Trials and Tribulations of Proficiency Testing for Malonylcarnitine (C3DC) in Dried-Blood Spots

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National Center for Environmental Health
Division of Laboratory Sciences

CDC

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
- NSQAP PT materials include 41 of the 42 disorders detectable by tandem mass spectrometry (MS/MS)

Table 2
Newborn screening panel: core panel and secondary targets

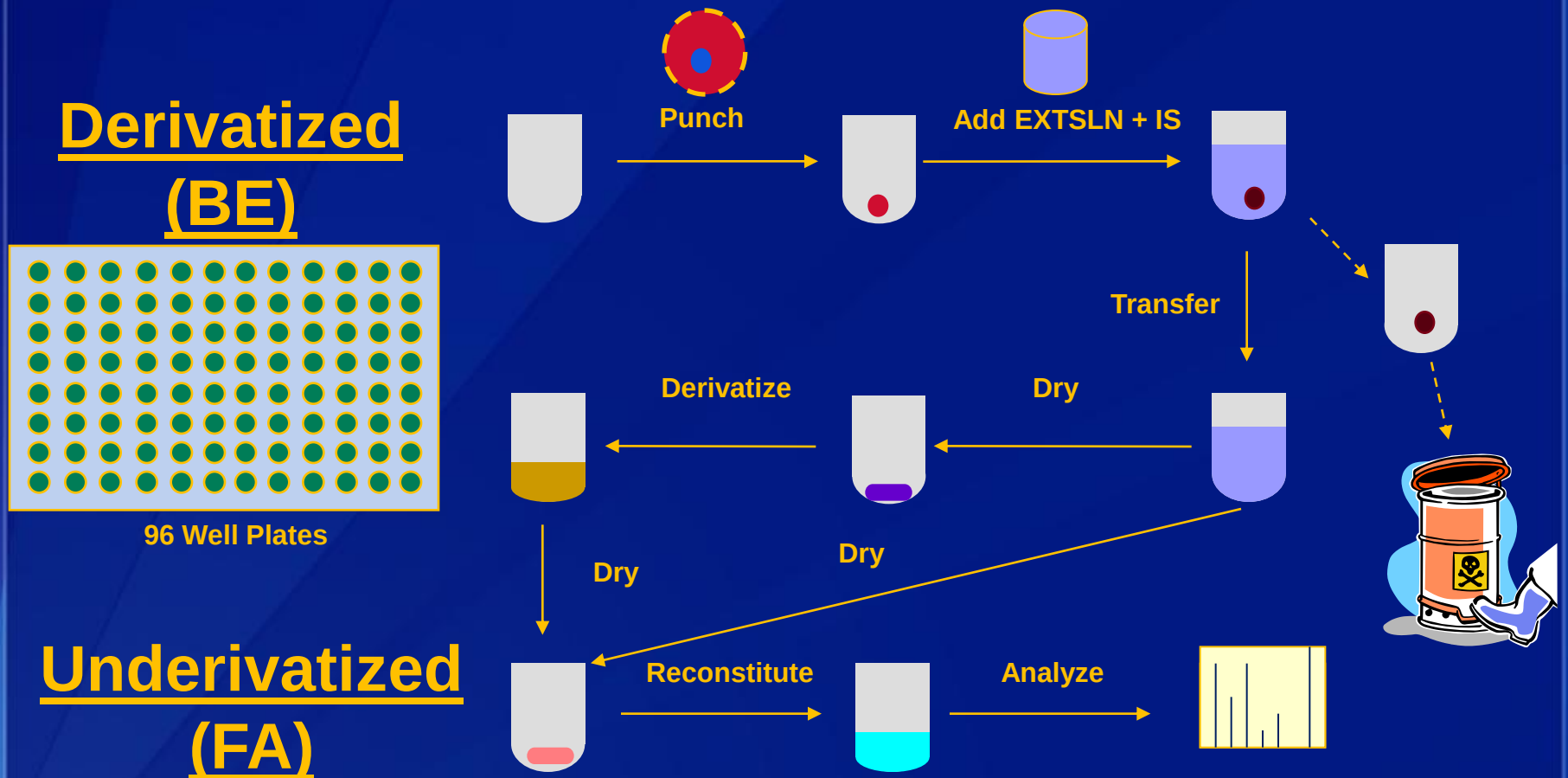
MS/MS				
Acylcarnitines		Amino acids		
9 OA	5 FAO	6 AA	3 Hb Pathies	6 Others
CORE PANEL				
IVA	MCAD	PKU	Hb SS*	CH
GA I	VLCAD	MSUD	Hb S/βTh*	BIOT
HMG	LCHAD	HCY*	Hb S/C*	CAH*
MCD	TFP	CIT		GALT
MUT*	CUD	ASA		HEAR
3MCC*		TYR I*		CF
Cbl A,B*				
PROP				
BKT				
SECONDARY TARGETS				
6 OA	8 FAO	8 AA	1 Hb 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.

