OUTCOMES FROM TANDEM MASS SPECTROMETRY (MS/MS) WORKSHOPS IN THE UNITED STATES AND THE PERFORMANCE EVALUATION OF MS/MS LABORATORIES

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Abstract. Disorders of fatty acid oxidation and organic acid metabolism produce serious clinical problems including death. Introduction of MS/MS technology for newborn screening allowed detection of these disorders in a single process, more than doubling the number of disorders that can be detected from dried-blood spots in newborn screening. Expanded newborn screening has become a critical issue with increased public awareness and demands. Screening by MS/MS is operational in several private testing and public health laboratories. Guidelines and quality assurance services are essential to enhance program expansions. The first of two workshops was organized in June 2000 and the second in September of 2001 to discuss procedures for integrating MS/MS into newborn screening programs. Both workshops addressed technical problems encountered with implementing MS/MS testing. One outcome of the first workshop was a pilot survey for assessing performance of MS/MS laboratories worldwide, which occurred in September 2000. This pilot survey led to the expansion of the proficiency testing services to include MS/MS testing. There are 32 participating laboratories, three of the ten countries represented are from the Asia-Pacific Region.

INTRODUCTION

The introduction of tandem mass spectrometry (MS/MS) technology into population-based newborn screening resulted in the ability to simultaneously detect numbers of disorders in fatty acid oxidation and in organic and amino acid metabolism from a single process using dried-blood spot (DBS) specimens (Millington *et al*, 1989; 1991). This powerful and versatile MS/MS technology enabled programs to screen for more than 20 metabolic and non-metabolic diseases for which early detection can result in significant improvement in health outcomes of newborns (Rashed *et al*, 1995). The increased awareness and demands from various groups to implement MS/MS technology into newborn screening have led to two recent workshops, along with other meetings, to address issues and provide guidelines for newborn screening laboratories worldwide. Screening by MS/MS is operational in several private testing and public laboratories (Chace *et al*, 1998). Guidelines and quality assurance (QA) services are essential to newborn screening program expansions.

The Newborn Screening Quality Assurance Program (NSQAP) at the Centers for Disease Control and Prevention (CDC) has a long history of involvement in QA activities and performance evaluations that serve and support newborn screening laboratories worldwide. In September 2000, NSQAP initiated a worldwide proficiency testing (PT) pilot program for operational MS/MS newborn screening laboratories in order to develop and refine an independent external assessment of MS/MS laboratory performance.

MATERIALS AND METHODS

In June 2000, the National Newborn Screening and Genetics Resource Center (NNSGRC), in collaboration with CDC, the Health Resources and Services Administration (HRSA), and the Association of Public Health Laboratories (APHL), organized and hosted the first MS/MS workshop Enhancing the Implementation of Tandem Mass Spectrometry in Newborn Screening Programs in San Antonio, Texas. This workshop was a working meeting of invited participants involved in MS/MS testing to discuss the operational procedures for effectively integrating MS/MS into the analysis of DBS routinely collected for newborn screening programs. Workshop participants identified needs, developed viable recommendations, and provided working guidelines for MS/MS operation in newborn screening. The workshop addressed technical problems encountered with implementing MS/MS testing: reagents, methodologies, and instrument protocol differences; cutoff decision criteria for selection of presumptive positive tests; quality

control; patient follow-up; diagnostics, treatment, and management; and overall program evaluation (Centers for Disease Control and Prevention, 2001). The same collaborators also cosponsored and helped organize a second MS/MS workshop, "Enhancing the Implementation of Tandem Mass Spectrometry for Newborn Screening Laboratories," on September 10-11, 2001, in Madison, Wisconsin. The proceedings from this workshop are in preparation.

The pilot proficiency testing (PT) program provided laboratories with three quarterly panels of blind-coded DBS specimens and to provide each laboratory an independent external assessment of its performance. All DBS specimens in the surveys were prepared from intact whole blood of 55% hematocrit. The first PT panels for MS/MS were distributed in April 2001 to participating domestic and international laboratories. The acylcarnitine DBS panel contained five specimens enriched with predetermined amounts of propionylcarnitine (C3), butyrylcarnitine (C4), octanoylcarnitine (C8), myristoylcarnitine (C14), and palmitoylcarnitine (C16). The amino acids panel contained five specimens enriched with predetermined amounts of phenylalanine (Phe), leucine (Leu), methionine (Met), tyrosine (Tyr), and valine (Val). All PT specimens were certified for homogeneity, accuracy, stability, and suitability for MS/MS analysis. Specimen sets were packaged in a zip-close metallized plastic bag with desiccant, and instructions for analysis and data-report forms were included separately in the shipping package and analytic performance was monitored and evaluated.

