

Supporting information

## A facile method for urinary phenylalanine measurements on paper-based lab-on-chip for PKU therapy monitoring.

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**Chemicals.** Phenylalanine Ammonia-Lyase from *Rhodotorula glutinis*, (2 U/0,28 ml), L-Phenylalanine ( $\geq 98\%$ ), *trans*-cinnamic acid ( $\geq 99\%$ ), sodium hydrogen phosphate ( $\geq 99\%$ ),  $\text{NH}_4\text{OH}$  28-30%, NaOH pellet ( $\geq 98\%$ ), Phenolphthalein powder and Ethanol ( $\geq 99.8\%$ ) were purchased by Sigma-Aldrich and used as received.

Extraction solution, isotopic labeled standard and flow solvent for MS-MS analysis were purchased by PerkinElmer as “NeoBase Non derivatized MS-MS kit”<sup>™</sup>

**Materials.** The Paper for microchamber substrate (Immobilon P Millipore) was purchased by Sigma/Aldrich. It was preactivated with alcohol methyl for 2 minutes before the protein immobilization. 96 microplate were purchased by Corning.  
Standard solutions of Phe, at concentration of 50, 300, 900 and 2500  $\mu\text{M}$  in buffer phosphate 10mM were prepared diluting a Phe stock solution (5 mM).

**Instruments.** All the analyses were carried out using the following equipments:

- Electrospray tandem mass spectrometer “Quattro Micro”, equipped with 1525 $\mu$  Binary HPLC pump and 2777C auto-sample manager, by Waters.
- Thermo-shaker “NCS Incubator”, by Wallac
- pHmeter “FiveEasy<sup>™</sup> FE20-KIT”, by Mettler Toledo
- Analytical balance “ML204”, by Mettler-Toledo
- Single ray Spectrophotometer Nanodrop ( $V_{\text{max}}=2\mu\text{L}$ , optical pathway 1 mm), by SpectraMax
- Q3-thermocycler developed by STMicronics.

## SI1 Paper-based lab-on-chip Fabrication

The lab-on-chip device is composed by different layers: a silicon part containing resistors for the heating and temperature control, a paper substrate glued to the first and finally a polycarbonate ring mounted upon the second layer to form the microchambers. A black plastic holder guarantee the easily device handling and a polycarbonate slide lid guarantee the microchamber sealing during the reaction.



**Fig. SI1** Paper-based Lab-on-Chip: a) components and b) cross section and c) full process on lab-on-chip

## SI2 UV-vis Spectrophotometric analysis

Kinetic study for PAL reaction were carried out by spectrophotometric technique, employed a PAL solution (0.156 units) in phosphate 10 mM at pH 8.31, the final L-Phe concentration was about 86  $\mu$ M.

In details: 2  $\mu$ L of a L-Phe 0.001M solution in 10 mM phosphate buffer at pH = 8.31 were added from time to time to 183  $\mu$ L of the PAL solution. After each addition, the optical absorption spectrum was acquired by a Nanodrop spectrophotometer. The absorbance values at wavelength of 273 nm, were corrected for the dilutions. After eight additions the final concentration of L-Phe was about 86  $\mu$ M. A comparison spectrum was performed with a solution of 86  $\mu$ M *trans*-cinnamic acid in 10 mM phosphate buffer at pH 8.31 obtained by diluting a stock solution 0.01M in the same buffer. The kinetics studies were carried out at temperature of 25°C and 35°C.

### **SI3 Preparation of urine mock sample, MS-MS analysis and evaluation of Phe concentration by proposed strategy on microplate wells**