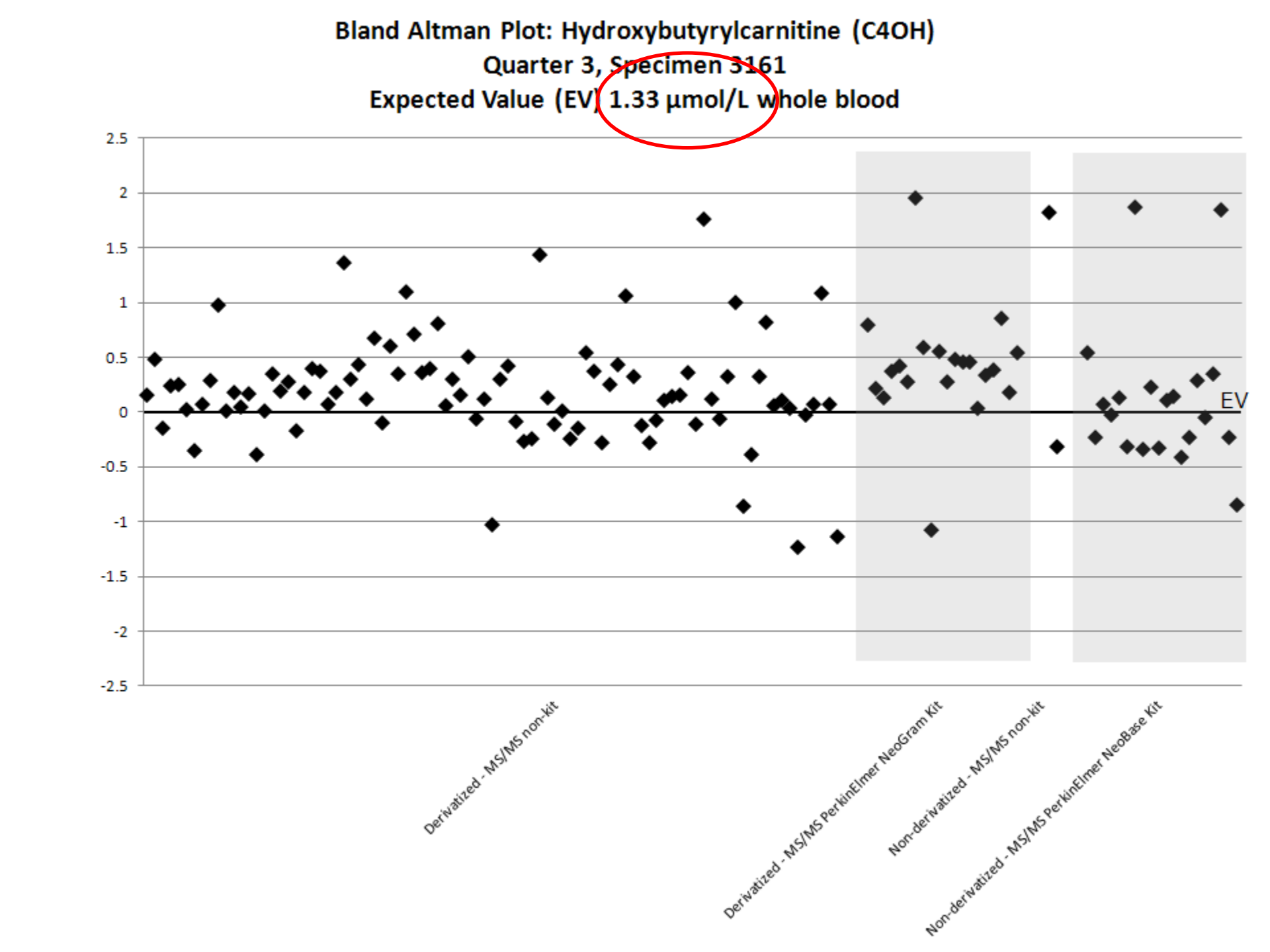
And then there were issues with C3DC analysis…