RESULTS AND DISCUSSION

Recommendations from the first working group meeting in June 2000 have been published (Centers for Disease Control and Prevention, 2001). This report included recommendations from various work groups of invited participants. The laboratory working group recommendations addressed issues in nomenclatures, specimen and standard preparation, MS/MS resources, instrument calibration, reduction in instrument-to-instrument variability, quality control activities, MS/MS data interpretation and results reporting, and storage of specimens and controls. The same short-term and long-term follow-up approaches were recommended for conditions detected by MS/MS method as with other newborn screening follow-ups. The work group made specific proposals in the area of diagnosis and treatment, and on the evaluation of MS/MS screening.

The second MS/MS meeting in September 2001 brought together over 200 participants with different levels of expertise and experience in newborn screening to continue addressing issues in implementing MS/MS technology. The second workshop reviewed the recommendations from the first MS/MS meeting, shared program experiences, and proposed new ideas. Publication of its proceedings is pending.

The MS/MS pilot PT survey led to the expansion of the NSQAP's services to disorders detectable by MS/MS testing. Listed in Table 1 are the 36 active participants in the PT program, 21 laboratories from 10 countries, and 15 laboratories in the United States. Of these laboratories, four are from the Asia-Pacific Region representing 3 countries. Two international laboratories and one domestic laboratory participated in the PT component for acylcarnitine analysis only.

Table 1. *Participants in the MS/MS proficiency testing program*

Country	Participants
Argentina	1
Australia	1
Canada	2
Denmark	1
Germany	3
Hong Kong	1
The Netherlands	1
Saudi Arabia	1
South Africa	1
South Korea	2
United Kingdom	7
United States	15
Total participants = 36	

Data were obtained over a 9-month period from three PT survey panels, and most participants measured all analytes in the PT challenges. Participants were requested to report both analytic values and cutoff values used for presumptive clinical assessments. Performance varied among the participants producing large 95% confidence intervals for the mean values for some analytes.

Tables 2 and 3 show data for mean, minimum, and maximum cutoff values of amino acids and acylcarnitines reported by MS/MS laboratories in April 2001. Table 2 shows that a wide range between minimum and maximum values were reported for tyrosine (Tyr) and leucine (Leu).

Table 2. Mean, minimum, and maximum cutoff values for amino acid reported by MS/MS laboratories.

	μmol/l Blood			mg/dl Blood		
Analyte	N	Mean cutoff value	Min/Max	N	Mean cutoff value	Min/Max
Phe	16	161	87-240	15	2.8	1.8- 4
Leu	10	356	245-535	10	4.9	3.2- 7
Met	11	78	30-201	11	1.2	0.5- 3
Tyr	12	300	100-561	11	6.1	1.8-10.2
Val	10	280	120-427	10	3.3	1.4- 5

Table 3. Mean, minimum, and maximum cutoff values reported by MS/MS laboratories for acylcarnitines.

	μmol/l Blood		
Analyte	N	Mean cutoff value	Min/Max
C3	15	7.7	2.8 -25
C4	12	1.4	0.5 - 2
C8	17	0.5	0.15- 1
C14	10	0.9	0.3 - 1.9
C16	14	9.7	4.6 -28

Table 3 also shows a wide range between minimum and maximum values reported for propionylcarnitine (C3) and palmitoylcarnitines (C16). The values reported for butyrylcarnitine (C4), octanoylcarnitine (C8), and myristoylcarnitine (C14) on the other hand, indicated narrow ranges between the reported minimum and maximum values.

Fig 1 shows the differences between the reported and expected values for acylcarnitines by domestic and international laboratories. In general, scattering over wide ranges is seen for international laboratories relative to the domestic laboratories for acylcarnitines, whereas the scatter was similar for the two groups for amino acid measurements. The three laboratories from the Asia-Pacific Region reported mean values near the expected values. Similar pattern was observed for the amino acids data (Fig 2) reported by Asia-Pacific Region laboratories with values near the expected values.

The measurements for octanoylcarnitine by MS/MS is important for the detection of medium-chain acyl-CoA dehydrogenase deficiency in newborns, the primary justification for the use of MS/MS technology in newborn screening. Fig 3 shows the reproducibility for the octanoylcarnitine measurement over three different surveys in 2000 and 2001 for four domestic (A-D) and two international laboratories. Selection of these laboratories for illustration in Fig 3 was based on the known duration of their experience with these MS/MS assays. For the selected, most experienced domestic laboratories the reproducibility was excellent from survey to survey. However, for three of four domestic laboratories, the mean values were consistently below the expected value. The selected international laboratory showed increased improvement in measurements with each survey approaching closer to the expected value. The other international laboratory from the Asia-Pacific Region demonstrated an excellent ability to reproduce values near the expected value over the three PT surveys.

