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A verification of the application of the non-derivatized mass spectrometry method in newborns screening of metabolic disorders

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Abstract

It is required that the clinical screening of metabolic disorders in newborns meet International Organization for Standardization 15189-2012 approval. The new tandem mass spectrometry (MS/MS) based screening system and its companion reagent should be independently authenticated before their implementation in clinical diagnosis laboratories.

Linearity, stability, accuracy, and precision evaluations were carried out to verify the performance of the Waters ACQUITY TQD MS/MS system with the NeoBase non-derivatized MS/MS PerkinElmer kit for detecting amino acids and acylcarnitine in newborns with metabolic disorders.

Statistically, the correlation coefficient (R^2) of 0.9982 to 0.9999 indicates good linearity. The measurements at the beginning and end of the reagent storage procedure were taken for stability verification. No significant difference was detected between the 2 periods. The amino acid exhibited a degree of bias in the range of 0% to 14.17%, with acylcarnitine's being was in the range of 0% to 14.84%; they consequently passed the quality assessment requirements for clinical laboratories of the China National Centre. The amino acids' within-run, between-run, and day-to-day run precision were 1.19% to 7.68%, 1.63% to 5.01%, and 4.77% to 12.48%, respectively, while the total imprecision was 5.55% to 13.33%. Acylcarnitine's within-run, between-run, and day-to-day run precision was 1.2% to 8.43%, 0.19% to 9.60%, and 2.33% to 10.74%, respectively, while it's total imprecision was 6.57% to 13.99%. The manufacturer declared that the total imprecision of the tests, using Multiple Reaction Monitoring, should be less than or equal to 25% of the coefficient of variation for the kit's high and low-quality control levels.

The performance of the non-derivatized MS/MS screening system in detecting the amino acids and acylcarnitines passed the test's requirements. It was maintained in accordance with the routine clinical chemical detection system.

Abbreviations: CV = coefficient of variation, IMD = inherited metabolic diseases, ISO = International Organization for Standardization, MRM = multiple reaction monitoring, MS = mass spectrometry, RSD = relative standard deviation, SD = standard deviation.

Keywords: accuracy, acylcarnitines, amino acids, linearity, precision, stability, tandem mass spectrometry

1. Introduction

Inherited metabolic diseases (IMD), also known as inborn errors of metabolism, refers to the enzymes, receptors, and cell membrane dysfunctions involved in and caused by genetic defects.[1] These diseases lead to the blockage of metabolic pathways, and an accumulation of intermediate or bypass products or a lack of terminal products, resulting in a variety of clinical symptoms. IMDs often remain undetected due to a frequent lack of clear symptoms during the early stages of neonatal development; symptoms are more commonly apparent during childhood, and may be progressively aggravated as the IMDs cause irreversible damage to the nervous system or even death. Consequently, it is often too late, by the time that clinical symptoms appear, to provide optimal treatment, such that the diseases lead to moderate-to-severe neuropsychological dysfunction, mental retardation, and even mortality.[2] Thus, neonatal disease screening or screening for congenital and inherited diseases causing serious harm in neonates can lead to their early diagnosis, avoiding or reducing the harm they cause through prevention and treatment.[3,4] The practice of screening for neonatal diseases began in the 1960s and has been employed for years to justify screening programs.[5,6] China has been developing such programs since the 1980s.

Tandem mass spectrometry (MS/MS) has been employed to extensively screen for numerous genetic metabolic neonatal diseases.[2,7] Recent advances in laboratory technology practices using MS/MS involving the application of single dried blood spot samples to filter paper have enhanced the identification of IMDs in

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newborns.[8] Due to the broad spectrum of diseases it covers, along with its specificity, ease of sample preparation, and high throughput,[2] MS/MS technology has led to the development in many countries of multidisorder newborn screening programs for 3 major types of genetic metabolic diseases, namely, amino acid disorders,[9,10] organic acidemias,[1] and fatty acid oxidation defects.[1,7]

According to the special guide for the International Laboratory Organization (ISO) ISO15189-2012, the "Quality and Ability Requirements for Medical Laboratories"[11] and the "Newborn Screening by Tandem Mass Spectrometry"[12] provided by the Clinical and Laboratory Standards Institute, USA, MS/MS and its companion reagents require independent verification by the user to confirm whether the detection system meets the laboratory's intended use. However, until now, few reports have been conducted on the comprehensive verification of related instruments and reagents used by other laboratories.[13,14] Even in the reported literature, local laboratory test samples were used. There remains, on the one hand, some doubt as to the reliability of the data obtained by the unverified equipment. Furthermore, a certain time is required to collect samples for use in clinical evaluations. A standardized method for universal laboratory use is difficult to establish.