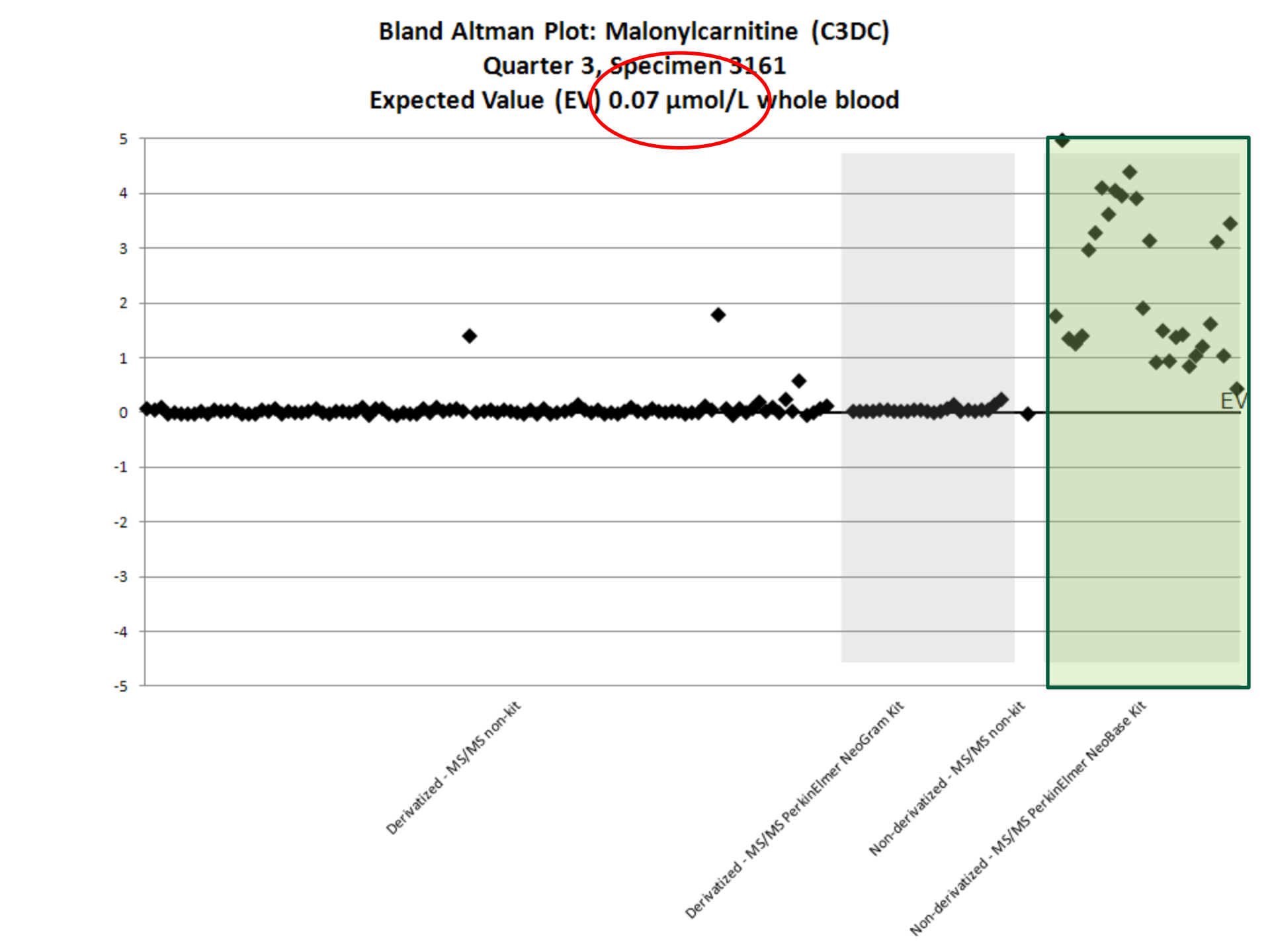
- **Non-derivatized assay**
  - Kit-, non-kit-based
  - Lower semi-quantitative results
- **Hydroxybutyrylcarnitine (C4OH)**
  - Introduced into NSQAP PT panels in 2010
  - Isobaric interference
    - m/z 248
- **PT misses ensued**
  - Increased corrective action reports
  - General feeling: WTH?

