Assessment of a CRISPR/Cas13a assay for detection of SARS-CoV-2 in Fecal Samples

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Abstract

SARS-CoV-2 is shed in the stool of some infected individuals, suggesting the possibility for fecal-oral transmission and raising safety concerns for patients receiving Fecal Microbiota for Transplantation (FMTs). In April 2020, the FDA issued a safety alert indicating that FMT products manufactured from stool donations provided after December 1, 2019, should not be administered to patients until a reliable screening method for SARS-Cov-2 is implemented. As such, our laboratory developed and validated an rRT-PCR assay for the detection of SARS-CoV-2 in stool with comparable sensitivity to respiratory testing methods. More recently, a SHERLOCK assay was produced as a kit to detect SARS-CoV-2 and may represent a faster, easier, and more standardized testing method. The SHERLOCK assay employs LAMP amplification combined with CRISPR/Cas13a based detection of viral RNAs. First, Reverse Transcriptase Loop-Mediated Amplification (RT-LAMP) is utilized to generate cDNA of the viral RNA targets ORF1ab gene and the Nucleocapsid (N) gene, as well as Human RNaseP POP7 gene as a positive control.[4] These cDNAs are then amplified by T7 driven transcription into RNA which enhances the detection of SARS-CoV-2 RNA in low copy number samples by crRNA-guided Cas13a-binding to target RNA, if present. Once Cas13a binds to SARS-CoV-2 targets, collateral cleavage activity activates fluorescent reporters which can be detected via a plate reader.[3] The SHERLOCK kit documentation claims a limit of detection (LoD) of 6.75 copies of viral RNA/uL (1.35 cp/µL for the N gene target) in as little as 40 minutes. This study seeks to validate the SHERLOCK kit for the detection of SARS-CoV-2 RNA in stool samples. Preliminary data from a dilution series using extracted iCoV-2 RNA at concentrations of 15, 10, 5, 3, and 1.5 cp/µL suggests that the SHERLOCK kit is effective at detecting samples of iCoV-2 RNA extracted from fecal spike-ins down to 15 cp/uL for the ORF1ab gene and 5 cp/uL for the N gene (4 positive out of 4 replicates). Moving forward, we will continue to collect data to determine the LoD and testing clinical samples taken from SARS-CoV-2 positive patients to further validate the kit's efficacy on stool samples.

Method

Here we introduce the use of a commercially available and peer-reviewed SARS-CoV-2 RNA CRISPR-based detection assay for its potential utility in screening fecal transplant material. This assay is based upon the detection of SARS-CoV-2 coronavirus nucleocapsid (N) or Orf1ab genes by gRNA-guided Cas13a interaction and cleavage activity which results in a fluorescent readout for a positive result.[2, 3]

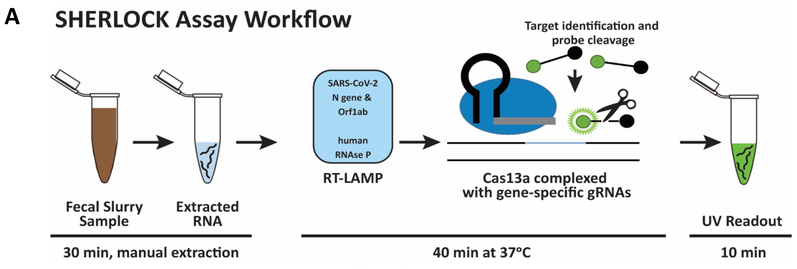

Figure 1.
A) Schematic representation of SHERLOCK CRISPR-based detection assay. Fecal samples are obtained from individuals potentially infected with SARS-CoV-2 virus. Total RNA is then extracted from the fecal sample slurry. This total RNA is then added to RT-LAMP master mix containing primers for SAR-CoV-2 N and ORF1ab, as well as the human Rnase P gene as an extraction control. RT-LAMP amplicons are added to a master mix consisting of T7 RNA Polymerase, crRNA for gene targets, and Cas13a. If target is present, collateral cleavage of the reporter by Cas13a results in fluorescence, detectable by UV readout.
B) Summary of LAMP Reaction. Schematic representation of Reverse Transcription Loop Mediated Amplification (RT-LAMP) of extracted SARS-CoV-2 RNA. RNA is incubated with gene target primers and LAMP mix. RT occurs to make cDNA, and primer regions bind to cDNA creating a loop. Strand-displacing DNA Polymerase recognizes primers in loop and transcribes dsDNA amplicon.

