Comparison of Derivatized and Non-Derivatized Tandem Mass Spectrometry Methods for the Detection of Metabolic Disorders in Newborns

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Project Overview

- **Pilot Project**: The California Genetic Disease Laboratory conducted a state mandated MS/MS research project (January 07, 2002 through June 13, 2003) to investigate the expansion of the existing newborn screening program.
- **Specimens**: The blood spot specimens were collected from the existing Newborn Screening Program.
- **Instrument Evaluation:** The four tandem mass instruments were evaluated and matched for their analytical performance by testing approximately 5,000 blood spot specimens.
- **Reference Ranges:** The reference ranges and cutoffs were established by analyzing 5,000 newborn specimens.

Project Overview

- **Newborns Tested**: A total of 354, 074 newborn blood specimens were tested to ascertain which of the 25 metabolic disorders of fatty acids, organic aciduria, amino acidopathies detectable by MS/MS Method could be included into our existing Newborn Screening Program.
- **Informed consent** was obtained for this additional use of the newborn blood specimens.
- **Disorders Identified**: Identified 50 cases of genetic disorders *using PerkinElmer Neogram MS/MS derivatized method*.
- **Follow-Up**: The patients were referred to genetic centers for follow-up care.

Project Overview

Total Cases (50) Detected Excluding PKU

Disorders	Detected	Detected by Revised Cutoff	Prevalence
MCADD	13		1/27, 000
SCADD/EMA	18		1/20, 000
MMA/PA	11		1/32, 000
LCHADD		1	1/354, 000
GA-I	1		1/354, 000
GA-II	2		1/177, 000
3-MCC	2		1/177, 000
Argininemia	1		1/354, 000
VLCADD	1	1 prenal-dx	1/354, 000
MSUD	1	1	1/354, 000
Total	50	3	1/6680 (No PKU)

Project Overview

- **Derivatized method** required a lengthy butanolic HCL extraction and utilized environmentally unfriendly chemicals.
- **Thus, a second study** was conducted in which two MS/MS methods, the derivatized and *the non-derivatized* were evaluated and compared for analytical performance and suitability for the screening program.
- **Newborns Tested**: A total 37, 149 paired newborn specimens were tested for comparison.

Comparison
Use of Ancillary Equipment

Derivatized Method
(based on 500 specimens)

1. Puncher (one set)
2. Incubator/Shaker (3 sets)
3. Evaporator (2 sets)
4. Plate Sealer (1 set)
5. Fume hood and Gas supply system
6. Microtiter plates (22)
7. Butanol-HCL

Non-Derivatized Method
(based on 500 specimens)

1. Puncher (one set)
2. Incubator/Shaker (1 set)
3. Not required
4. Not required
5. Not required
6. Microtiter plates (6)
7. Not required

Specimen Extraction

Derivatized Method

1. Extract a 3.2 mm blood spot with methanol/:water (75:25) extraction solution containing stable-isotope labeled internal standard (30ºC; 30min).
2. Transfer and air dry the extract at 60ºC.
3. Derivatize w/butanolic HCL for 30 min at 60ºC.

Non-Derivatized Method

1. Extract a 3.2 mm blood spot with methanol/:water (75:25) extraction solution containing stable-isotope labeled internal standard (30ºC; 30min).
2. Perform analysis.

Specimen Extraction

Derivatized Method	Non-Derivatized Method
4. Dry the samples at 40°C using air as drying agent.	4. Not required.
5. Reconstitute.	5. Not required.
6. Perform analysis.	6. Not required.