MS/MS NBS Assay Scheme

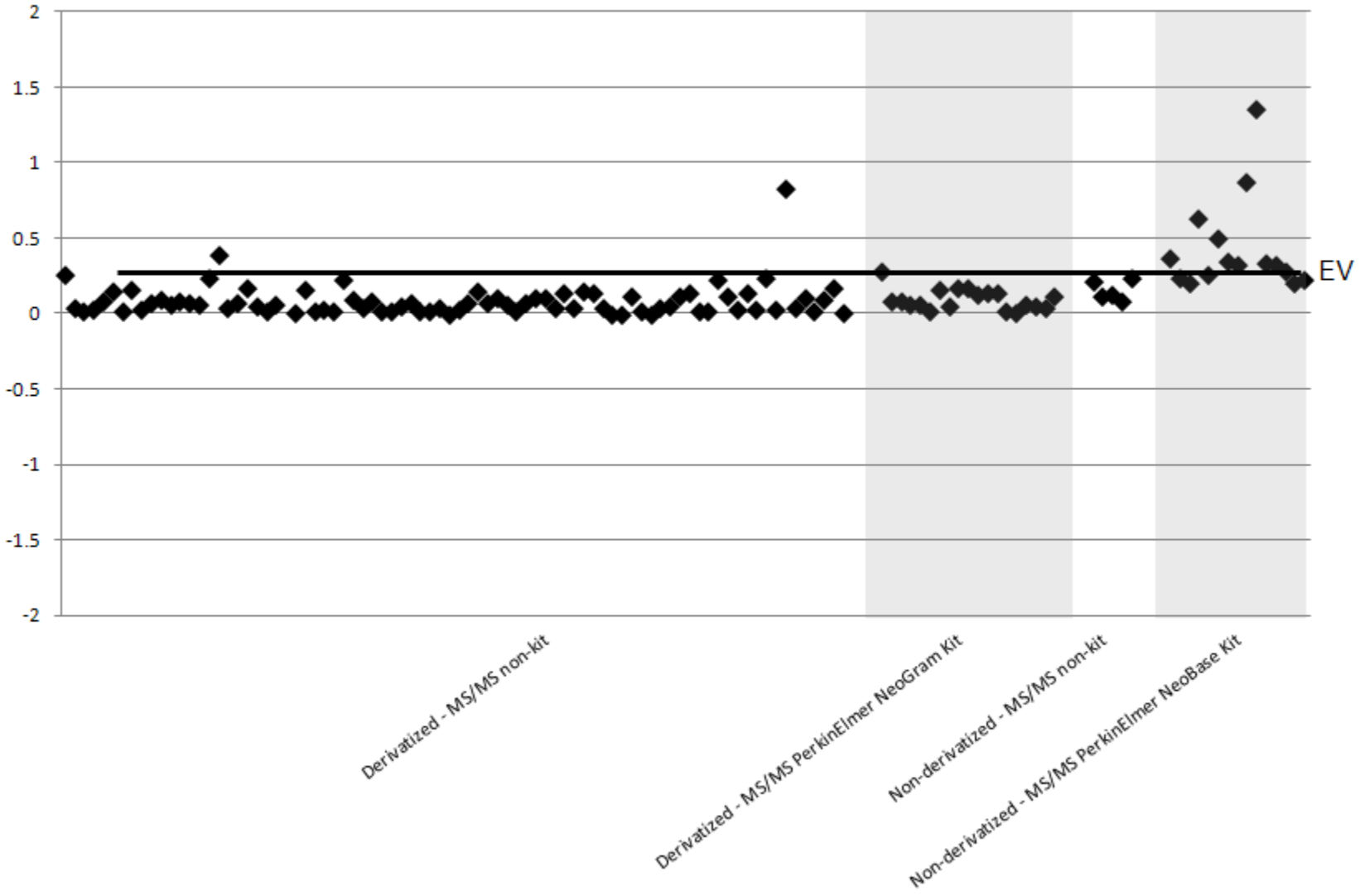


De Jesús VR, Chace DH, Lim TH, Mei JV, Hannon WH. Comparison of Amino Acids and Acylcarnitines Assay Methods Used in Newborn Screening Assays by Tandem Mass Spectrometry. Clinica Chimica Acta 2010; 411: 684-689.