Based on this situation, the laboratory used amino acid and acylcarnitine interstitial blood test samples from the Inspection Testing Centre of China's National Institute of Health and Family Planning Commission as an evaluation sample for the detection of neonatal genetic metabolic disease. Amino acid and acylcarnitine screening systems consisting of ACQUITY TQD MS/MS from Waters and its supporting reagents, the NeoBase non-derivatized MS/MS kit from PerkinElmer Inc, MA, were validated according to the most current standards. The verification criteria included linear range, reagent stability, accuracy, and precision. These verification indices were all indicators included in the interstitial sample quality assessments performed by the clinical inspection center.

2. Material and methods

2.1. Samples

This study was approved by the Hospital Ethics Review Board and the approval number is Bath 2018, No. 072. Filter paper dried blood spot specimens were used to evaluate all of the programs. Quality assessment samples of amino acid and acylcarnitine by MS/MS were employed by the Inspection Testing Centre of the China National Institute of Health and Family Planning for the screening of neonatal genetic metabolic diseases.

2.2. Instruments and reagents

The MS/MS system of the Waters Corporation (Massachusetts) includes an MS/MS ACQUITY TQD, a high-performance liquid chromatography (1525 μ) and a 2777 compact sample manager (2777C). The reagents were from the non-derivatized assay kit (PerkinElmer) for a variety of amino acids, and acylcarnitine and succinylacetone screening. The microplate incubated shaker Mb100-4a was from Allsheng Instruments Co, Ltd (Hangzhou, China).

2.3. Linearity evaluation

According to the linearity evaluation of the CLSI EP6-A quantitative measurement procedures and compared with the quality control blood spot sample from the Clinical Inspection Centre (2017), L was used for the first amino acid concentration, while acylcarnitine was employed in the second concentration spot as L and the fifth concentration as H. The scheme followed is as follows in Table 1.

Table 1

Linearity evaluation formula.

Concentration gradient	Optimum ratio
1	201711 (L)
2	0.833L + 0.167H
3	0.667L + 0.333H
4	0.500L + 0.500H
5	0.333L + 0.667H
6	0.167L + 0.833H
7	201715 (H)

A 75 μL sample was transferred each time from the U- to the V-plate, covered by an aluminum foil film, and then assessed by the machine. From the response intensity ratio of the sample to the internal standard as the abscissa, X, and the corresponding solution concentration ratio as the ordinate, Y, the regression equations were obtained.

2.4. Stability test

In order to measure the consistency between the 2 working solutions of newly prepared reagent and the stable ending reagent, samples of each concentration were repeatedly measured at least 6 times to calculate their average value. The newly prepared and stable ending working fluids were measured in the same analytical run so as to minimize instrumental imprecision. The consistency of the average values between the 2 groups was applied by the T test. The test results were analyzed by SPSS statistic software (IBM, New York) using the T test.

2.5. Accuracy evaluation

The accuracy of the MS/MS system was evaluated in 2017 by the Department of Health in a quality assessment using the ventricular blood spot sample. The results were calculated according to the scoring standards for external quality assessments.

2.6. Precision evaluation

2.6.1. The relative standard deviation (RSD) of the within-run. Three sets of samples, each at high, medium and low concentrations, were appraised according to the proposed analytical method. The samples were measured sequentially, each in its own analytical run, after the instrument was turned on. The standard deviation (SD) and RSD values of each concentration sample were calculated.

2.6.2. The RSD of the between-run. Three sets of samples, each at high, medium and low concentrations, were assessed according to the proposed analytical method. Three such experiments were completed within 1 business day. The SD and RSD values of each concentration sample were calculated.

2.6.3. The RSD of the day-to-day run. The same batch of reagents and calibrators were used to perform 2 experiments in 1 business day. Each sample was measured twice in each

Table 2

Results of the linearity evaluation of the amino acids.