In the first PT survey, the specimen panel included a dose-response specimen set for octanoylcarnitine, the mean reported values should have been close to or equal to the line of identity with an ideal slope of one. For domestic (not shown) and international laboratories the data patterns were similar. Fig 4 shows the data for the international laboratories and the only Asia-Pacific Region laboratory in this

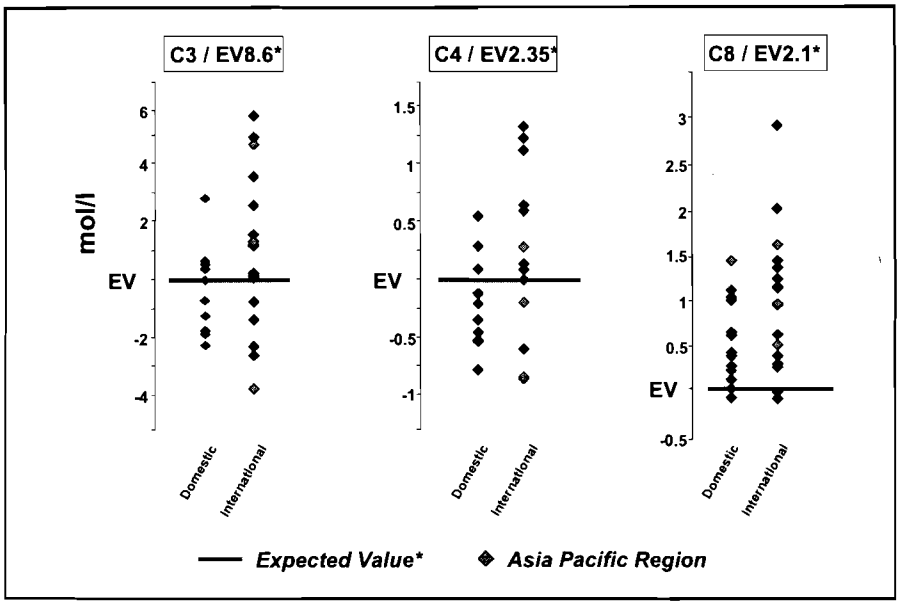

Fig 1. Difference between the reported and expected values for acylcarnitines, by domestic and international laboratories.

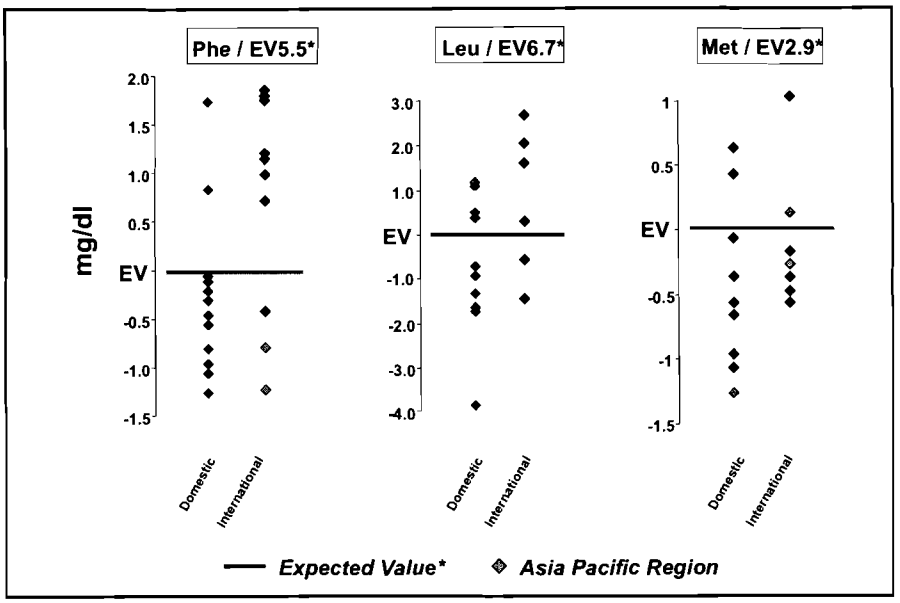

Fig 2. Difference between the reported and expected values for amino acids, by domestic and international laboratories.

survey. The mean reported values for international laboratories showed a large scatter around the line of identity. In Fig 4, the reported data for the laboratory from the Asia-Pacific Region indicated that the values were close to the line of identity across the dose-response curve.