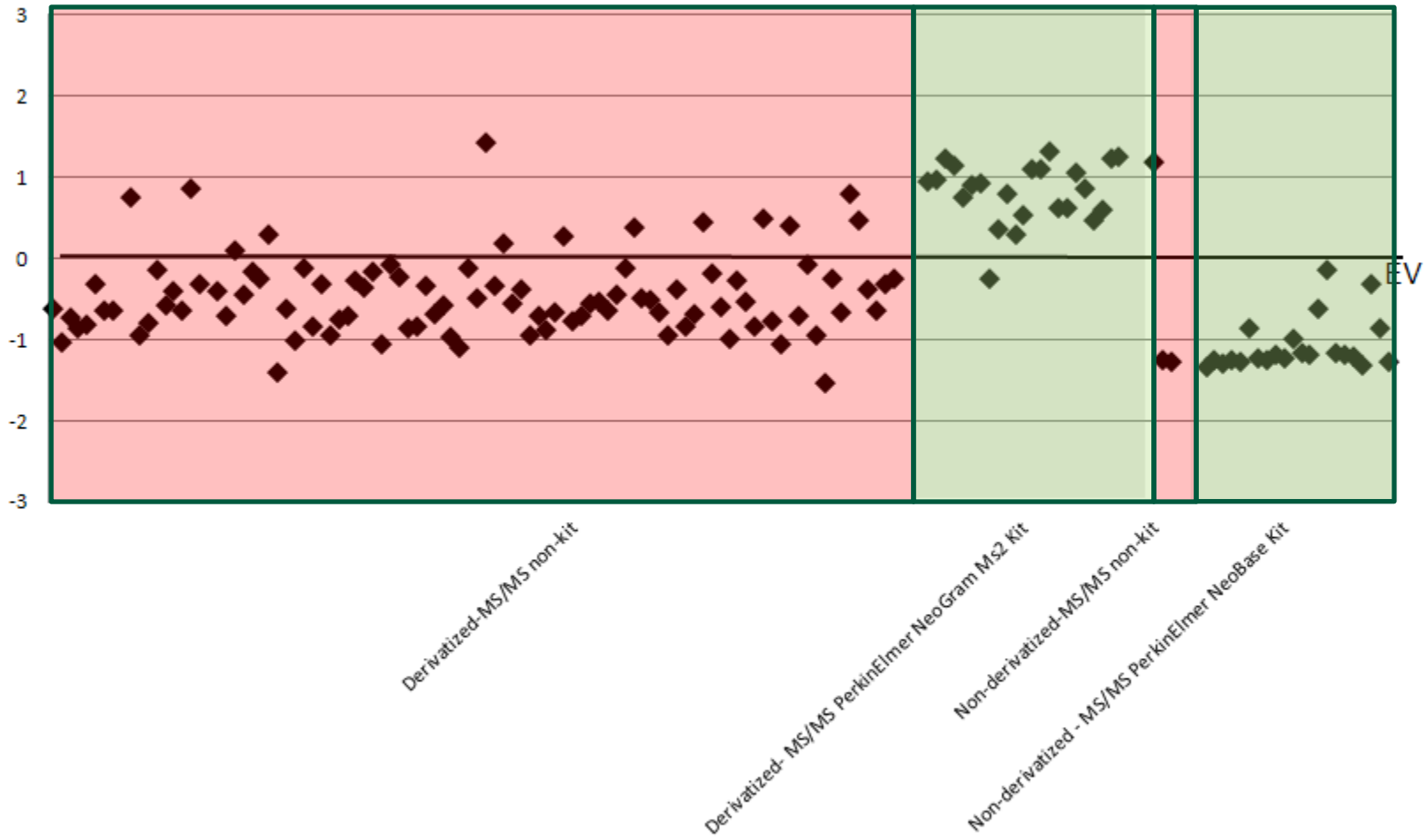

Bland Altman Plot: Hydroxybutyrylcarnitine (C4OH)
Quarter 3, Specimen 3161
Expected Value (EV) 1.33 µmol/L whole blood
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
EV
Derivatized - MS/MS non-kit
Derivatized - MS/MS PerkinElmer NeoGram Kit
Non-derivatized - MS/MS non-kit
Non-derivatized - MS/MS PerkinElmer 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



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



Ionization Efficiency

MS/MS Performance Metrics 2005 - 2010

Domestic False Positive Rates (%) for 2005-2010

Disorder/Analyte	Year					
	2005	2006	2007	2008	2009	2010
Phenylketonuria (Phe)	0.4	0.2	0.5	0.7	1.8	0.1
Maple Syrup Urine Disease (Leu)	0.0	0.6	3.1	0.7	0.6	0.1
Homocystinuria (Met)	0.2	0.2	0.4	0.0	0.7	0.2
Tyrosinemia I, II, III (Tyr)	0.0	0.0	0.3	0.0	0.3	0.3
Maple Syrup Urine Disease (Val)	1.8	2.2	1.7	0.4	0.7	0.1
Citrullinemia (Cit)	0.0	1.1	0.0	0.2	0.4	0.4
C3 Screen	0.2	0.0	0.1	0.3	0.8	0.3
C3DC Screen	N/A			0.8	1.6	2.9
