Newborn Screening by Tandem Mass Spectrometry: A Comparison of Underivatized Methods

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Introduction

PerkinElmer Life Sciences recently released an MS/MS chemistry kit (The NeoGram™ AAAC kit) for the measurement of amino acids, free carnitine and acylcarnitines from dried blood spots. Use of the kit and the corresponding data acquisition method with the PerkinElmer system is intended to provide analyte concentrations and relationship profiles that aid in the identification of elevated blood amino acid, free carnitine and acylcarnitine levels in newborns. Quantitation and interpretation of these elevated levels and the relationships between them has enabled the detection of a variety of metabolic disorders. "Certain disorders require complex metabolic profiles and intermetabolic relation to detect disease with low false-positive and no false-negative rates" (1). It is only through MS/MS technology that *screening* for many of the disorders is possible.

The NeoGram™ AAAC kit provides the reagents and method for measuring amino acids, free carnitine and acylcarnitines by using a sample derivatization process. PerkinElmer is currently developing a method and reagents for the measurement of amino acids, free carnitine and acylcarnitines that does not require the derivatization of sample material. Sample preparation is much less complex, requires less equipment, and reduces personnel exposure to harsh chemicals. Sample acquisition time is ~2 minutes.

Summary

The PerkinElmer Underivatized assay was evaluated for method comparison and bias estimation following NCCLS guidelines. The purpose of the study was to compare the assay to a method currently in use for routine amino acid and acylcarnitine profiling in a newborn screening environment. The Wisconsin Newborn Screening Lab performs newborn sample analysis using an underivatized MS/MS method for routine screening. Comparison between the NeoGram and Wisconsin methods include C0, C2, C3, C4, C6, C8, C10, C12, C14, C16 and Leu, Met, Phe, Tyr and Val.

Results

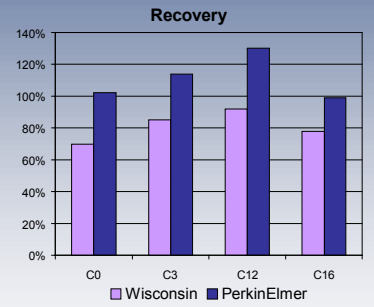
Identical sample sets, consisting of 50 samples each and representing reportable ranges, were assayed in duplicate by the NeoGram and Wisconsin methods. The comparison values for C2, C4, C6, C8, C10, C14, Leu, Met, Phe, Tyr, and Val ranged from zero to twenty percent difference between methods. The comparison for analytes C0, C3, C12 and C16 show greater than twenty percent difference. This difference was further evaluated by calculating recovery. Although both methods show good recovery, variation on this parameter affect the correlation between methods (See Figure 1). Table 1 shows the observed correlation between the methods.

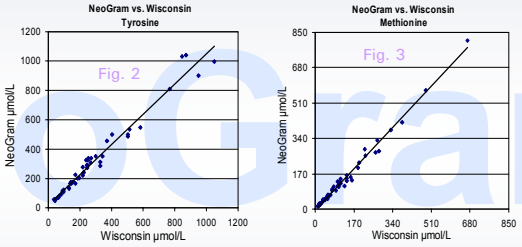
Table 1. Correlation Between Methods

	Slope/Intercept	R^2		Slope/Intercept	R^2
C0	1.43x + 0.04	0.98	**C14**	1.00x + 0.45	0.94
C2	1.17x + 1.09	0.96	**C16**	1.23x + 0.06	0.96
C3	1.31x + 0.21	0.98	**Leu**	1.20x + 25.4	0.98
C4	1.18x + 0.04	0.94	**Met**	1.17x - 5.10	0.99
C6	1.12x + 0.50	0.92	**Phe**	0.93x + 11.6	0.97
C8	0.97x + 0.35	0.95	**Tyr**	1.02x + 20.7	0.97
C10	1.02x + 0.07	0.92	**Val**	1.02 x + 17.4	0.97
C12	1.40x + 0.77	0.95			

Figure 1. Recovery Data

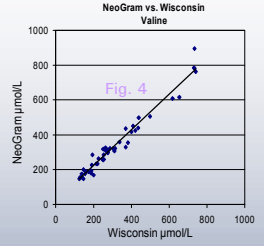
Differences seen in C0, C3, C12, and C16 results are clarified by the calculated recovery from each method for these analytes.





