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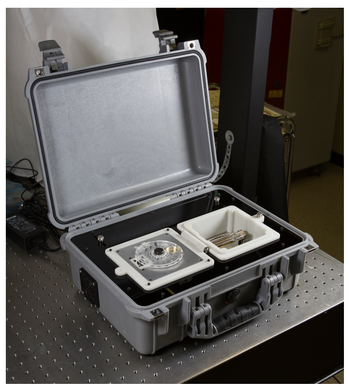
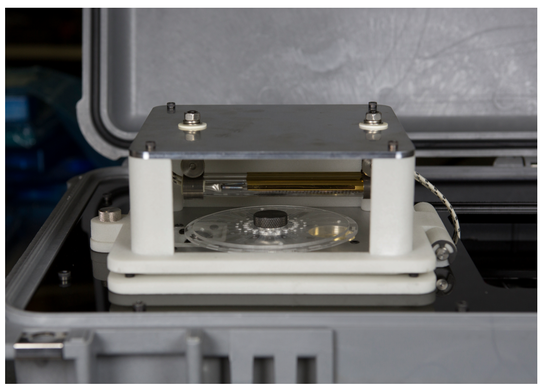
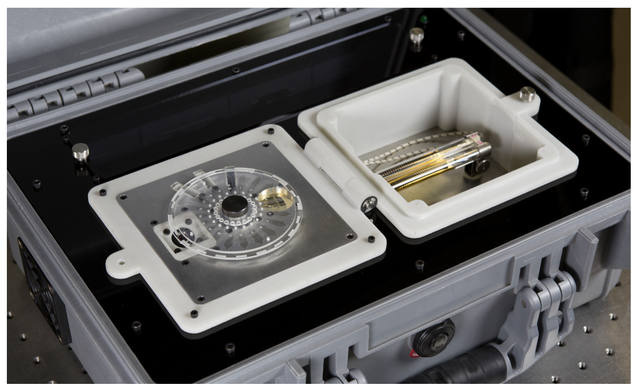
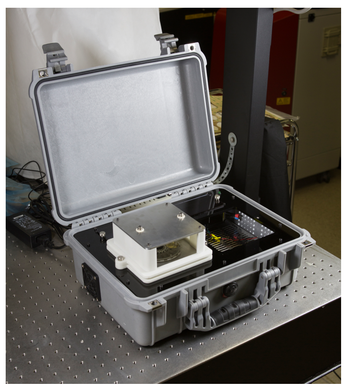
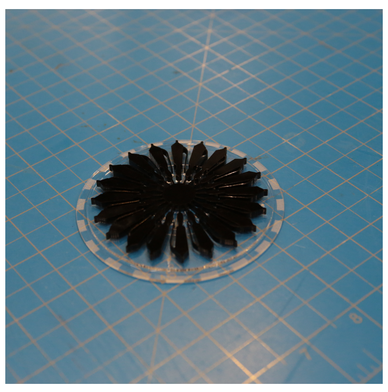

Surface Passivation of Microfluidic Device for Real-Time Nucleic Acid Amplification Tests

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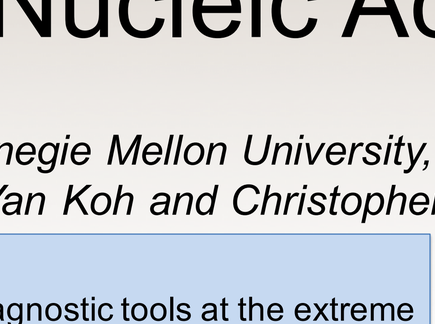
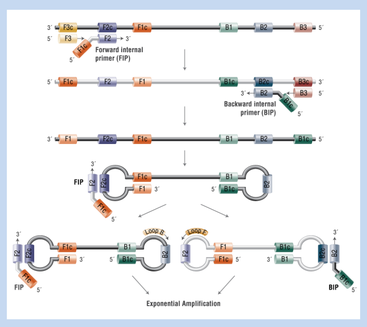
ABSTRACT

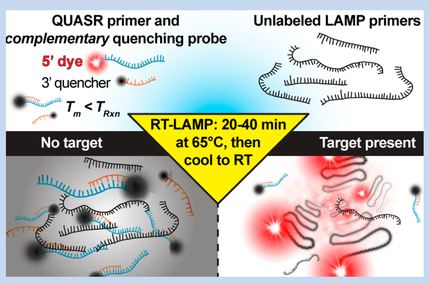
Globally, there is a need for rapid diagnostic tools at the extreme point-of-care. The SpinDx platform has been experimentally expanded to be able to perform loop mediated isothermal amplification (LAMP). Traditionally, we have used a QUASR LAMP method, however it is limited to end-point detection only. Here we have pursued a real-time LAMP detection that will enable us to observe the rise of positive samples and to end the reaction when it reaches a predefined threshold (decreasing the runtime). We have modified the disc surface with a Teflon coating to prevent adsorption of the fluorescent dye that is used in the LAMP reaction.

INTRODUCTION

Nucleic acid amplification tests offer earlier diagnosis compared to serological diagnostic methods. PCR has been the gold standard for diagnostic nucleic acid amplification tests, however it involves expensive and bulky components and requires the sample to be processed, making it unideal for deployment in developing countries. Alternatively, LAMP is performed at a single temperature, simplifying the required instrumentation. In addition, the reaction is more robust than PCR, allowing the use of unprocessed samples, while retaining high sensitivity and specificity. This is especially true using the QUASR technique, developed here at Sandia, to improve the signal-to-noise ratio of detection.







