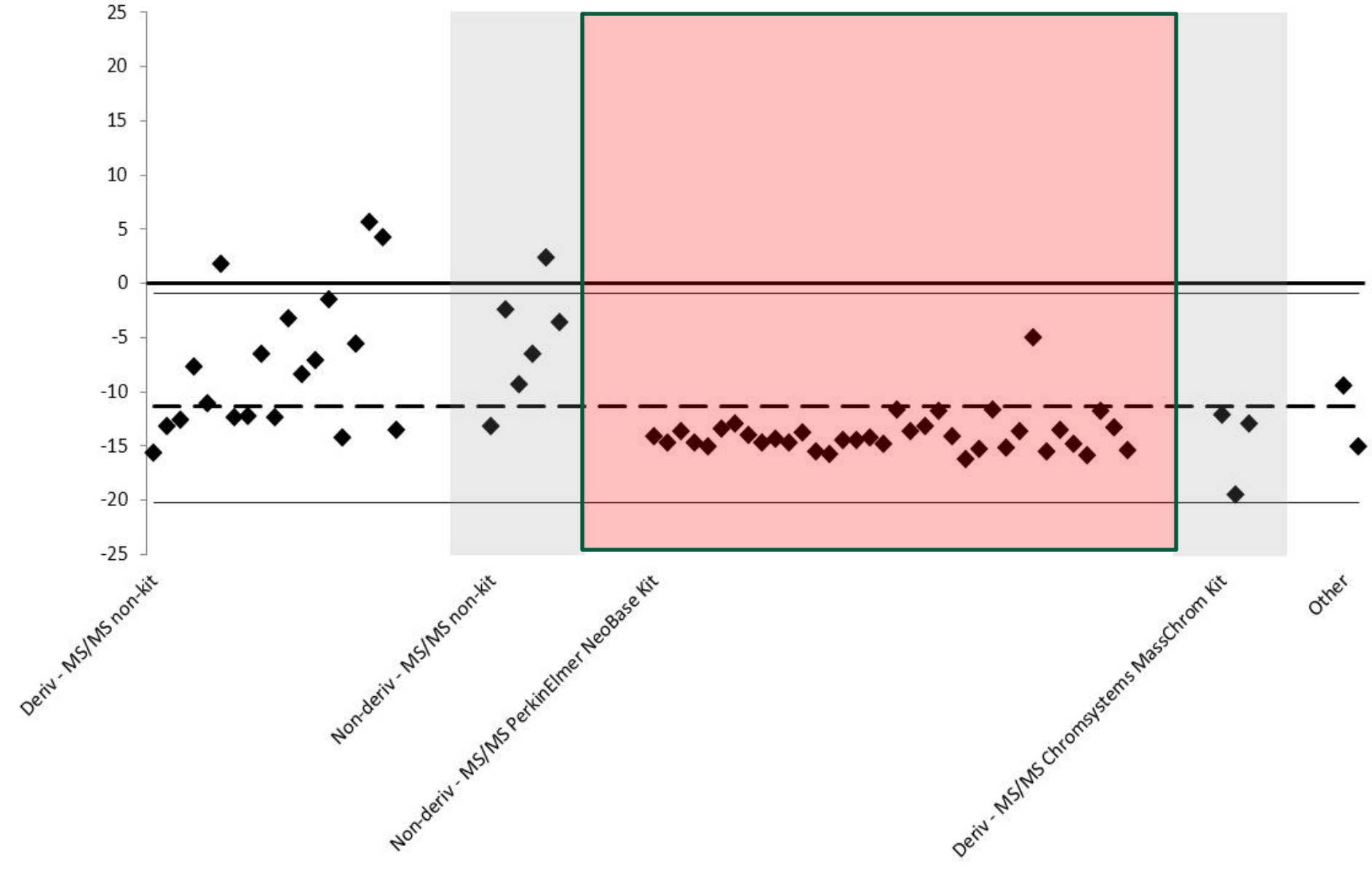


Figure 33. Bias Plot of Octanoylcarnitine (C8) Values by Method
Quarter 3, Specimen 5
Expected Value (EV) 3.10 µmol/L whole blood



Bias Plot of Succinylacetone Values by Method
Quarter 1, Specimen 11232
Expected Value 20.27 μmol/L whole blood