Index	Linear equation	Correlation coefficient (R^2)	Linear range
Alanine (ALA)	$y=0.8973x-0.0687$	0.9994	194.08–863.82
Arginine (ARG)	$y=0.8477x-0.0019$	0.9982	7.01–31.54
Citrulline (CIT)	$y=1.1429x-0.0249$	0.9993	50.42–392.08
Leucine (LEU)	$y=1.0295x-0.0879$	0.9998	162.34–707.81
Methionine (MET)	$y=1.0329x-0.0351$	0.9985	20.41–370.33
Phenylalanine (PHE)	$y=0.9189x-0.0285$	0.9998	39.60–559.26
Tyrosine (TYR)	$y=0.8976x-0.0387$	0.9998	55.66–928.05
Valine (VAL)	$y=0.8976x-0.0519$	0.9997	111.51–831.66

Table 3

Results of linear evaluation of acylcarnitine.

Index	Linear equation	Correlation coefficient (R^2)	Linear range
Free carnitine (C0)	$y=0.9402x+0.004$	0.9998	1.9–253.17
Acetylcarnitine (C2)	$y=0.8072x+0.1712$	0.9998	1.1–165.70
Propionylcarnitine (C3)	$y=0.9174x-0.0025$	0.9996	0.03–19.50
Butyrylcarnitine (C4)	$y=0.9092x+0.0294$	0.9999	0.03–18.72
Hydroxybutyrylcarnitine (C4OH)	$y=0.8857x+0.0039$	0.9987	0.05–3.81
Isovalerylcarnitine (C5)	$y=0.8571x+0.0106$	0.9995	0.03–4.75
Glutarylcarnitine (C5DC)	$y=0.973x+0.0115$	0.9995	0.03–4.37
Hexanoylcarnitine (C6)	$y=0.9603x+0.006$	0.9992	0.01–2.71
Octanoylcarnitine (C8)	$y=0.9072x+0.002$	0.9996	0.02–2.77
Decanoylcarnitine (C10)	$y=0.8971x+0.0096$	0.9999	0.01–5.75
Lauroylcarnitine (C12)	$y=0.9147x+0.0003$	0.9999	0.08–5.59
Myristoylcarnitine (C14)	$y=0.7183x+0.0284$	0.9982	0.07–8.29
Palmitoylcarnitine (C16)	$y=0.8539x+0.0666$	0.9999	0.21–51.82
Octadecanoylcarnitine (C18)	$y=0.9259x+0.005$	0.9999	0.74–5.39

experiment, to a total of 5 business days. Further analysis was performed to find the mean coefficient of variation (CV) with no significant difference being found between any 2 or more results.

3. Results and discussions

The linearity evaluation was carried out according to the CLSI EP6-A quantitative measurement method.[15] The correlation coefficient (R^2) was 0.9982 to 0.9999, indicating that the linearity was good. The results are shown in Tables 2 and 3. The verification determined that the linear ranges of the testing items provided by the manufacturer were too high to cover the horizontal ranges of the daily test specimens. For example, the linear range of C0 as declared by the manufacturer was 51 to 2930 μmol/L. The lowest level of C0 in our laboratory specimen was 1.07 μmol/L, which was lower than the limit for the linear range. Similar results were found in the other testing items. During this verification, we expanded the linear range of all the indicators to make them conform better to the daily testing requirements as shown in Tables 2 and 3.

A significant difference was found to exist between the 2 groups of data if $P<.05$. As shown in Tables 4 and 5 below, there was no significant difference between the results measured at the beginning and end of the kit storage period. The stability period of the reagents in the kit as declared by the manufacturer was 4 weeks. This study verified that the reagents passed the stability index.

The first dried blood spots specimens for the inter-laboratory quality assessments conducted in 2017 were tested in this research and the degree of bias calculated, as shown in Tables 6 and 7 below. The results were calculated according to the scoring standard of external quality assessment at the Ministry of Health. The passing rate was 100%, indicating that all indices underwent accuracy evaluation.

The precision evaluation results were expressed as % CV. The within-run precision of the amino acid index was 1.19% to 7.68%, while the between-run precision was 1.63% to 5.01% and the day-to-day run was 4.77% to 12.48%. The total imprecision of the amino acid was 5.55% to 13.33%. The within-run precision of the acylcarnitine index was 1.2% to 8.43%, the between-run was 0.19% to 9.60% and the day-to-day run was 2.33% to 10.74%. The total imprecision of acylcarnitine was 6.57% to 13.99%.

Table 4

Stability evaluation results for the amino acids.