CONCLUSION AND RECOMMENDATIONS

Recommendations, developed by the workshop participants has been useful to newborn screening programs for planning, guidance, program evaluation, harmonization, and comparison with other MS/MS programs, and for

defining the quality assurance and evaluation parameters necessary to ensure high-quality testing. The performance of the Asia-Pacific Region laboratories in the pilot PT study was similar to that of laboratories in the United States and worldwide and reported PT values from the Asia-Pacific Region were similar to those reported by the most experienced MS/MS laboratories in the United States.

The quarterly published summary reports for performance evaluation from NSQAP are an invaluable resource for MS/MS laboratories to assess and guide improvements in performance. The NSQAP provides immediate contact with laboratories to help resolve quality performance issues and to offer consultative services. The reporting of PT data by MS/MS laboratories is scheduled as a future enhancement to the NSQAP Internet Website. This electronic reporting process will permit faster access to PT data for international participants and a more timely focus on corrective actions. NSQAP is designed to help screening laboratories achieve excellent technical proficiency and maintain confidence in their performance while processing large numbers of

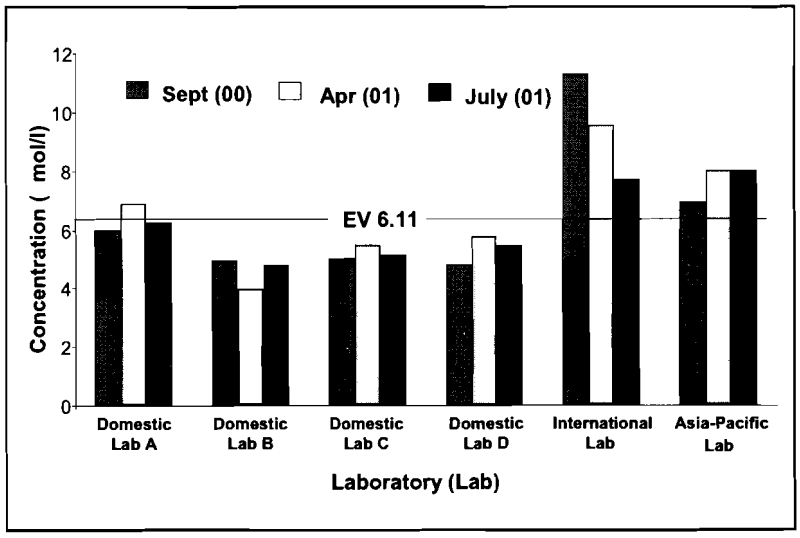

Fig 3. Reproducibility for octanoylcarnitine measurements.

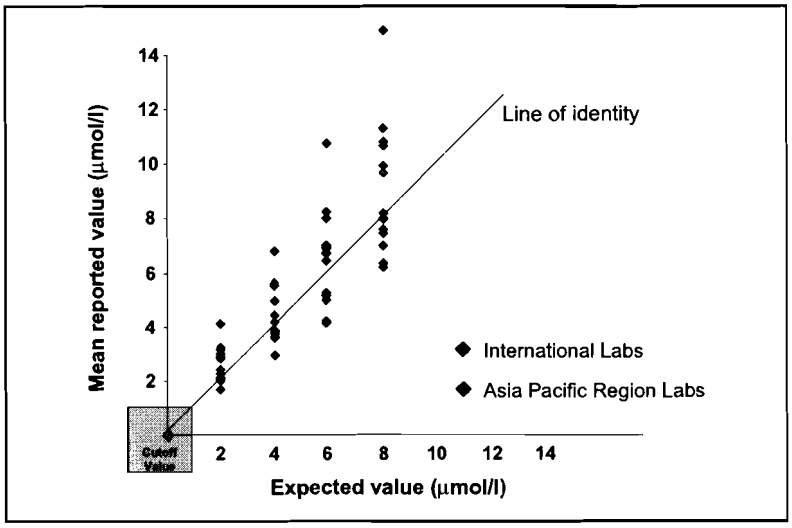

Fig 4. Results reported for a blind-coded specimen set containing a dose-response curve for octanoylcarnitine (C8).

specimens daily. The NSQAP will continue to expand its services and focus on helping to improve the quality of testing and the NSQAP and the NNSGRC will continue efforts to enhance implementation of MS/MS in newborn screening programs seeking assistance.

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