Malonylcarnitine (C3DC): The beginning

- Dicarboxylic acylcarnitine biomarker used to screen for malonic acidemia (MAL)
  - IEM caused by congenital deficiency of malonyl-CoA decarboxylase
  - (2nd target panel)
- Introduced into NSQAP PT panels in 2008

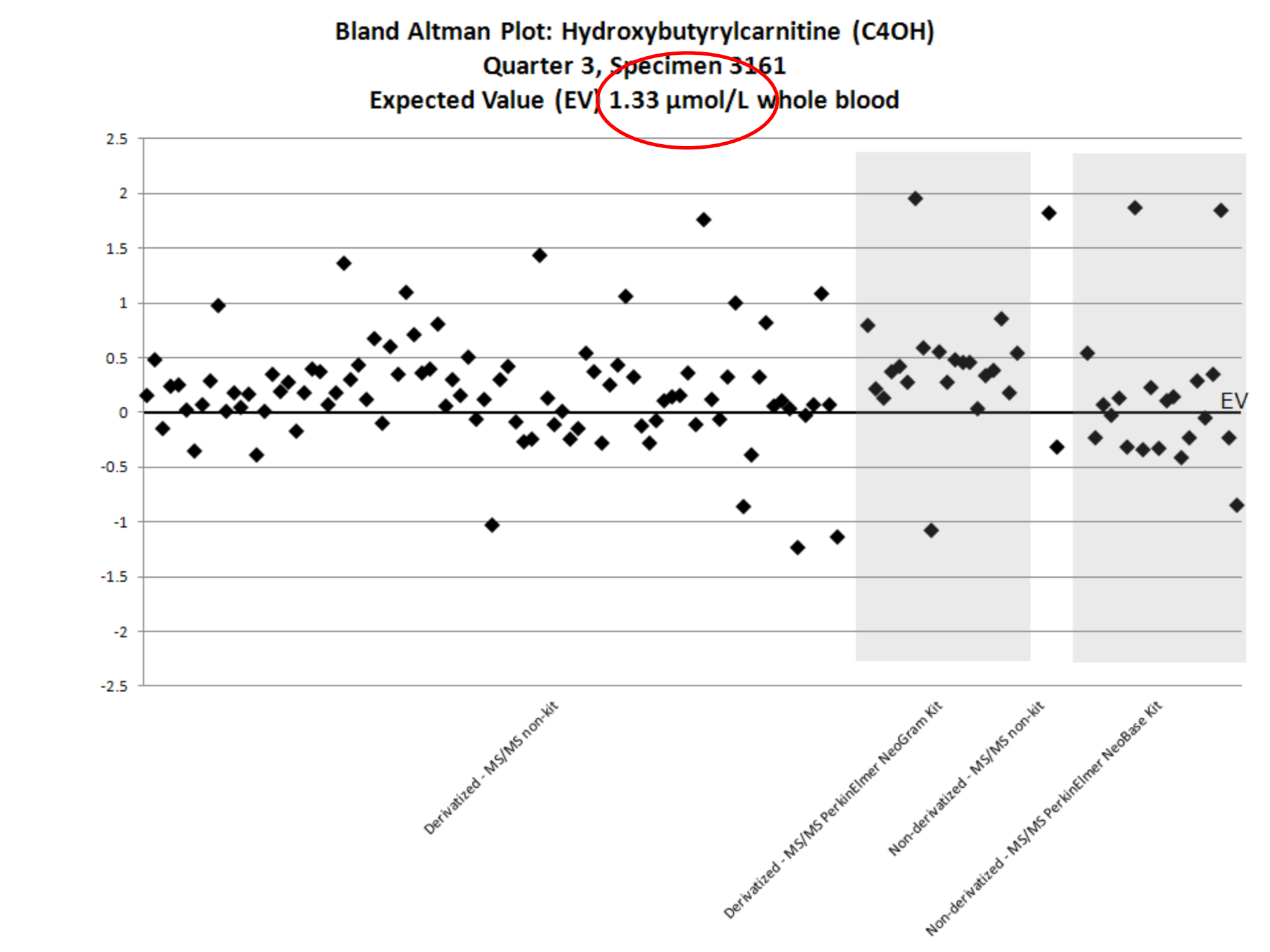
Table 2
Newborn screening panel: core panel and secondary targets