SHERLOCK Kit Detection of SARS-Cov-2 RNA

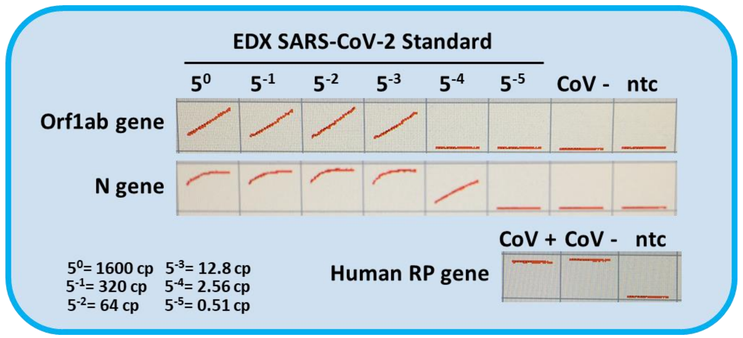

Figure 2. Fluorescent readout for SHERLOCK assay detection of a dilution series of SARS-CoV-2 RNA. Input was a dilution series using EDX-SARS-CoV-2-Standard positive control (Exact Diagnostics). Starting concentration of control is 200 cp/µL of synthetic SARS-CoV-2 RNA and 75 cp/µL of human gDNA. Detection of either the SARS-CoV-2 Orf1ab or N gene, as well as the human RNase P (RP) gene was carried out using a BioRad plate reader. Readout of fluorescent reporter is graphed at each concentration as signal units (Y-axis) over time in minutes (X-axis).

cp/µL	N	ORF1ab	RNaseP
200	5/5	5/5	5/5
40	1/1	2/2	n/a
8	1/1	2/2	n/a
0.32	0/1	1/2	n/a
0.06	0/1	0/2	n/a

Table 1. Verification of the LoD of SARS-CoV-2 using the SHERLOCK assay. EDX-SARS-CoV-2-Standard positive control genetic material (Exact Diagnostics) was added to the SHERLOCK assay in a series of dilutions from 200 cp/µl to 0.06 cp/µl. Human RNase P is used as an internal control for spike-ins and RNA extraction. Any fluorescent readout was recorded as a positive result for the presence of SARS-CoV-2 RNA.

References

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Detection of iCoV-2 RNA in Fecal Samples

Inactivated SARS-CoV-2 RNA detection from fecal sample matrix workflow:

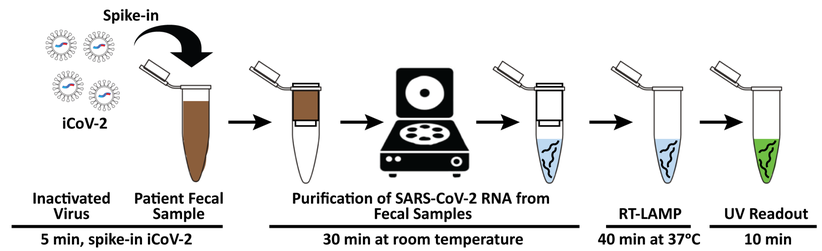

Figure 3. Workflow schematic for iCoV-2 spiked-in to stool, RNA isolation and detection from fecal samples. Heat-inactivated virus was diluted to desired concentrations and spiked into donor stool slurry. RNA is purified from the stool slurry using QIAamp Viral RNA mini kit by Qiagen. Purified RNA is added to RT-LAMP reaction and incubated at 37°C for 40 minutes. 5 µL of the RT-LAMP Amplicon is added to 20 µL of Cas13a master mix and mixed well before a 10 minute UV readout. Samples containing detectable levels of target genes will have a positive fluorescence readout.

	iCoV-2 Spiked into Stool			iCoV-2 Direct Extraction		
cp/µL	N	ORF1ab	RNaseP	N	ORF1ab	RNaseP
15	4/4	4/4	0/4			
10	4/4	1/4	n/a	6/6	4/4	0/3
5	4/4	1/4	n/a	6/6	4/4	n/a
3	0/4	3/4	n/a	0/6	4/4	n/a
1.5	0/4	0/4	n/a	0/6	0/4	n/a

Table 2. Determining the sensitivity of the SHERLOCK assay to detect SARS-CoV-2 from fecal sample matrices. RNA extractions were performed on dilutions of heat-inactivated SARS-CoV-2 virus either directly or after they had been spiked into stool slurry. Dilutions were used in SHERLOCK kit protocol to test for presence of all three gene targets. The LoD for N gene was 5 cp/µL in both samples. Non-spiked viral RNA was able to be detected consistently at lower concentrations than RNA extracted from fecal samples. Internal control (RNaseP gene) was not able to be detected for either condition. All positive results are indicated out of the total number of assays performed.

Results & Future Direction

Results:

- The SARS-CoV-2 SHERLOCK assay (IDT) can detect SARS-CoV-2 N and Orf1ab gene targets from heat-inactivated virus spiked into stool, but at a lower sensitivity than pure inactivated virus samples (not spiked-in to stool).
- Initial findings suggest an approximate LoD of SARS-CoV-2 RNA from virus that was spiked in and extracted from the fecal matrix for the Orf1ab gene as 15cp/µL, and the N gene as 5 cp/ul.
- We were unable to detect the human RNaseP gene from the stool samples spiked with SARS-CoV-2 using the CRISPR assay.

Future Direction:

We intend to further validate the ability of this technology to detect SARS-CoV-2 RNA in clinical stool samples to determine if CRISPR/Cas-based detection would be a sensitive and efficient option for detection of viral RNA in stool.

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