

# Comparison of Phenylketonuria Screening with a Fluorimetric Method and with Tandem Mass Spectrometry

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## INTRODUCTION

Early detection of diseases by newborn screening (NBS) is necessary for correct and timely clinical decisions. One of the early methods for NBS of phenylketonuria (PKU) was a fluorimetric method for quantification of phenylalanine (Phe). This method is still used in many countries in south-eastern Europe. The introduction of expanded NBS with tandem mass spectrometry (MS/MS) enabled screening for many diseases, including PKU. Slovenia is now in a unique position to compare the methods because it screens for PKU with a fluorimetric method detecting Phe and also using MS/MS for expanded NBS, also measuring Phe and tyrosine.

## METHODS

Fluorimetric method was performed using the Neonatal Phenylalanine kit from Perkin Elmer. The expanded NBS method is performed on a MS/MS in MRM mode, samples prepared using a nonderivatised kit NeoBase™ 2 Non-derivatized MS/MS kit from Perkin Elmer. Cut-off of Phe for both methods was 120  $\mu\text{mol/L}$ , on the MS/MS we have an additional ratio Phe/Tyr for PKU screening with the cut-off 2.15.

We compared measurements of nearly 7000 newborn blood spots. The descriptive statistics, Bland-Altman analyses and Spearman correlation coefficient were calculated. The numbers of true positives and false positives for both methods were compared.

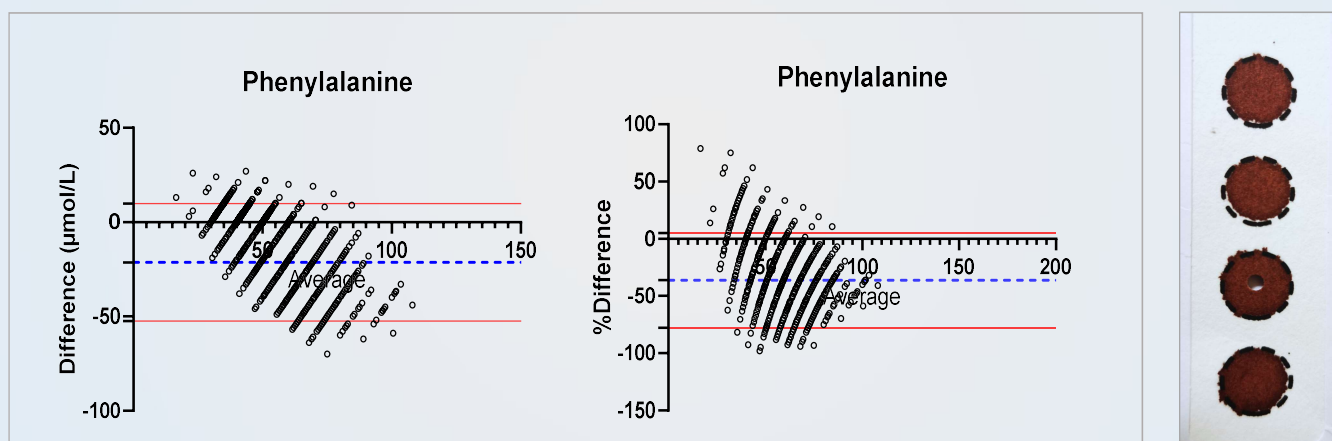


Figure 1: Bland – Altman plot between MS/MS method and fluorimetric method in  $\mu\text{mol/L}$  and %.

Picture 1: Dried blood spots.

Amino acids (n=6868)	Mean differences (%)	SD (%)	95% Limits of agreement (%)	Mean differences ( $\mu\text{mol/L}$ )	SD ( $\mu\text{mol/L}$ )	95% Limits of agreement ( $\mu\text{mol/L}$ )	Spearman correlation coefficient
Phenylalanine	-36	21.1	- 77.6 - 5.2	-21	15.9	- 52.4 - 9.8	0.49

Table 1: Comparison between MS/MS method and fluorimetric method: Bland-Altman (mean differences, SD and limits of agreement between methods in % and  $\mu\text{mol/L}$ ) and power of linear relationship between the two methods (Spearman correlation coefficient).

## RESULTS

Phe concentrations were 21 – 296  $\mu\text{mol/L}$ , with the mean of 46  $\mu\text{mol/L}$  (MS/MS method) and 10 – 280  $\mu\text{mol/L}$ , with the mean of 67  $\mu\text{mol/L}$  (fluorimetric method). Spearman correlation coefficient had a value of 0.49. Bland-Altman analysis comparing MS/MS method with the fluorimetric method in absolute values had a bias 21  $\mu\text{mol/L}$  (SD 15.9  $\mu\text{mol/L}$ ), in percent difference the bias was 36 % (SD 21 %). The fluorimetric method yielded 16 positive results, one of the patients being a true positive. The MS/MS method flagged only the one PKU patient as positive.

## CONCLUSION

The MS/MS and the fluorimetric method have a moderate correlation and the Bland-Altman analyses show that the fluorimetric method gives higher results. Nevertheless, the cut-off for MS/MS was the same as on the fluorimetric method, because the literature data and laboratory experiences do not warrant lower cut-offs. There were no false positives by MS/MS, which will occur after screening more newborns. Both methods did not have any false negatives. Our results show that MS/MS is more suited for NBS of PKU and as Phe and Phe/Tyr are screened as part of the expanded NBS, MS/MS screening for PKU is also more cost and time effective.