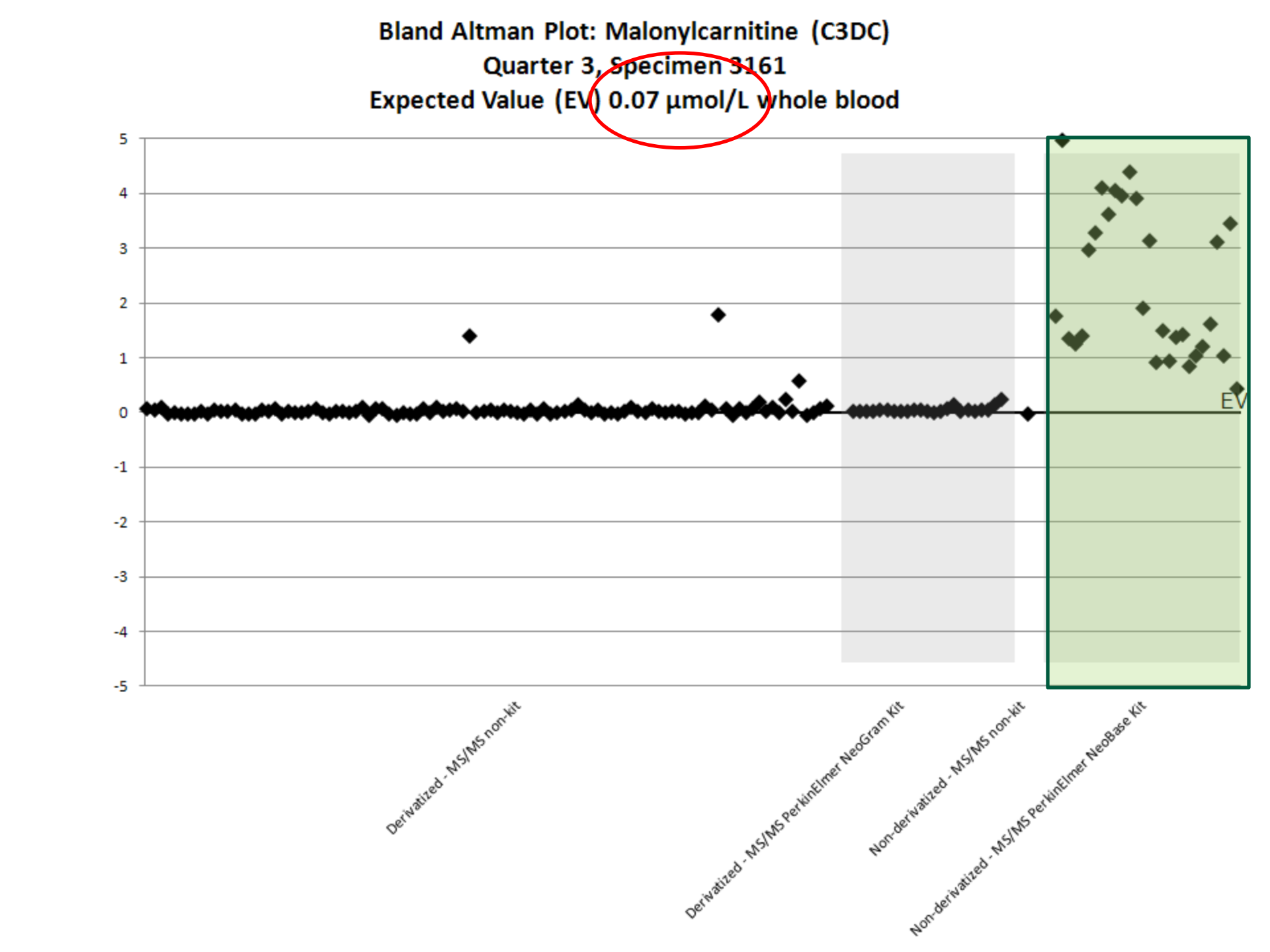
MS/MS				
Acylcarnitines		Amino acids		
9 OA	5 FAO	6 AA	3 Hb Pathies	6 Others
CORE PANEL				
IVA	MCAD	PKU	Hb SS*	CH
GA I	VLCAD	MSUD	Hb S/βTh*	BIOT
HMG	LCHAD	HCY*	Hb S/C*	CAH*
MCD	TFP	CIT		GALT
MUT*	CUD	ASA		HEAR
3MCC*		TYR I*		CF
Cbl A,B*				
PROP				
BKT				
SECONDARY TARGETS				
6 OA	8 FAO	8 AA	1 Hb Pathies	2 Others
Cbl C,D*	SCAD	HYPER-PHE	Var Hb*	GALK*
MAL	GA2	TYR II		GALE
IBG	M/SCHAD	BIOPT (BS)		
2M3HBA	MCKAT	ARG		
2MBG	CPT II	TYR III		
3MGA	CACT	BIOPT (REG)		
	CPT IA	MET		
	DE RED	CIT II		