C4 Screen	1.2	0.6	0.2	1.1	1.0	0.9
C5 Screen	0.2	0.0	0.0	0.9	1.0	0.1
C5DC Screen	0.6	0.0	0.0	0.0	1.0	0.0
C6 Screen	0.2	0.2	0.6	0.3	0.9	0.1
C8 Screen	0.2	0.1	0.3	0.0	0.8	0.1
C10 Screen	0.9	0.0	1.0	0.4	1.4	0.1
C16 Screen	0.0	0.0	0.1	0.0	0.4	0.2

MS/MS Performance Metrics 2005 - 2010

Domestic False Negative Rates (%) for 2005-2010

Disorder/Analyte	Year					
	2005	2006	2007	2008	2009	2010
Phenylketonuria (Phe)	0.8	0.6	0.0	1.1	0.5	0.8
Maple Syrup Urine Disease (Leu)	0.0	0.0	0.0	0.0	1.1	0.5
Homocystinuria (Met)	1.4	0.0	0.0	0.0	0.6	0.7
Tyrosinemia I, II, III (Tyr)	0.0	1.6	0.7	3.3	1.0	1.5
Maple Syrup Urine Disease (Val)	0.0	0.0	0.0	0.0	0.9	1.1
Citrullinemia (Cit)	0.0	0.0	0.0	0.0	1.7	0.5
C3 Screen	0.0	1.9	0.0	0.0	2.1	0.7
C3DC Screen	N/A			0.0	4.0	19.4
C4 Screen	0.0	0.4	0.0	0.0	3.1	0.0
C5 Screen	1.7	0.8	0.0	0.0	4.0	0.5
C5DC Screen	0.0	3.7	0.0	0.0	1.7	1.0
C6 Screen	0.0	0.0	0.6	0.0	3.4	0.7
C8 Screen	0.6	0.6	0.0	0.0	1.2	0.7
C10 Screen	1.1	1.3	0.0	0.0	2.1	0.7
C16 Screen	0.0	0.6	0.0	0.0	8.9	1.0