#### *Preparation of mock samples in human urine.*

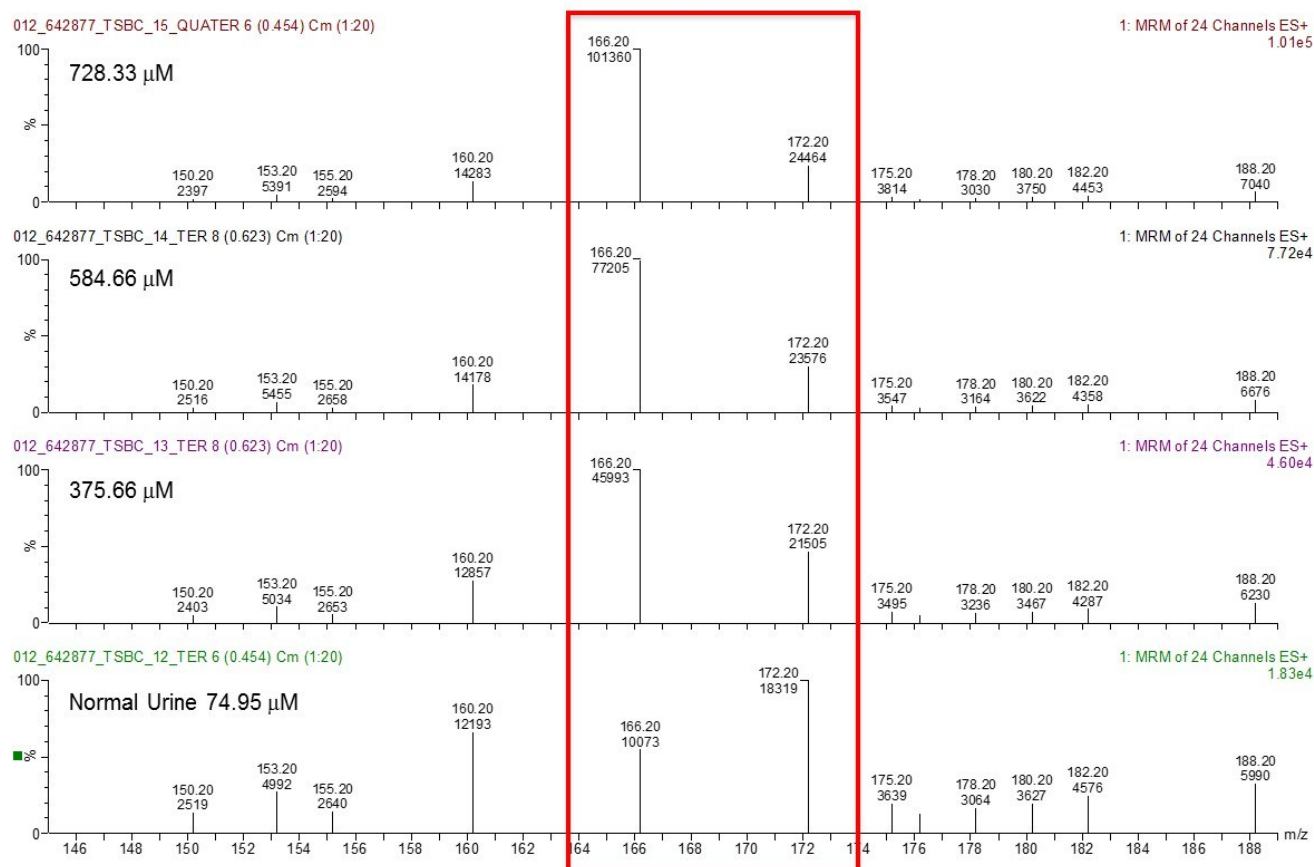
Urine sample was collected by a healthy donor. The mock sample with a Phe concentration of 295, 492 and 688  $\mu\text{M}$  were created diluting 5.9, 9.8 and 13.8  $\mu\text{L}$  of concentrated Phe stock solution (5 mM in deionized water) with urine containing endogenous Phe (74.95  $\mu\text{M}$ ) in a total volume of 100  $\mu\text{L}$ .

#### *Mock sample MS-MS measurements*

The quantitative measurement of Phe by MS-MS on mock samples, normal urine and urine of PKU treated patient were carried out using the NeoBase Non-derivatized MSMS kit (PerkinElmer 3040-0010). Urine specimens for mass spectrometry measurements were firstly centrifuged (1500 rpm, five minutes), then 3  $\mu\text{L}$  of supernatant were added to 97  $\mu\text{L}$  of extraction solution containing the internal standard of the L-Phe labeled with  $^{13}\text{C}$ . An aliquot of the final solution was injected into the mass spectrometer. The urine of healthy subjects and patients was obtained after at least three hours of fasting.

