

A COMPARISON OF NON-DERIVATIZED AND DERIVATIZED METHODS FOR NEWBORN SCREENING BY MS/MS

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INTRODUCTION

PerkinElmer Life Sciences has developed a non-derivatized MS/MS method for the analysis and profiling of free carnitine, acylcarnitines, and amino acids extracted from newborn dried blood spots. In this study, we compare the new PerkinElmer non-derivatized assay to the non-derivatized assay used by the Wisconsin State Department of Hygiene as well as to the current PerkinElmer derivatized method.

METHODS AND MATERIALS

SAMPLES

Identical sample sets, consisting of fifty samples each and representing reportable ranges were assayed by the NeoGram and Wisconsin methods. In addition to the 50 samples, CDC Proficiency Testing (PT) samples were tested and the Non-derivatized and Derivatized results were compared to the added analyte amounts and the values reported to the CDC from global laboratories. C5DC was tested separately.

REAGENTS

- PerkinElmer's NeoGram Non-derivatized Amino Acids and Acylcarnitines Reagent Kit - development materials.
- PerkinElmer's NeoGram Derivatized Amino Acids and Acylcarnitines Reagent Kit - production materials.
- Wisconsin's In-house method and reagents for combined amino acids and acylcarnitines analysis.

RESULTS AND DISCUSSION

PERKINELMER'S NON-DERIVATIZED vs. DERIVATIZED

The comparison between PerkinElmer's derivatized and non-derivatized methods indicate acceptable correlation with the slopes of linear regression analyses showing estimated differences ranging from 1.0% to 18.0% (average 8%) for 18 of the 24 analytes studied. Of the six analytes that showed consistent bias (C0, C2, C10, C16, Gly and Arg), there were four instances in which the bias was due to better recovery by the non-derivatized method (C2, C10, C16 and Arg). For these four analytes, the non-derivatized results were clearly closer to the expected values of the samples.

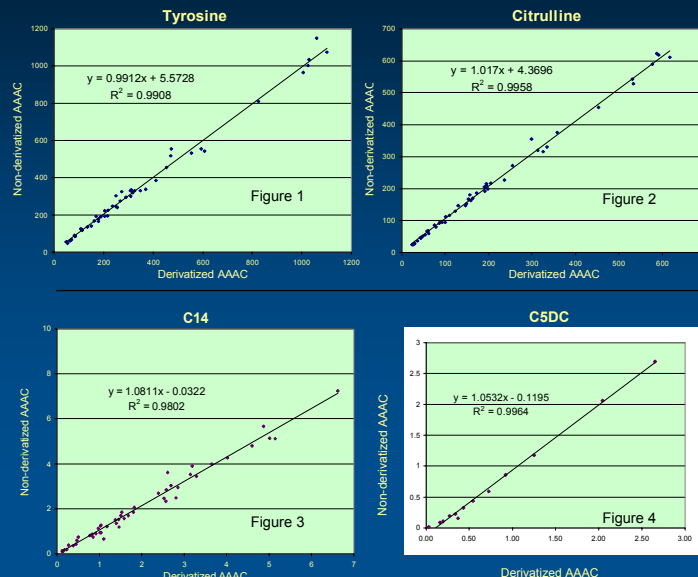
For C0 the significant bias ($y = 0.76x - 7.7$) was as expected given the fact that derivatization causes some degree of hydrolysis on the acylcarnitines. This hydrolysis produces free carnitine (C0) and thus the higher recoveries observed with the derivatized method. As for the remaining analyte Gly, the non-derivatized method yields only recoveries of about 50%. Gly is a small molecule and it requires larger collision energies to efficiently fragment and produce the desired neutral loss of 46 amu for measurement. In contrast, the derivatized method employs the loss of the butyl group (derivative) to measure the abundance of Gly. The energetic requirements are much more favorable for the loss of the butyl group from the glycine-butyl ester than for the concerted loss of H₂O and CO (loss of 46 amu) from the amino acid (Table 1 and Figures 1 - 4).

It is important to highlight that the performance of C5DC is remarkably similar when measured by either the non-derivatized or the derivatized assay. As shown in Figure 4, there is virtually no compromise when measuring C5DC by the non-derivatized method.

