

## Development of a paper-based diagnostic platform for the detection of diarrhea causing pathogens

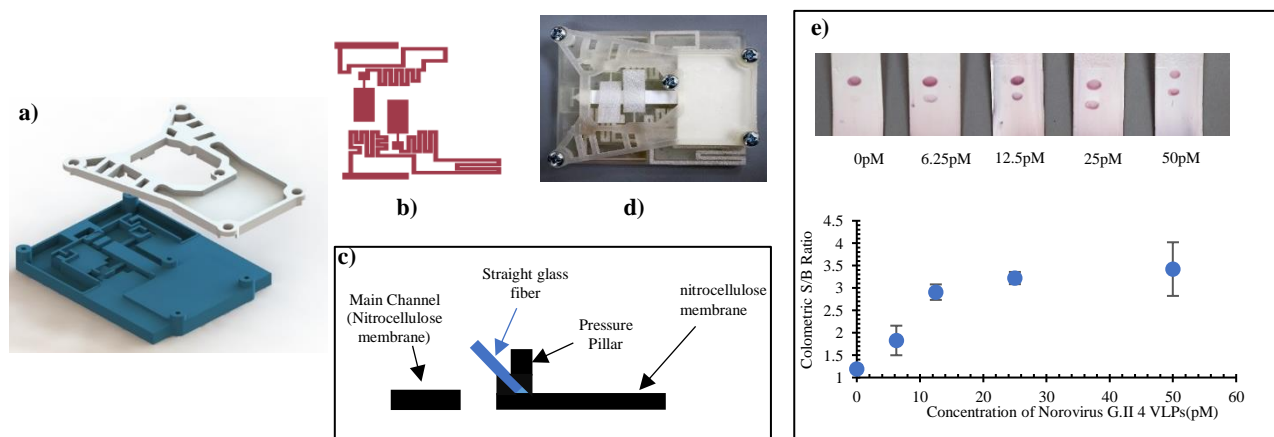
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**Introduction:** Diarrheal diseases or gastroenteritis is the second leading cause of death among children in developing countries. Current methods to diagnose gastroenteritis require expensive instrumentation, as well as trained professional both of which are not easily available in poor countries. A rapid, inexpensive, and easy to use point-of-care diagnostic device is needed to correctly detect the diarrhea causing pathogens in order to provide proper treatment and prevention to the patients.

**Materials and Methods:** Our diagnostic platform is a microfluidic paper analytical device ( $\mu$ PAD) which is impregnated with antibodies for norovirus along with goat anti-mouse antibody (GAM) which serve as control. Norovirus is the most common cause of diarrhea outbreaks in the US. The  $\mu$ PAD is integrated with a 3D printed fixture as shown in Fig 1a). Our device consists of three different inlets for- detecting reagents, wash buffer, and signal amplification reagents, which are time delivered in sequence to the reaction area of the  $\mu$ PAD. This time delivery of the reagents was achieved by two strategies. In the first strategy (Fig 1b), following the addition of the detecting reagents, the flow of wash buffer and signal amplification reagents was delayed by letting them pass through a glass fiber based serpentine like network (2-Dimension Geometry-Based Programmable Network). The delay between the two reagents was controlled by varying the length of the serpentine network. The second strategy--3-Dimension Geometry-Based Programmable Network utilized a piece of glass fiber material used as a fluidic triggering element which was placed at a fixed angle over the  $\mu$ PAD (Fig 1c). This technique utilized gravity to move the trigger down and contact the  $\mu$ PAD initiating flow. The two techniques were combined on our 3D printed fixture to detect the presence of VLPs for Norovirus (Fig 1d).

**Results:** Using the two aforementioned strategies, we were able to control the sequence and time delivery of different reagents on the  $\mu$ PAD. The flow of wash buffer on to the  $\mu$ PAD was delayed by 7 mins and signal amplification reagents by 11 mins. This delay provided ample time for detecting reagents to interact with the antibodies impregnated in reaction area of the  $\mu$ PAD. We were able to show positive signals for both norovirus capture spot as well control GAM spot. We also generated a dose response curve (Fig 1e) which showed that with increasing concentration of norovirus, the signal intensity increased. The lowest concentration at which our diagnostic platform can detect a signal for norovirus was 6.5pM.



**Figure 1: A paper based diagnostic platform for the detection of Norovirus**

**Conclusion:** We have demonstrated that our diagnostic platform operates with a single actuation step which triggers timed delivery of multiple immunoassay reagents and signal enhancers and it can successfully detect norovirus at extremely low concentrations. Future directions for this work will involve multi-plexing, where multiple anti-antigen capture antibodies are embedded on the nitrocellulose membrane for detecting different, but closely-related pathogens that cause similar symptoms.