

MS/MS Based Expanded Newborn Screening: Instrument Platform Considerations

Yijun Li, Alex Cherkasskiy, Hari Nair and Blas Cerda.

Perkin Elmer Life and Analytical Sciences – Genetic Screening R and D, 940 Winters Street, Waltham, 02451



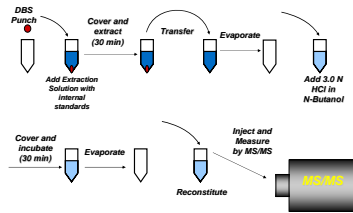
1 Introduction

The outcome of the isotope dilution mass spectrometry based MS/MS assays used for newborn screening is expected to be independent of the triple quadrupole mass spectrometry platform used. However, in principle, factors such as isotope effects can influence the performance of the mass spectrometer in a platform dependant fashion.

We report the results from a study that compared the performance of two MS/MS platforms commonly used for newborn screening: The Sciex API2000 and Waters Quattro Micro triple quadrupoles. The study consisted of an analytical evaluation in which performance characteristics of both the platforms were compared as well as a clinical comparison in which newborn and enriched samples were measured in parallel on both platforms.

Detailed comparison of the experimental precision, linearity and recovery as well as clinical sensitivity and specificity between the two platforms revealed that the two platforms were analytically and clinically equivalent.

2 The assay: Extraction and Quantitation of AA and AC



3 Analytical Equivalence

The objective of this study was to determine the analytical equivalence when measuring dried blood spots with the NeoGram Amino Acid and Acylcarnitines Derivatized kit on both the Sciex and the Waters tandem mass spectrometry platforms.

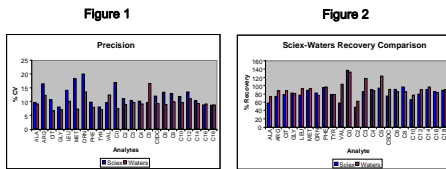


Figure 1: The total imprecision observed on Sciex (Blue) and Waters (Red) platforms. Precision was evaluated in parallel on both platforms. For these studies, dried blood spots at three different concentrations of amino acids and Acylcarnitines were analyzed in triplicate using the derivatized assay (three runs per day for a total of 5 days). The data from these experiments clearly indicate that both platforms provide adequate precision (MRM mode). The total imprecision observed on either platform was within 20% CV with most of the analytes with 10% CV or less.

Figure 2: The results of recovery studies for Sciex (Blue) and Waters (Red). With exception of Val, C3, and C5 where the Waters platform showed higher recoveries, there is very good correlation between both platforms. This level of correlation is not surprising as the extraction protocol used to prepare the samples was the same for both platforms. The differences observed are thus due to potential relative positive bias on the Waters Instrument VS the Sciex system for Val and C5.

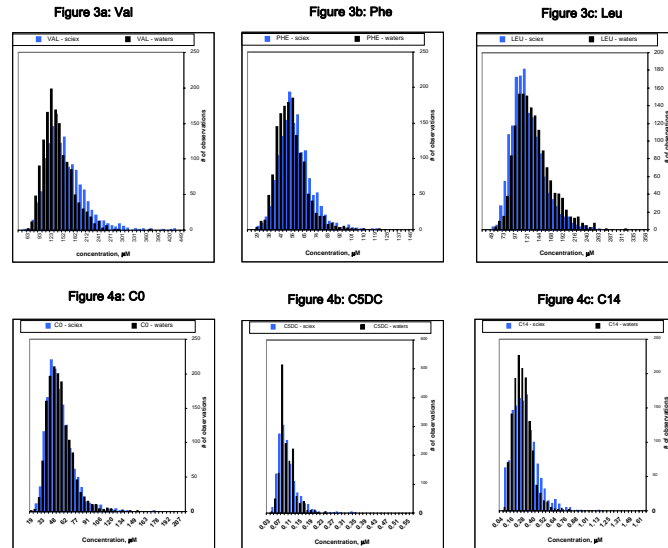
4 Clinical Equivalence: Objective

The objective of this study was to determine a correlation of results obtained from the clinical point of view when measuring dried blood spots with the NeoGram Amino Acids and Acylcarnitines Derivatized kit on both the Sciex and the Waters tandem mass spectrometry platforms.

Study Design: A clinical equivalence study was conducted with a sample set that consisted of 1514 unknown patient samples and 24 samples spiked (measured repeatedly) with different levels of C0, C2, C3, C4, C5, C5DC, C6, C8, C10, C12, C14, C16, C18, ALA, ARG, GLY, CIT, LEU, MET, ORN, PHE, TYR and VAL. The clinical analysis was performed by setting a predetermined reference point for each analyte that was taken to be the "cut off" for the study. The sample set was enriched with the spiked samples in order to increase the number of samples that were closer to the cutoff and thus in turn increasing the degree of challenge for the analysis of the platforms. The first level of analysis was a comparison of the mean and standard deviation for each analyte based on the patient cohort. The second level of analysis consisted on a comparison in which the result from each sample from each platform was determined to be on one side or another of the set cutoff. Comparisons were made on the number of concordant determinations between platforms.

