Paper-based Device for Gastroenteritis Detection Integrated with Sample Preparation Cartridge

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Introduction: In developing countries, gastroenteritis is a common and often deadly disease caused by several diverse pathogens typically resulting in symptoms such as diarrhea, vomiting and abdominal cramping.[1] Treatment decisions beyond oral rehydration therapy require advanced diagnostic testing by well-trained medical personnel using costly instrumentation and facilities that are not always available in resource-poor settings. Thus, a need remains for inexpensive, easy-to-use, rapid, portable and highly sensitive detection assays for gastroenteritis.

Materials and methods: We designed a microfluidic paper analytical device (µPAD)[2] which uses colorimetric signal enhancement of gold nanoparticles in a multiplexed lateral-flow immunoassay array, capable of detecting up to six different diarrheal pathogens. We integrated the µPAD with an early prototype of the *Paratus SDS®* *Cartridge* which simplified sample preparation (Figure 1b), allowing for rapid sample extraction from a swab and pre-incubation with detecting reagents prior to introduction onto the µPAD. The µPAD consisted of four different parts, including (i) a sample pad for wicking sample fluids from the cartridge, (ii) a hydrophobic pad fabricated by wax printing to restrict fluid from unwanted areas, (iii) a reaction pad with embedded capture antibodies within a 3 x 3 microarray spot pattern, and (iv) an absorbent pad to draw fluids over the capture area by capillary force (Figure 1a). The µPAD assay was initially tested with a single target, Norovirus GII.4, which is the main cause of outbreaks of gastroenteritis in the US, at a concentration range of 0 – 1000 pM non-infectious virus-like particles spiked in buffer. The full assay time sequence was 30 minutes from sample collection, extraction, and µPAD capture with amplification.

Results and discussions: A representative µPAD test with a positive result in the presence of 500 pM Norovirus is shown in Figure 1c. Here, the built-in assay controls all generated a clear red/purple color in the back row of the array indicating that the test functioned properly, while the only other clear spot was in the bottom left array location where the anti-norovirus capture antibody was embedded indicating the positive reaction of Norovirus antigen within the sample. A surface plot further illustrates the spatial location and intensity of each colorimetric signal (Figure 1d). Positive signals were seen with as little as 50 pM virus-like particles with little, or no cross-

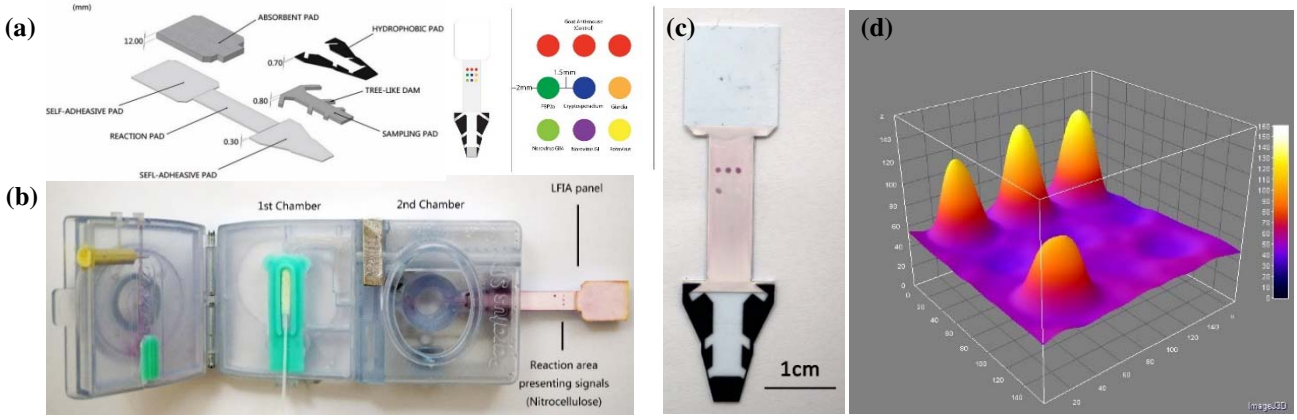

Figure 1. Integration of µPAD with swab-based sample preparation cartridge for detection of Norovirus reactivity with other immunoassay reagents within the µPAD.

Conclusions: We have demonstrated a rapid, highly sensitive, sample-to-answer method for detection of Norovirus antigens. Additional testing in stool and expansion to other pathogens is currently underway with the goal of providing a low-cost differential diagnostic test for acute gastroenteritis.

References:

1. R. Dolin, G. L. Mandell, J. E. Bennett. *Principles and practice of infectious diseases* (7th ed.) ISBN 0-443-06839-9
2. J. L. Osborn, B. Lutz, E. Fu, P. Kauffman, D. Y. Stevens and P. Yager, *Lab on a chip*, 2010, 10, 2659-2665.