Index	Low	Medium	High
ALA	0.129	0.191	0.802
ARG	0.974	0.069	0.553
CIT	0.083	0.078	0.727
LEU	0.091	0.245	0.966
MET	0.233	0.163	0.909
PHE	0.008	0.164	0.936
TYR	0.314	0.331	0.775
VAL	0.714	0.144	0.650

ALA = alanine, ARG = arginine, CIT = citrulline, LEU = leucine, MET = methionine, PHE = phenylalanine, TYR = tyrosine, VAL = valine.

Table 5

Stability evaluation results for acylcarnitine.

Index	Low	Medium	High
C0	0.088	0.606	0.276
C2	0.889	0.937	0.686
C3	0.184	0.898	0.307
C4OH	0.423	0.524	0.114
C4	0.184	0.680	0.332
C5	0.225	0.301	0.266
C5DC	0.184	0.837	0.585
C6	0.184	0.944	0.221
C8	0.184	0.501	0.541
C10	0	0.458	0.344
C12	0.225	0.913	0.145
C14	0.423	0.336	0.980
C16	0.118	0.804	0.154
C18	0.053	0.765	0.065

The manufacturer declared that the total imprecision of the tests using the MRM method should be at or below 25% CV at the low and high-quality control levels in the kit. Through the precision verification, we found that the amino acid and acylcarnitine detection systems consisting of Waters ACQUITY TQD MS/MS and its associated reagent NeoBase non-derivatized MS/MS Kit had a large bias range for the different testing items. The high- and low-value biases of the same test item were also irregular, which may be related to the stability of the internal standard of each test. The results of the precision test are shown in Tables 8 and 9:

Table 6

Accuracy evaluation results for the amino acids.

Index	Bias (%)					Score
	201,711	201,712	201,713	201,714	201,715	
ALA	0.65	−4.01	−4.12	−1.1	−10.11	100%
ARG	−13.27	−10.39	−14.17	−12.56	6.63	100%
CIT	3.07	0.72	−0.46	10.29	−3.29	100%
LEU	1.24	−1.35	−3.38	4.45	−7.45	100%
MET	6.9	4.32	0	4.91	−5.56	100%
PHE	7.9	−0.35	−3.38	3.54	−7.79	100%
TYR	6.83	−7.11	−7.84	−0.41	−11.56	100%
VAL	1.43	−2.14	−6.07	2.52	−9.13	100%

ALA = alanine, ARG = arginine, CIT = citrulline, LEU = leucine, MET = methionine, PHE = phenylalanine, TYR = tyrosine, VAL = valine.

Table 7

Accuracy evaluation results for acylcarnitine.

Index	Bias (%)					Score
	201,711	201,712	201,713	201,714	201,715	
C0	6.84	−5.16	9.04	−5.74	−12.33	100%
C2	11.45	−4.08	0.26	−11.77	−10.54	100%
C3	13.5	−1.6	11.08	0.27	−5.33	100%
C4	0	−0.65	10.21	0.34	−10.95	100%
C4OH	0	−7.23	0.56	−11.71	−13.91	100%
C5	0	0	7.56	0	−4.63	100%
C5DC	0	−13.22	0.82	−12.64	−11.45	100%
C6	0	0	10.95	−8.96	−8.86	100%
C8	0	−5.19	4.9	−8.92	−10.83	100%
C10	0	−3.81	8.26	−7.83	−10.96	100%
C12	0	−2.83	10.08	−6.88	−10.73	100%
C14	−12.22	−11.92	−11.59	−9.89	−7.62	100%
C16	0	−2.73	8.78	−7.36	−10.96	100%
C18	4.05	−5.91	2.12	−11.54	−14.84	100%

Table 8

Precision evaluation results for the amino acids (% CV).

Index	ALA	ARG	CIT	LEU	MET	PHE	TYR	VAL
Low concentration								
RSD of within-run	1.88	7.68	1.19	1.80	2.76	1.84	2.02	3.45
RSD of between-run	2.11	5.56	4.59	3.31	5.01	3.82	2.20	4.97
RSD of day-to-day run	4.77	6.51	5.66	5.15	5.16	4.93	5.95	4.84
Total imprecision	5.55	11.23	8.03	6.59	9.55	6.95	8.11	7.67
Medium concentration								
RSD of within-run	1.99	1.64	5.06	2.75	2.36	2.02	3.32	1.82
RSD of between-run	1.64	3.05	3.80	2.64	3.34	3.30	1.63	3.15
RSD of day-to-day run	7.95	4.99	6.84	5.40	12.48	12.35	9.36	5.85
Total imprecision	11.21	7.72	10.22	8.07	13.33	12.97	10.32	8.39
High concentration								
RSD of within-run	5.08	6.33	4.61	4.30	5.57	4.72	5.11	5.55
RSD of between-run	1.72	2.27	2.29	2.33	2.04	1.74	2.54	2.59
RSD of day-to-day run	8.60	7.97	6.23	8.91	8.92	8.25	7.28	8.35
Total imprecision	10.51	10.79	8.36	10.35	10.96	9.84	9.16	10.64

