Three-dimensional origami paper-based microfluidics device for creatine analysis in urine: a disposable tool for identifying urine sample adulteration by dilution

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The attendees will be receive detailed information on the use of a novel tech to control the integrity of urine samples using paper-based microfluidics technology. In the present case this technique will be applied to the analysis of creatinine, the most common analyte to control urine adulteration by dilution.
Because of the low cost and portability of the developed devices, this method has a potential application outside the specialized laboratories and particularly to control urine integrity immediately at the site of collection in doctor's offices, in small laboratories and in occupational medicine centers.
The relevance of the problem of sample tampering is well-known in forensic toxicology and sample dilution is the most used method to cheat toxicological controls. The prevalence of this phenomenon with a long history in analytical toxicology, is reported also in recent papers [1–3]. Among the criteria to assess urine integrity by excluding dilution, the quantification of creatinine probably represents the most popular method.
Although the majority of analytical methods offer adequate sensitivity and specificity, at present creatinine analysis requires laboratory instrumentation which hinders the possibility of direct test of urine at the collection site. This hinders the immediate interaction with the urine donor, which could be important to prevent claims of post-collection counterfeiting.
Since the first introduction by Whitesides et al. [4], the use of paper-based microfluidic devices (μPADs) for the development of chemical sensors has been extensively reported [5]. Among several approaches for producing μPADs, the use of commercial wax printers proved to be inexpensive and straightforward in the fabrication of the device. The procedure is based on two steps: (i) patterning chromatography paper into hydrophilic channels by fabricating hydrophobic barriers, and (ii) addition of the reagent to the hydrophilic portion of the paper support. The sample is driven through the reagent zone as results of the wicking capacity of the paper without external assistance. This approach does not require highly qualified personnel nor expensive instrumentation. Also, it can be performed on-site, enabling a prompt analytical response also in less-equipped environments. The advantages of μPADs have provided forensic science with reliable tools to face different forensic issues.
On the grounds of the above considerations, the goal of the present work was to develop a low-cost device able to provide a rapid and sensitive colorimetric detection of creatinine in urine samples. This presentation will provide details of the developed procedure which was conceived as a first-line screening potentially to be confirmed with laboratory instrumentation.
The proposed microfluidic devices were designed as a three-dimensional origami pattern. The test was based on three specific reactions for the detection of creatinine using picric acid, 3,5-dinitrobenzoic acid and Nessler's reagent. The urine sample is transferred without any treatment directly onto the hydrophilic portion of the paper, and colorimetric reactions are developed in few minutes. The color change is measured in terms of "RGB distance" by using a simple and free software for smartphone cameras. The device was also validated for quantitative determinations in terms of accuracy and precision. The optimized method was tested on real urine samples (n=53) using as reference a clinical chemistry method performed on immunoassay instrument.
In conclusion, the perspective usefulness of paper-based microfluidics as a low-cost and easy to use technique in forensic toxicology will be presented with a specific focus on its possibilities of on-site analysis to prevent urine adulteration.

Keywords
Microfluidic paper-based devices (μPADs); Urine adulteration; forensic toxicology

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