Table 1. Non-Derivatized vs. Derivatized

Analyte	Non-Derivatized	Derivatized
Ala	$y = 0.89x + 38.9$ R ² = 0.9617	$y = 1.39x - 0.3$ R ² = 0.9801
Arg	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
Asp	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
Cit	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
Glu	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
Gly	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
Leu	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
Met	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
Orn	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
Phe	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
Tyr	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
Val	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C0	$y = 1.06x + 3.8$ R ² = 0.9829	$y = 0.94x + 0.4$ R ² = 0.9922
C2	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C3	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
C4	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
C6	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
C8	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C10	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
C12	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C16	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
C18	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
C20	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
C22	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C24	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
C26	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C28	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
C30	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
C32	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
C34	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C36	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
C38	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C40	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
C42	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
C44	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
C46	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C48	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
C50	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C52	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
C54	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
C56	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
C58	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C60	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
C62	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C64	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
C66	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
C68	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
C70	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C72	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
C74	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C76	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
C78	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
C80	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
C82	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C84	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
C86	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C88	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704
C90	$y = 1.04x + 7.3$ R ² = 0.9885	$y = 1.13x + 0.03$ R ² = 0.9589
C92	$y = 0.99x + 5.6$ R ² = 0.9762	$y = 1.18x + 0.3$ R ² = 0.9636
C94	$y = 1.04x + 28.2$ R ² = 0.9548	$y = 1.11x + 0.2$ R ² = 0.9548
C96	$y = 0.76x - 7.7$ R ² = 0.9663	$y = 1.56x - 9.8$ R ² = 0.915
C98	$y = 0.94x + 0.4$ R ² = 0.9922	$y = 1.56x - 9.8$ R ² = 0.915
C100	$y = 0.99x + 15.9$ R ² = 0.9704	$y = 1.15x - 0.3$ R ² = 0.9704

Figures 1 - 4. Tyr, Gly, C8 and C5DC Derivatized vs. Non-derivatized



NON-DERIVATIZED vs. WISCONSIN

The correlation between the methods is satisfactory and the relationship is linear for each of the 15 analytes (Table 2). The comparison values for C2, C4, C6, C8, C10, C14, Leu, Met, Phe, Tyr and Val ranged from zero to twenty percent difference between the Wisconsin and non-derivatized methods. One example is shown as Figure 5 below. The comparison for analytes C0, C3, C12 and C16 show differences greater than twenty percent (23-43%). These differences were further evaluated by calculating the recovery from the samples. The increase in method bias for these analytes is due to greater recovery by the NeoGram method (Figure 6).

Table 2. Non-Derivatized vs. Wisconsin

Analyte	Slope + Intercept	R ²	Analyte	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	0.96	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	1.02x + 17.4	0.97
C12	1.40x + 0.77	0.95			

Figure 5. Methionine Results Non-derivatized vs. Wisconsin

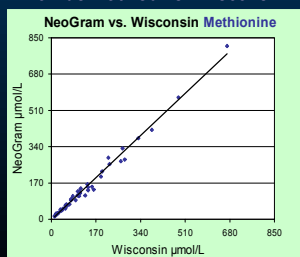
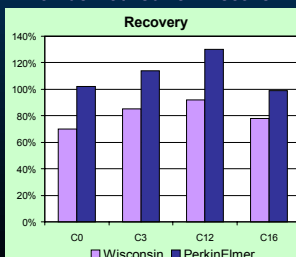


Figure 6. C0, C3, C12, C16 Non-derivatized vs. Wisconsin



CDC COMPARISON

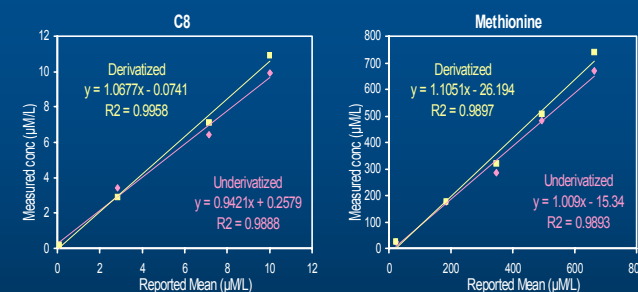
PerkinElmer participates in the CDC Newborn Screening Quality Assurance Program. Both the derivatized and non-derivatized NeoGram methods were used to analyze the tandem mass spectrometry PT samples. The results of the first quarter 2002 samples show good correlation for the measured values compared to the added amount of analytes. The slopes of regression line comparisons between the measured values and the CDC spiked levels ranged from 0.81 to 1.28 for the derivatized method and 0.89 to 1.3 for the non-derivatized method (Table 3). The results also show good comparison to the mean values reported to the CDC from other MS/MS laboratories, most of which use a derivatized method of sample preparation.

Table 3. PerkinElmer Measured vs. CDC Added

ANALYTE	DERIVATIZED		UNDERIVATIZED	
	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 non-derivatized assay performs well in comparison to an established newborn screening MS/MS method that also does not require derivatization. The correlation between the methods was satisfactory and suggests that the NeoGram non-derivatized assay will perform appropriately when used for the application of newborn screening.

The observed correlation between the NeoGram derivatized and non-derivatized methods indicates that the non-derivatized method performs comparably to the more established derivatized assay. Furthermore, the good correlation between the NeoGram non-derivatized method results against CDC MS/MS PT added values shows that the non-derivatized method provides acceptable results that are quite comparable to the values reported from other laboratories using a variety of methods.

ACKNOWLEDGEMENTS

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