The MS-MS system consist of a mass spectrometer QuattroMicro (Waters) equipped with triple quadrupole analyzer and an ESI source. The mass spectrometer is coupled online to a system of HPLC pumps and an auto-sampler. The experimental conditions have been set as follows: 633L/hr nitrogen stream, 120  $^{\circ}\text{C}$  temperature, 350  $^{\circ}\text{C}$  desolvation temperature, 4KV capillary voltage, 24V cone voltage, RF lens 0.1. For each analysis, 20  $\mu\text{L}$  of solution were injected. The data were acquired using MassLynx 4.0 software, using the MRM (multiple reaction monitoring) mode. Phe concentrations were evaluated by internal standard method (Neolynx 4.0 software).

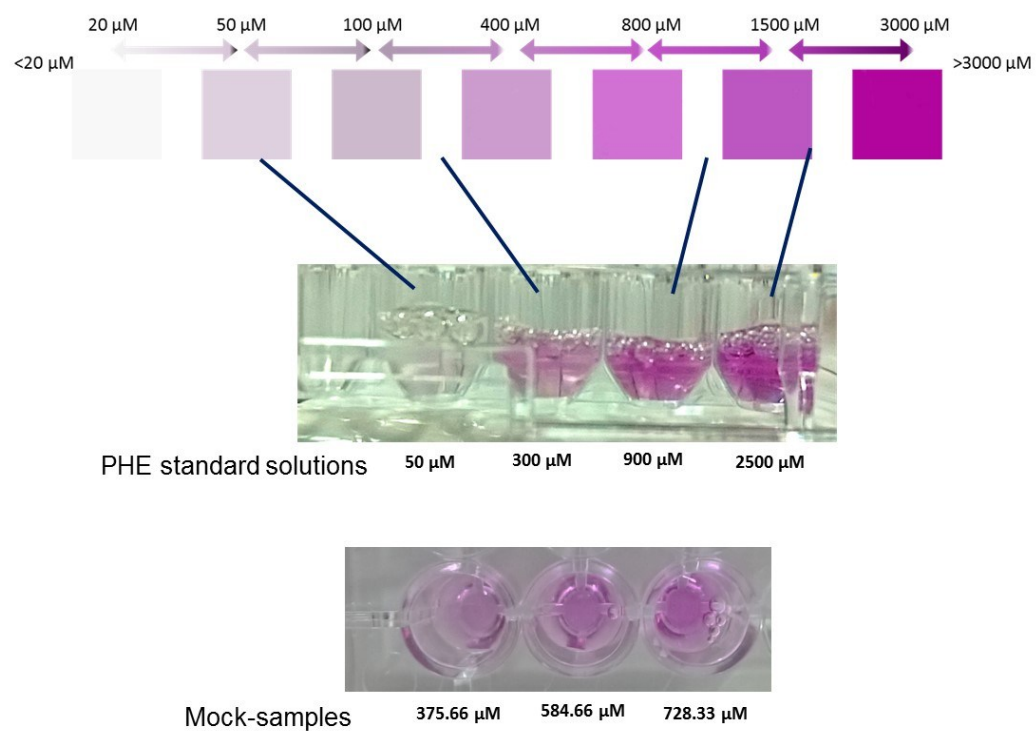
The MS-MS measurement report for all mock samples a Phe concentration of 375.66, 584.66 and 728.33  $\mu\text{M}$ , and a Phe concentration of 74.95  $\mu\text{M}$  for the normal urine containing endogenous Phe (Fig SI2).