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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journal homepage: www.elsevier.com/locate/clinchim

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

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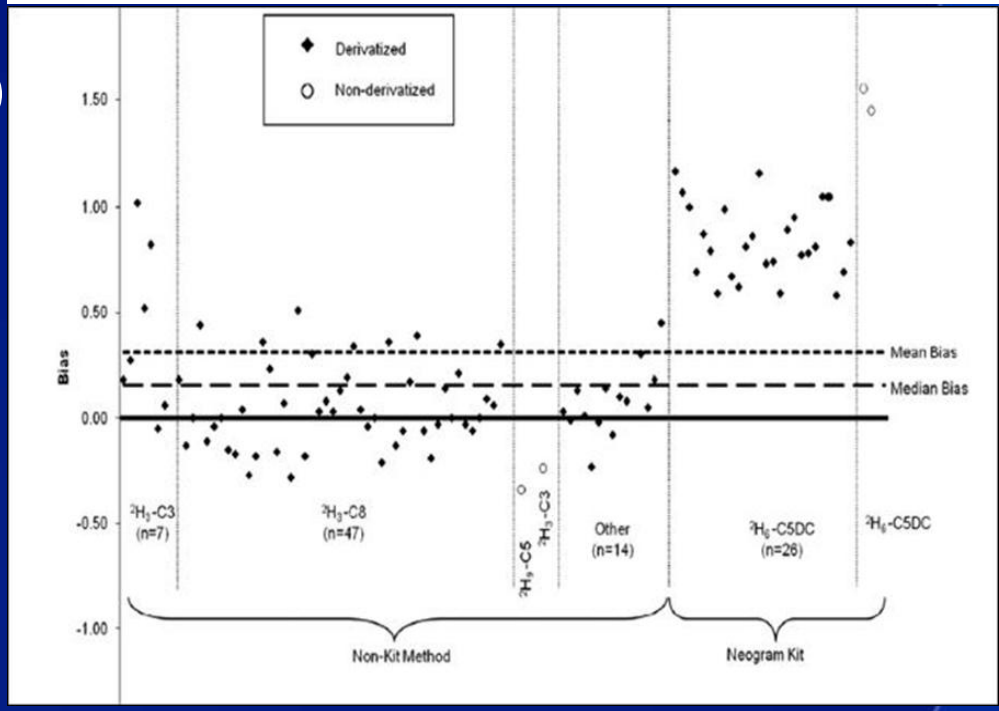


NSQAP adapts to ensure high-quality screening

- **PT Testing**
  - NSQAP new category: C3DC + C4OH
  - Allows for reduced corrective action reports
  - No double-dipping!
  - On-line reporting category: live in January 2012 (as of 11-07-2011)
  - Instructions will be provided as soon as web site changes are completed
- **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
- **Many challenges remain for C3DC 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

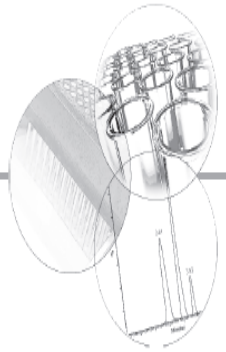
NSQAP Web Site: http://www.cdc.gov/labstandards/nsqap.html

Why Must We Assure Assay Quality in Newborn Screening Labs?

- ✓ Early and accurate detection of congenital disorders saves lives!