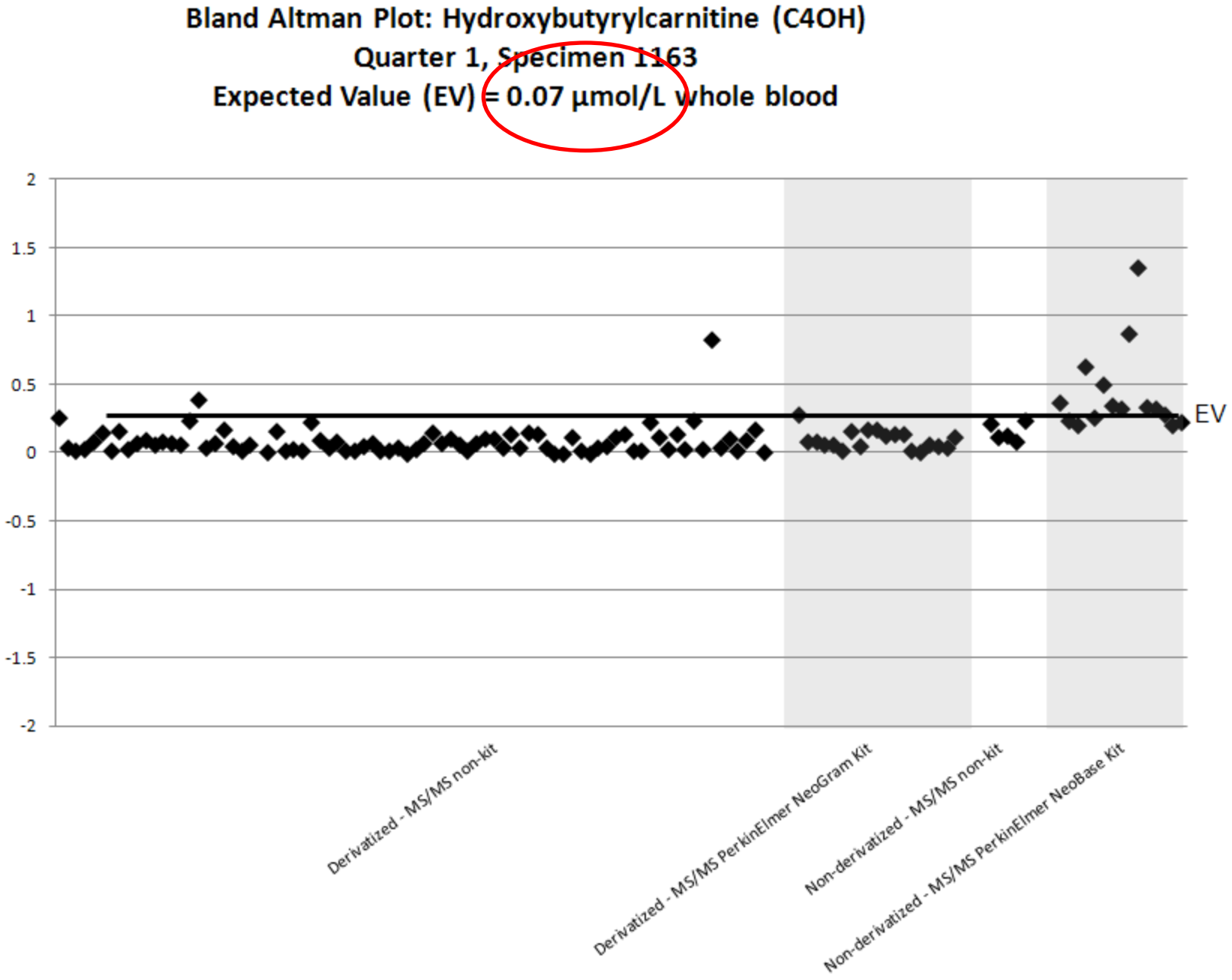
NOTE: Codes are as follows: OA, disorders of organic acid metabolism; FAO, disorders of fatty acid metabolism; AA, disorders of amino acid metabolism; Hb Pathies, hemoglobinopathies.
* Identifies conditions for which specific discussions of unique issues are found in the main report.

