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

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

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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