**Fig. SI2** MS-MS spectra for mock sample and normal urine.

#### *Evaluation of Phe concentration by proposed strategy on Microplate wells.*

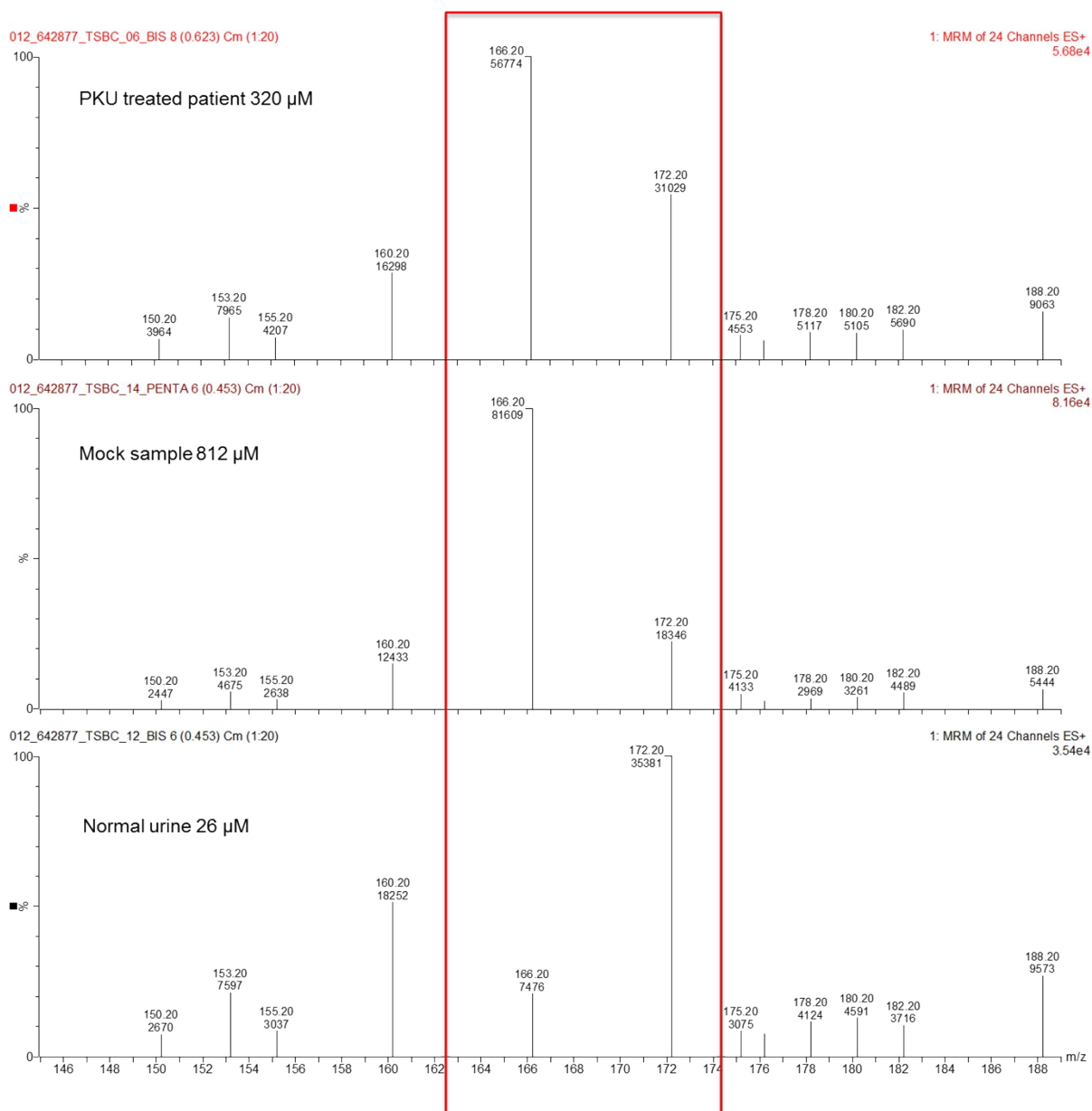
Standard Phe solutions and mock samples were tested by our proposed method on standard 96 microplate, employing the following procedure: 10  $\mu$ L of sample was added to 75  $\mu$ L of phosphate buffer 10mM, 5  $\mu$ L of NaOH 0.1M and finally 10  $\mu$ L of PAL (2U/0.28  $\mu$ L). The solutions were mixed and heated at 35  $^{\circ}$ C for 20 minutes. Then a volume of 3  $\mu$ L of phenolphthalein was added at each well and its color compared with the chromatic scale as reported in figure SI 3.



**Fig S13** Evaluation of Phe concentration for standard solutions and mock samples on microplate.

## SI4 Urine from PKU treated patients MS-MS measurements

The urine from PKU treated patient, the normal urine containing endogenous Phe and a mock samples for comparison were analyzed by MS-MS technique finding a Phe concentration of 320, 26 and 812  $\mu\text{M}$  respectively (Fig SI3).



**Fig. SI4** MS-MS spectra for urine sample of PKU treated patient, mock sample and normal sample.