And then there were issues with C3DC analysis…

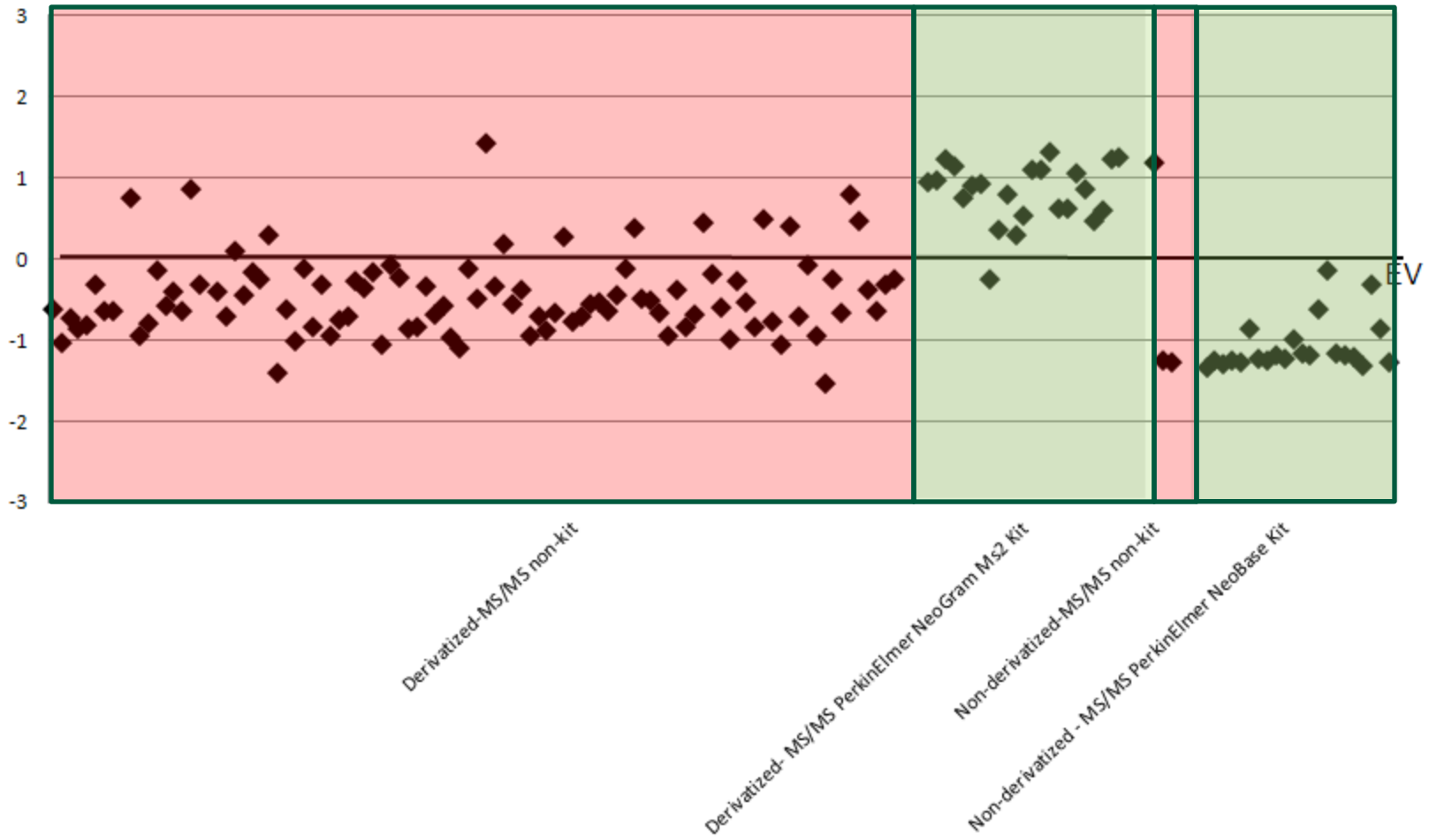
- Non-derivatized assay
  - Kit-, non-kit-based
  - Lower semi-quantitative results
- Hydroxybutyrylcarnitine (C4OH)
  - Introduced into NSQAP PT panels in 2010
  - Isobaric interference
    - m/z 248
- PT misses ensued
  - Increased corrective action reports
  - General feeling: WTH?