FOREWORD

SPECIAL FOCUS: DRIED BLOOD SPOTS



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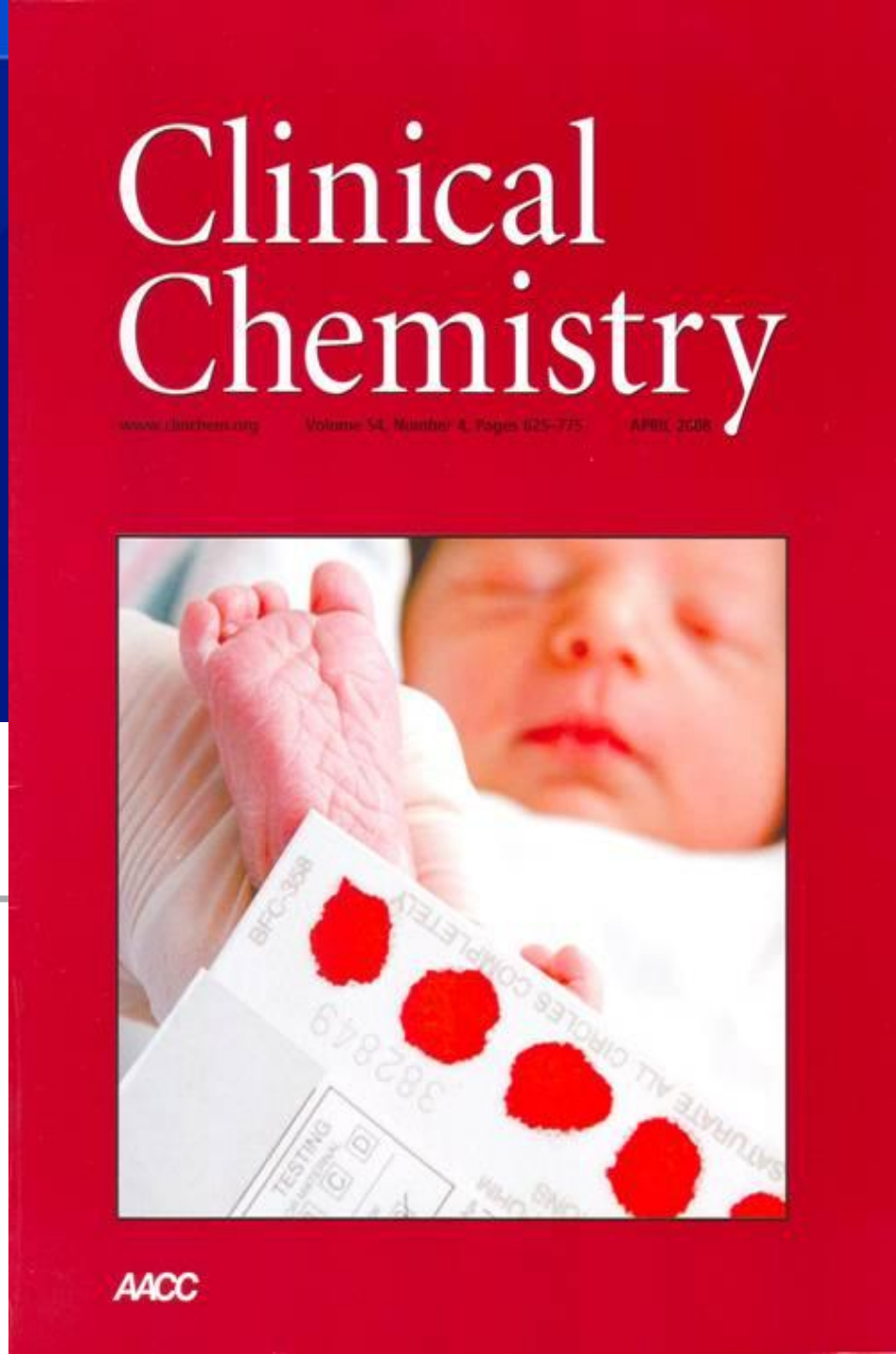
A glowing future for dried blood spot sampling

"...a number of factors have recently come together to encourage this industry to break out of its shell and look for suitable alternatives to traditional plasma sampling."



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  - W. Harry Hannon, PhD
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- **Collaborators**
  - Association of Public Health Laboratories
  - US newborn screening laboratories
  - International newborn screening laboratories

Thank you for your attention!