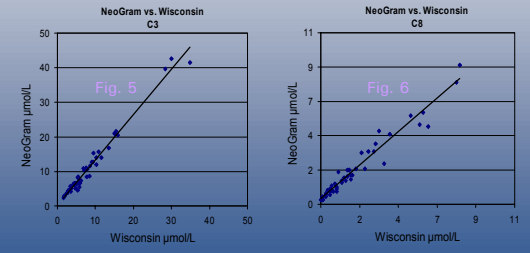
Figures 3 - 4.

Graphs of Tyrosine, Valine and Methionine display examples of the linear relationships and good correlation obtained from the comparison data.



Figures 5 & 6.

Graphs of Propionyl carnitine and Octanoyl carnitine display examples of linear relationships and good correlation obtained from the comparison data.



Additional Comparison Data: CDC Results

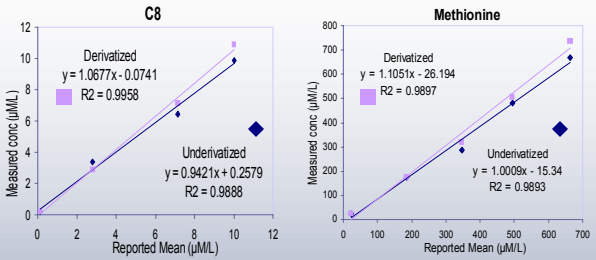
PerkinElmer participates in the CDC Newborn Screening Quality Assurance Program. The tandem mass spectrometry samples are tested by both the derivatized and underivatized NeoGram methods. The results of the first quarter 2002 samples show good correlation both for the measured values compared to the added amount of analytes as well as in comparison to the mean values reported to the CDC from other MS/MS laboratories. A comparison of the measured NeoGram results to spiked analyte amounts is made in Table 2 below.

Table 2. Measured vs. Added

	DERIVATIZED		UNDERIVATIZED	
ANALYTE	SLOPE	R^2	SLOPE	R^2
C3	0.84	0.999	1.22	0.99
C4	1.25	0.978	0.89	0.943
C8	1.28	0.988	1.13	0.974
C14	1.19	0.991	0.99	0.997
C16	0.81	0.982	1.3	0.997
Val	0.86	0.96	0.96	0.98
Tyr	1.02	0.993	1.17	0.98
Leu	1.04	0.993	1.08	0.97
Phe	1.23	0.997	1.1	0.98
Met	1.05	0.99	0.95	0.99

Examples of regression line comparisons between measured analyte values and the mean values reported to the CDC are displayed below in figures 7. and 8.

Figures 7 and 8. Measured vs. CDC Reported Values



Conclusions

The present study shows that the NeoGram Underivatized assay performs well in comparison to an established newborn screening MS/MS method that also does not require derivatization. The correlation observed between the methods was satisfactory and suggests that the NeoGram Underivatized assay will perform appropriately when used for the application of newborn screening. Furthermore, the good correlation between the NeoGram Derivatized and Underivatized methods against CDC MS/MS samples, indicates that the underivatized method performs comparably to the more established derivatized assay. Additionally, the underivatized method brings the added benefit of less complexity in sample preparation and time and cost savings associated with this. The NeoGram Underivatized method is still preliminary and further development is underway.

References 1. U.S. Department of Health and Human Services CDC. Using Tandem Mass Spectrometry for Metabolic Disease Screening Among Newborns. MMWR April 13, 2001; Vol. 50, No. PR-3.

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