The figure on the left shows how the amplification in LAMP occurs. The figure on the right shows how QUASR LAMP utilizes the primer/quencher combination to more easily discern positive and negative results.

The real-time detection in LAMP allows for quantitative diagnostic detection. In the LAMP reaction Syto9 is used as a fluorescence dye to indicate a positive result. In the current discs we have seen surface interaction issues with the small molecule dye and the PMMA surface of the disc. We have investigated surface modifications to eliminate the adsorption of the dye onto the surface of the disc.

References:

1. Ball, Cameron S., Yooli K. Light, and Chung-Yan Koh. "Quenching of Unincorporated Amplification Signal Reporters in Reverse-Transcription Loop-Mediated Isothermal Amplification Enabling Bright, Single-Step, Closed-Tube, and Multiplexed Detection of RNA Viruses." *Analytical Chemistry* 88.7 (2016): 3562-568. *ACS Publications.*

2. Priye, Aashish, Sara W. Bird, Yooli K. Light, Cameron S. Ball, Oscar A. Negrete, and Robert J. Meagher. "A Smartphone-based Diagnostic Platform for Rapid Detection of Zika, Chikungunya, and Dengue Viruses." *Scientific Reports* 7 (2017): 44778.

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METHODS

1. Disc components are masked and sprayed with a 1:2 solution of Teflon AF 1600 and FC40

2. Then, the components are inversely masked and exposed to UV-ozone

3. The two parts of the disc are thermally bonded

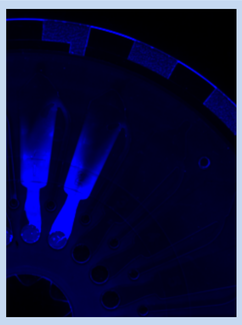
4. Finally, the assembled disc is treated with an amphiphile solution to ease loading

RESULTS

Surface Treatment

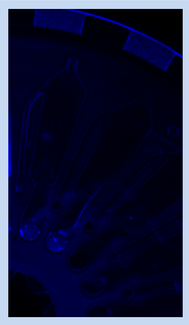
The images to the right show that compared to an untreated disc (left image), the Teflon and RainX are effective at preventing dye adsorption on the disc.

Preliminary results indicate that RainX is also effective in preventing dye adsorption, which would be an easier-to-use and more affordable alternative to Teflon.



Untreated disc, showing the adsorption of the dye on the disc

Teflon treated disc, showing no surface adsorption of the dye

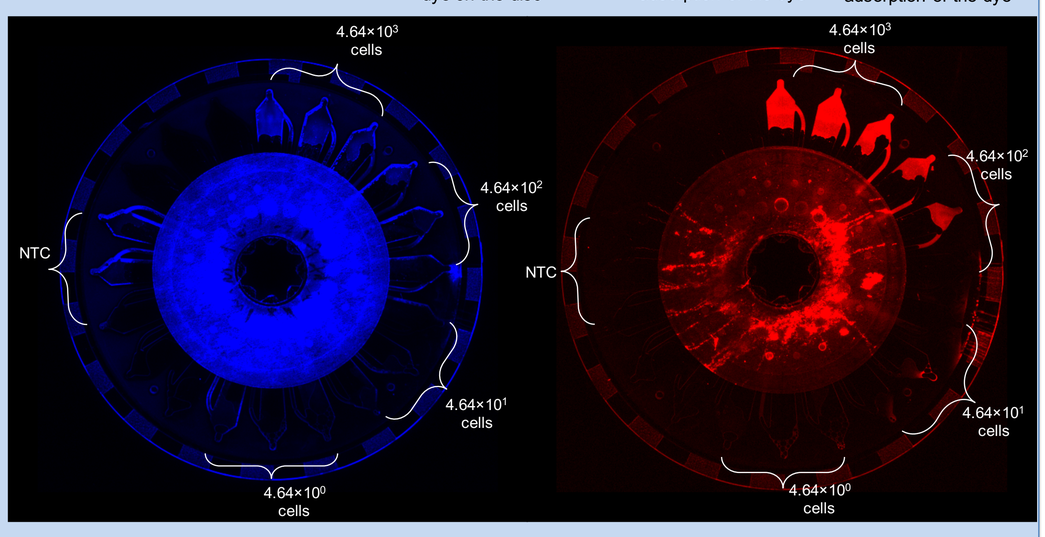


RainX treated disc, showing no surface adsorption of the dye

Real-Time LAMP Test

Using four concentrations and negative controls LAMP was used to test Campylobacter jejuni.

The blue scan image (left) shows the detection of Syto9 fluorescence in the LAMP reaction. The bright chambers are positive detections of C. jejuni. The negative control chambers show some staining around the edges where potentially the Teflon was not evenly coated onto the surface. The red scan image (right) detects the Cy5 fluorescence in the QUASR LAMP and was used to confirm that the reaction worked. The bright chambers show positive detection, which align with the positive detection from the Syto9 scan.



Gel imager scan of the Syto9 dye in the LAMP reaction

Gel imager scan of Cy5 dye

CONCLUSION

The Teflon surface coating allows the discs to be compatible with the dye used in the LAMP reaction. We have seen that the LAMP real-time method is able to detect the presence of C. jejuni. Future studies include determining the limit of detection, experimenting the diagnostic method with a larger panel of diseases, and investigating an easier approach to making the discs compatible with the small molecule fluorescence dye.

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