5 Clinical Equivalence: Comparison of Distributions

In this portion of the study, the results from the measurement of 1514 unknown patient samples are compared in the form frequency distributions. Overlapping the distribution of the results between platforms provides a measure of correlation by comparing the mean and spread of the data between platforms.



Examples of the frequency distributions obtained are provided in Figures 3 and 4. From the data displayed, it was noted that for many analytes the frequency distributions on the Waters platform are often narrower than that of the Sciex instrument. Apparently, for those analytes, the precision on the Waters platform is slightly better. However, the data indicate that there is excellent correlation between platforms for all analytes.

7 Percent Agreement in Clinical Decision Between Platforms for Amino acids and Acylcarnitines

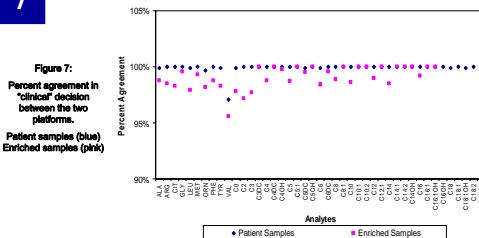


Figure 7: Percent agreement in "clinical" decision between the two platforms.

In order to determine the percent agreement between platforms, the paired results for each sample measured were compared against the corresponding analyte cutoffs. In this procedure, the potential outcomes for each pair of results are such that both measurements are on the same side of the corresponding cutoffs (concordant pairs) or not (discordant pairs). The percent agreement was determined by dividing the number of concordant pairs over the total number of paired measurements. This analysis was performed at two levels. In the first level the comparison is made using only the data from the unknown patient samples. In the second level the data from the *borderline* (enriched) samples is included in the analysis. It is in this second set where the test is most stringent because the enriched samples were very close to the set cutoffs. Overall, there is an excellent agreement between two platforms. The 95% agreement observed in the enriched data set is remarkable considering the fact that 25% of the samples in this set were borderline and thus very close or at the corresponding cutoffs.

6 Clinical Equivalence: Comparison against Cutoffs

This portion of the study focused on the correlation of results between platforms in relation to a cutoff. In these experiments, predetermined reference points were used to represent cutoffs for each of the analytes. Samples were measured repeatedly on both platforms and the results were compared against the corresponding cutoff. Each sample was chosen so that its expected concentration for a particular analyte was at or very close to the predetermined cutoff (i.e. borderline samples).

The expectation from these studies is such that the repeated paired measurement will result in a statistically random distribution around the cutoffs from which comparisons can be made to obtain indication of biases, reproducibility, and consistency in results between platforms.

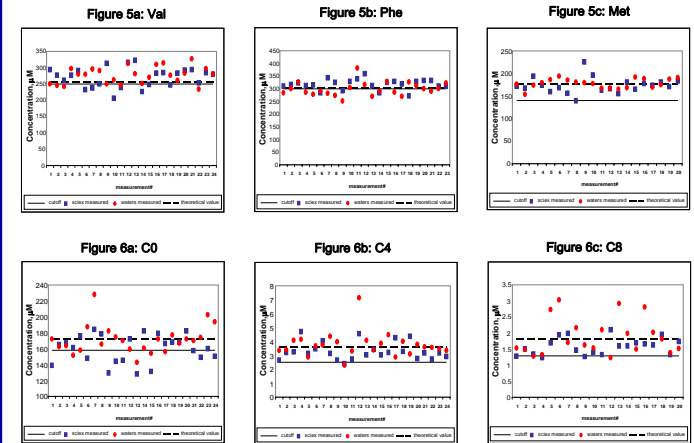


Figure 5 and 6 show the distribution of exemplary cases for these "borderline" sample tests. In most cases the results are statistically random distributions around the cutoff or expected value. These results are thus an indication that there are no significant biases between platforms and that both platforms can consistently provide reproducible results. The paired data indicate that in all cases there is a high degree of agreement between the platforms on the determinations made (that is, whether a data point is on one side or another of the corresponding cutoff).

8 Conclusion

- Analytical comparisons between the two platforms that included estimation of analytical bias, measurement of precision and accuracy, and comparison of measurements in multiple instruments reveal that the two platforms display analytical equivalence.
- The results of rigorous clinical evaluation indicate that both platforms, Waters and Sciex systems, are clinically substantially equivalent.