Bland Altman Plot: Hydroxybutyrylcarnitine (C4OH)
Quarter 3, Specimen 3161
Expected Value (EV) 1.33 µmol/L whole blood
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


Bland Altman Plot: Malonylcarnitine (C3DC)
Quarter 3, Specimen 3161
Expected Value (EV) 0.07 µmol/L whole blood
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


Bland Altman Plot: Hydroxybutyrylcarnitine (C4OH)
Quarter 1, Specimen 1163
Expected Value (EV) = 0.07 µmol/L whole blood
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
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

Bland Altman Plot: Malonylcarnitine (C3DC)
Quarter 1, Specimen 1163
Expected Value (EV) = 1.57 µmol/L whole blood



Ionization Efficiency

MS/MS Performance Metrics 2006 - 2011

Selected Domestic False Positive Rates (%) for 2006-2011

Disorder/Analyte	Year					
	2006	2007	2008	2009	2010	2011
Phenylketonuria (Phe)	0.2	0.5	0.7	1.8	0.1	0.3
Maple Syrup Urine Disease (Leu)	0.6	3.1	0.7	0.6	0.1	0.2
Tyrosinemia I, II, III (Tyr)	0.0	0.3	0.0	0.3	0.3	0.0
Maple Syrup Urine Disease (Val)	2.2	1.7	0.4	0.7	0.1	1.6
Citrullinemia (Cit)	1.1	0.0	0.2	0.4	0.4	0.3
C3 Screen	0.0	0.1	0.3	0.8	0.3	0.0
C3DC Screen	N/A		0.8	1.6	2.9	0.3
C5 Screen	0.0	0.0	0.9	1.0	0.1	0.0
C5DC Screen	0.0	0.0	0.0	1.0	0.0	0.0
C8 Screen	0.1	0.3	0.0	0.8	0.1	0.3
C16 Screen	0.0	0.1	0.0	0.4	0.2	0.0

MS/MS Performance Metrics 2006 - 2011

Selected Domestic False Negative Rates (%) for 2006-2011

Disorder/Analyte	Year					
	2006	2007	2008	2009	2010	2011
Phenylketonuria (Phe)	0.6	0.0	1.1	0.5	0.8	0.0
Maple Syrup Urine Disease (Leu)	0.0	0.0	0.0	1.1	0.5	0.0
Tyrosinemia I, II, III (Tyr)	1.6	0.7	3.3	1.0	1.5	0.0
Maple Syrup Urine Disease (Val)	0.0	0.0	0.0	0.9	1.1	0.0
Citrullinemia (Cit)	0.0	0.0	0.0	1.7	0.5	0.0
C3 Screen	1.9	0.0	0.0	2.1	0.7	0.0
C3DC Screen	N/A		0.0	4.0	19.4	11.7
C5 Screen	0.8	0.0	0.0	4.0	0.5	0.0
C5DC Screen	3.7	0.0	0.0	1.7	1.0	0.6
C8 Screen	0.6	0.0	0.0	1.2	0.7	0.0
C16 Screen	0.6	0.0	0.0	8.9	1.0	2.0

What to do?