Specimen Throughput

Derivatized Method

1. Run wash samples in between assay plates to reduce system clogging.
2. 510 tests per instrument in 24 hours.

Non-Derivatized Method

1. Wash samples not required.
2. 765 tests per instrument in 24 hours.

Results

Comparison of Linearity (EP 10)

Amino Acids				
Derivatized Method			**Non-Derivatized Method**	
	Regression Line	**R^2**	**Regression Line**	**R^2**
PHE	**y=1.247x+88.962**	**0.9992**	**y=1.194x+88.893**	**0.998**
TYR	**y=0.905x+84.759**	**0.9987**	**y=1.0255x+84.389**	**0.9996**
LUE	**y= 0.7236x+165.19**	**0.9996**	**y= 0.9031x+270.74**	**0.999**

Comparison of Linearity (EP10)

- **Acylcarnitines**

Derivatized Method			Non-Derivatized Method	
	Regression Line	R^2	Regression Line	R^2
C0	y=1.2164x +49.989	0.9999	y=0.9961x+35.089	0.9995
C3	y=1.2844x+2.3219	0.9999	y=1.3014x+2.3772	1.000
C8	y=1.014x+0.3457	0.9984	y=1.0782x+0.3394	0.9876
C18	y=0.9014x+0.6753	0.9913	y=0.9696x+1.2951	0.9973

Recovery (EP 10)

Amino Acids			Acylcarnitines		
Analyte	Derivatized Method	Non-Derivatized Method	Analyte	Derivatized Method	Non-Derivatized Method
Valine	75%	74%	C0	121%	98%
Alanine	71%	74%	C2	85%	123%
Citrulline	104%	131%	C3	127%	130%
Phenylalanine	128%	115%	C4	99%	126%
Tyrosine	94%	99%	C8	108%	129%
Leucine	70%	91%	C12	107%	114%
Methionine	116%	100%	C14	76%	111%
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Precision as Coefficient of Variation (EP10)

Amino Acids			Acylcarnitines		
Analyte	Derivatized Method	Non-Derivatized Method	Analyte	Derivatized Method	Non-Derivatized Method
Valine	19%	13%	C0	6%	16%
Alanine	10%	21%	C2	8%	15%
Citrulline	5%	16%	C3	7%	16%
Phenylalanine	11%	14%	C4	23%	34%
Tyrosine	11%	20%	C8	25%	29%
Leucine	10%	15%	C12	26%	22%
Methionine	18%	23%	C14	21%	16%
			C16	45%	27%
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Carryover

- **The MS/MS instruments have shown statistically insignificant ($p<0.01$) carryover for each method.**

Operational cost

Analysis of 500 Specimens	Derivatized (D) Method	Non-Derivatized (N) Method	Cost Reduction for N
Punching Time	120 min	120 min	---
Extraction Process Time	216 min	52 min	~76% reduction in time
Ancillary Equipment (AE)	Incubator/Shaker (3sets) Evaporator (2 sets) Plate sealer (1set) Fume hood (1set) Microtiter plates (22) Gas supply system	Incubator/Shaker (1set) Not required Not required Not required Microtiter plates (6) Not required	~ 90% reduction of AE
Reagents for Sample Derivatization	Butanol/HCL	Not required	Less cost for reagents

Operational cost

Analysis of 500 Specimens	Derivatized (D) Method	Non-Derivatized (N) Method	Cost Reduction for N
Specimen Throughput (ST)	24 hrs	20 hrs	~ 17% increase in ST
Preventive Maintenance (PM)	Incubator/Shaker (3) Evaporators Fume hood Gas supply system	Incubator/Shaker (1) Not required Not required Not required	~ 40% less PM
Staff/Training (ST)	Man power (3 chemists, 2 laboratory assistants) Training (20 days)	Man power (2 Chemists) Training (18 days)	~ 10% less ST
Safety (S)	Storage and disposal of environmentally unfriendly chemicals	Not required	~ 5% less requirements of S

Conclusions

- Both assays are highly correlated.
- The distributions are log normal.
- Both methods have shown equivalent analytical performance.
- The cutoffs for each analyte will be different.
- The study recommends that the screening results of an analyte should be reported based on more than one cutoffs, e.g., use of ratios and secondary markers.
- Both methods are suitable for screening newborn specimens.
- The Non-Derivatized method is rapid and cost effective compared to the Derivatized MS/MS Method.