

**Electronic supplementary information**

**Nanostructured paper-based platform for phenylalanine neonatal screening by  
LED-induced fluorescence**

**Analytical Methods**

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## Electronic supplementary information

### Sample extraction

Neonatal samples and control samples were provided by the Blood Spot PHENYLALANINE NEONATAL-MW Kit. The manufacturer's instructions specifies how these samples were obtained: blood samples were spotted in filter paper number 903 in the center of a 1 cm circle.

The samples were pretreated following the procedure proposed by Seia et al.<sup>1</sup> with some modifications.

Briefly, the surface of the filter paper N° 903 with the blood sample was dried at room temperature for 12 h, avoiding heat and sunlight. then the samples were stored at 4 °C until the time of the studies.

For the tests, control samples of the Phenylalanine Blood Spot MW enzyme analysis kit and neonatal samples were used. In the quantification of Phe, a disc of filter paper with the bloodstain was sectioned and placed in an Eppendorf tube with 200 µL in glycine buffer pH 8.00 to 0.2 M and treated to a sonication procedure for 2 minutes. Finally, the contents of all the tubes were aspirated and the eluted samples were stored at 4 °C until use.

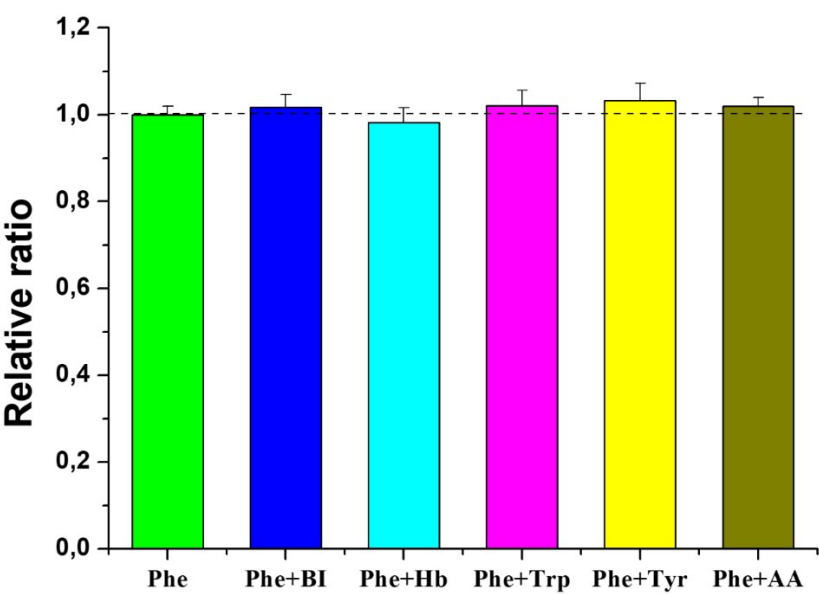
**Table 1.** Within-day and between-day precision for three levels of controls.

<sup>a</sup> Within-day (µM)	<sup>a</sup> Mean (µM)	<sup>a</sup> SD (µM)	CV (%)
Low (60)	60.62	2.273	3.75
Medium (420)	419.4	21.93	5.23
High (900)	899.6	37.07	4.12
<sup>a</sup> Between-day (µM)			

Low (60)	66.52	3.412	5.13
Medium (420)	425.4	24.76	5.82
High (900)	886.1	59.11	6.67

<sup>a</sup> Phe concentration (μM)

**Figure S1.** Selectivity study at normal physiologic levels of AA (66  $\mu$ M).



## References

- 1 M. A. Seia, P. W. Stege, S. V. Pereira, I. E. De Vito, J. Raba and G. A. Messina, *Anal. Biochem.*, 2014, **463**, 31–37.