

Extraction and Detection of SARS-CoV-2 RNA from Saliva in a Microfluidic Device

SenSIP Algorithms and Devices REU

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ABSTRACT

- Point-of-care diagnostics provide for the possibility of increasing ease and access to rapid healthcare results in a cost-effective manner
- Saliva provides for a wealth of potential data for a variety of diseases, including those to which can be detected and quantified from nucleic acids.
- Extraction and amplification/detection of specific nucleic acid sequences from saliva, especially those composed of RNA, can be troublesome outside of a laboratory setting
- Using a portable, easy to use, low equipment requirement variant of solid-phase silica extraction built into a microfluidic device, we hope to detect SARS-CoV-2, the causative agent of Covid-19, in a fluorescent reader using RT-LAMP with intercalating fluorophores

MOTIVATION

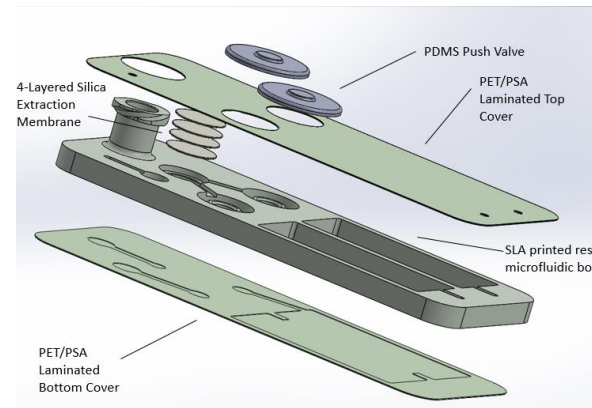
Many areas of lower resources are underserved regarding access to healthcare and timely or affordable laboratory testing. Point-of-care devices continue to offer the promise of improvements to access and cost in these types of areas. Extraction from challenging matrices such as saliva or blood could vastly expand both the range of accuracy of any potential tests and assays based upon nucleic acids.

PROBLEM STATEMENT

- Many available methods for extracting nucleic acids.
- Extracting nucleic acids without substantial amounts of expensive laboratory equipment and electricity is not so easy
- Detecting those nucleic acids of questionable purity in a handheld fluorescent reader is particularly challenging.

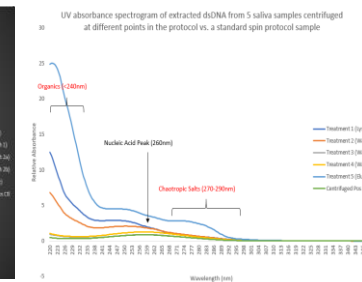
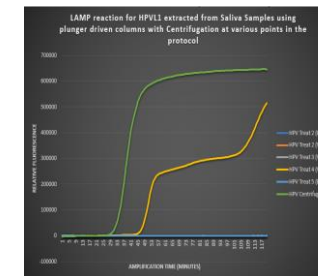
EXPERIMENTAL METHODS: UV SPECTROSCOPY

- Preliminary results involve the extraction of SARS-CoV-2 heat deactivated whole genome spiked RNA from water and human saliva using commercial silica columns modified with adaptors to allow plunger driven forced fluid extraction
- Compared directly to standard centrifuge protocol
- Later results involve a microfluidic extraction cartridge complete with silica columns and plunger driven samples.
- UV spectrum analysis (Nanodrop UV spectrometer) for determination of concentration and potential contaminants.
- LAMP and qPCR performed to compare relative performance and LOD for assay vs directly analyzed pure samples of known concentration



PRELIMINARY RESULTS

- Plunger results with centrifuging at different points
- Lamp is very sensitive to inhibitors such as ethanol and Chaotropic salts



REFERENCES

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