ALA = alanine, ARG = arginine, CIT = citrulline, CV = coefficient of variation, LEU = leucine, MET = methionine, PHE = phenylalanine, RSD = relative standard deviation, TYR = tyrosine, VAL = valine.

Table 9

Precision evaluation results for acylcarnitine (%).

Index	C0	C2	C3	C4OH	C4	C5	C5DC	C6	C8	C10	C12	C14	C16	C18
Low concentration														
RSD of within-run	7.25	4.16	5.97	6.83	4.57	4.74	8.65	6.60	4.64	5.01	6.53	7.87	4.76	1.33
RSD of between-run	6.68	9.60	7.32	8.81	7.04	6.38	7.74	4.74	5.10	5.65	2.52	4.95	2.49	3.24
RSD of day-to-day run	9.26	7.59	9.15	10.74	7.22	6.14	9.60	8.49	7.54	7.07	7.54	6.08	3.28	5.26
Total imprecision	14.10	13.99	12.12	13.71	9.94	10.27	12.57	11.45	9.76	9.25	9.44	13.69	8.29	7.55
Medium concentration														
RSD of within-run	4.49	5.61	3.89	8.43	8.34	6.41	2.34	3.89	5.39	3.53	5.03	2.67	3.13	2.73
RSD of between-run	4.45	5.06	3.10	4.10	4.65	6.38	3.43	5.03	5.93	6.34	4.44	1.85	4.00	5.46
RSD of day-to-day run	9.12	7.26	8.32	4.25	6.29	9.15	8.72	8.67	7.51	9.52	8.01	8.27	7.51	7.96
Total imprecision	11.41	10.46	9.72	11.22	11.93	13.24	9.65	10.74	11.04	12.07	10.46	9.08	9.07	10.09
High concentration														
RSD of within-run	3.84	1.45	1.20	2.30	1.36	5.48	3.43	5.62	5.76	3.19	2.27	4.56	3.07	3.42
RSD of between-run	0.19	2.66	2.81	2.98	4.54	2.51	4.25	3.50	4.14	2.84	2.26	2.76	2.51	2.18
RSD of day-to-day run	4.95	7.73	4.86	2.33	5.68	4.25	2.97	3.26	6.92	5.22	4.37	10.07	5.31	4.89
Total imprecision	7.66	8.67	6.82	6.57	8.78	8.33	8.55	8.18	10.75	8.52	8.16	13.45	8.53	8.69

RSD = relative standard deviation.

4. Conclusions

This study verified the linearity, accuracy, and precision of Waters' newborn screening MS/MS platform and the PerkinElmer NeoBase non-derivatized MS/MS kit reagent, with a linear correlation coefficient of 0.9985 to 0.9998. In this project, some of the performance parameters provided by the manufacturer, such as those for free carnitine, were found to have narrow linear range such that it was unable to cover the detection range of the daily sample concentration levels. This verification test expanded the linear range of all the indicators to better align with the scope of the daily testing, with the possibility for further expansion. A laboratory should establish a linear range according to the actual situation. In the accuracy evaluation, the amino acid bias was 0% to 14.17% while that for acylcarnitine was 0% to 22.22%. The bias range greatly differed for diverse items, with even high and low concentrations of the same indicator having irregular biases; this may be related to the stability of the internal standard. However, the results were calculated according to the scoring standard from the Ministry of Health's external quality assessment, demonstrating that all indices underwent the accuracy evaluation.

In general, the precision evaluation for each index meant that the day-to-day run precision of the clinical chemical detection system was greater than that of the between-run precision, which itself was greater than that of the within-run batch. Each index was found to meet the performance parameters stated by the manufacturer.

In summary, this study revealed that the performance of the neonatal genetic metabolic disease screening system, which consists of Waters AQUITY TQD MS/MS and PerkinElmer non-derivatized amino acids and the acylcarnitine detection kit, meets the manufacturer's declared parameters. This detection system can be managed in accordance with the relevant standards for conventional clinical chemistry testing systems.

Author contributions

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