Paper-based multiplexed colorimetric device for the simultaneous detection of salivary biomarkers

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The possibility to perform near-patient medical analyses with real-time results has made point-of-care tests (POCTs) of great interest in the healthcare system. Among the wide range of POCT substrates, paper appears very appealing thanks to the easy folding that allows the creation of suitable channels where the flow of biological samples can be finely controlled. Most of the paper-based POCT devices enable the detection of a single target analyte, like for the Covid-19 rapid antigen test. On the contrary, performing a multiplexed assay allows the identification of a combination of analytes or different biomarkers in a single analysis, giving a more informative test result and improving its clinical value. In addition, integrating nanoparticles (NPs) in POCTs can further boost the device performance and increase its stability. Noble metal NPs, in particular, present remarkable advantages due to their tunable plasmonic properties and nanozyme activities. Thanks to the high sensitivity provided by NPs integrated in POCTs, the detection of biomarkers in non-invasive fluids can be achieved despite the concentrations are hundreds of times lower than those in blood. In this work, we developed a monolithic and fully integrated paper-based device for the simultaneous detection of three salivary biomarkers. The structure of the device is achieved in a single-step process through CO_2 laser cutting, which allows for remarkable parallelization and rapidity (ca. 5 sec/device). The pattern includes a central sample deposition zone and three identical arms, which contain a pre-treatment and a test zone. The detection of the biomarkers, in case of above-physiological concentrations, is obtained through a blue-to-pink color change. This colorimetric readout is due to the target-induced reshaping of plasmonic multibranched gold NPs that leads to a clear spectral shift. The colorimetric response, appreciable both by naked-eye and smartphone-based readout, is achieved within 10 minutes. We also report the development of a prototype kit for the multiplexed POCT, providing robustness and easy handling of the device.