- **Derivatized assay can resolve C3DC and C4OH!**
  - C3DC, C5DC analysis enhanced by derivatization
  - Choice of IS (Chace et al 2009)
  - If unable to derivatize, establish ratios, work with other labs
  - Follow-up procedures for correct screening classification (i.e., cutoffs)

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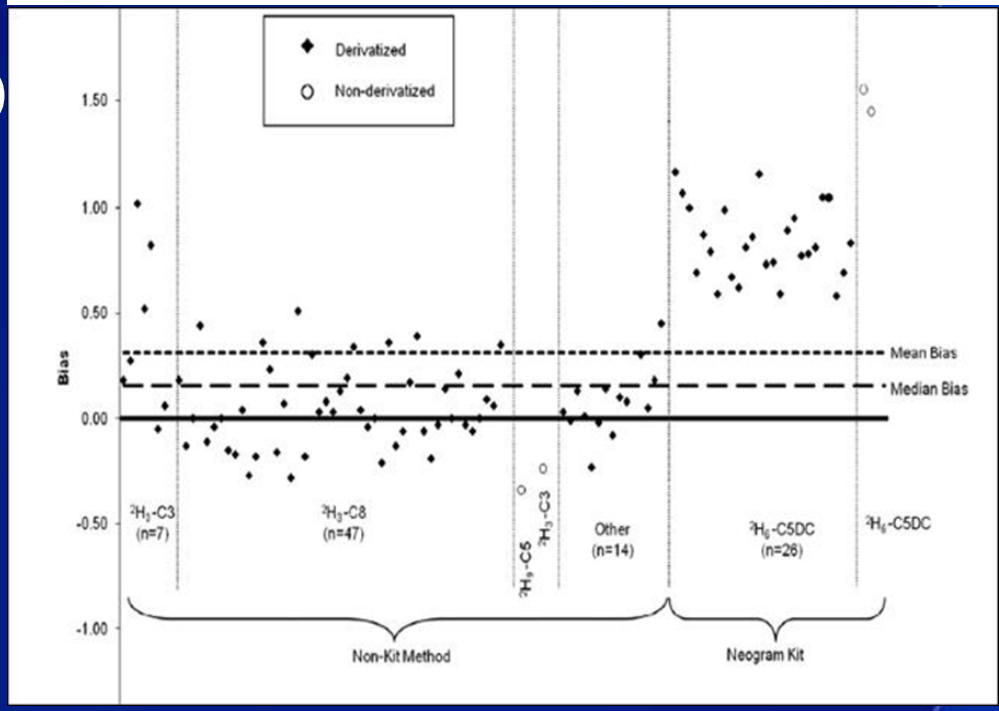
Quantification of malonylcarnitine in dried blood spots by use of MS/MS varies by stable isotope internal standard composition

Donald H. Chace [a,*], Timothy Lim [b], Christina R. Hansen [a], Barbara W. Adam [b], W. Harry Hannon [b]

[a] Pediatrix Analytical, The Center for Research and Education, Pediatrix Medical Group, Inc., 1301 Concord Terrace, Sunrise, FL 33323, United States
[b] Newborn Screening Quality Assurance Program, Centers for Disease Control and Prevention (CDC), 4770 Buford Highway, NE, Atlanta, GA 30341, United States



NSQAP adapts to ensure high-quality screening

- **PT Testing**
  - NSQAP new category: C3DC + C4OH
  - Allows for reduced corrective action reports
  - No double-dipping!
  - On-line reporting category: live in January 2012

- **QC Materials**
  - Two characterization sheets for AA, AC QC materials
  - No changes to reporting scheme

Summary

- **Newborn screening by tandem mass spectrometry is a successful public health program**
  - >95% of newborns screened in US
  - 42/56 RUSP disorders are MS/MS-detectable
- **Many challenges remain for DER-UND screening**
  - Understanding assay and metabolite limitations is key
  - Establish proper procedures to eliminate false positives and negatives
- **NSQAP is a comprehensive resource for laboratory services**
  - New PT reporting reflects current practices in the field

For More Information

Víctor R. De Jesús, PhD
Team Lead, Biochemical Mass Spectrometry Laboratory
Newborn Screening and Molecular Biology Branch
(770) 488-7963 (o)
(770) 488-7459 (f)
vdejesus@cdc.gov

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