Importance of Assessing Clinical Specimen Quality in SARS-CoV-2 RT-PCR Testing

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Abstract

COVID-19, caused by the SARS-CoV-2 virus, is a disease that has affected people across the globe. With so many infections and families losing loved ones to the disease, detecting this virus is key to controlling its spread. Avoiding false negative results in tests is a major part of achieving this goal. This study used two different RT-PCR assays one with and one without a cellularity control to assess specimen quality. The results suggest that those tests that do not control for the presence of human cells could potentially unknowingly release a false negative result due to low cellularity.

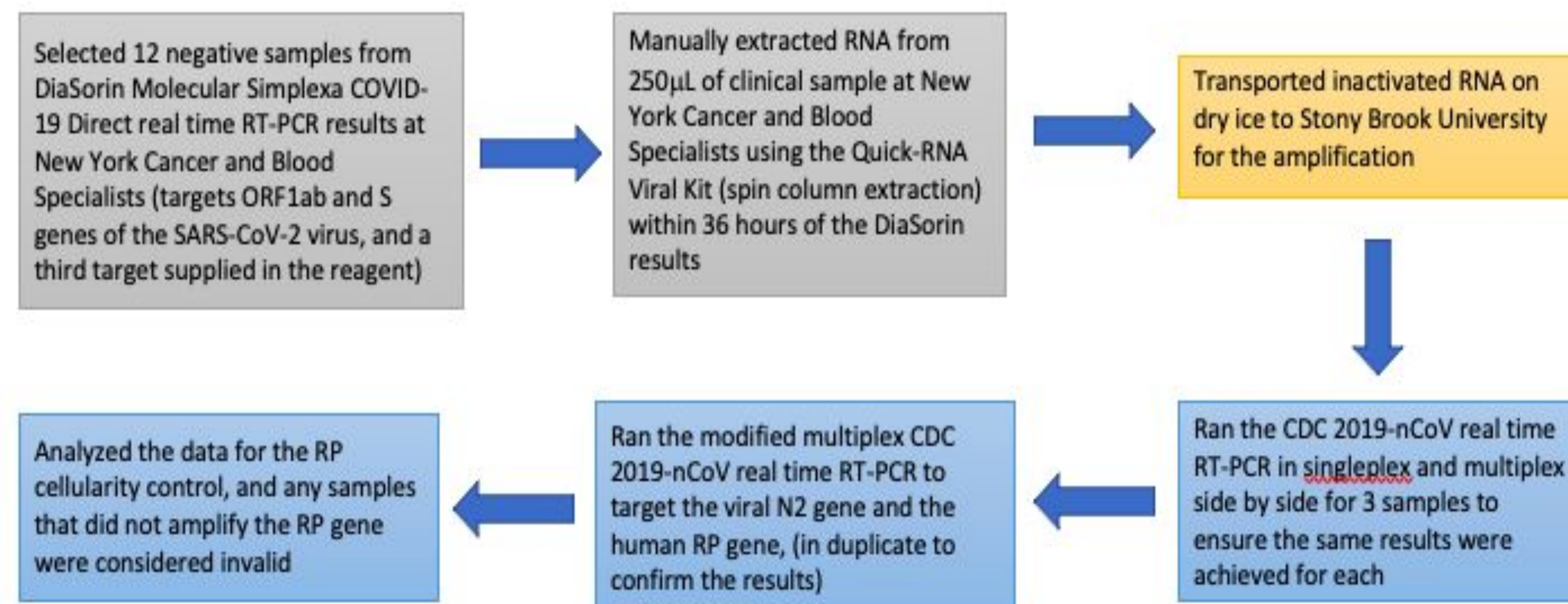
Introduction

The SARS-CoV-2 virus was discovered in late 2019 and has caused 145 million cases of COVID-19 and 3.1 million deaths worldwide as of April 23, 2021. Detecting this highly contagious virus is paramount in controlling its spread. Several tests have been FDA-approved for emergency use, but many do not control for the presence of human cells in the clinical specimen (cellularity control). This is important because viruses live within cells. If not enough cells are present in the specimen, false negative results can occur. By using the DiaSorin Molecular Simplexa COVID-19 Direct real time RT-PCR that doesn't have a cellularity control and the CDC 2019-nCoV real time RT-PCR that targets the human RNase P (RP) gene as a cellularity control, a comparison can be made to see if the samples will have a valid result using both methods.

Objective

This study aims to determine whether detecting human cells in clinical specimens is necessary to avoid false negative results.

Method



Results

Table 1 shows the results comparison of the two test methods. A valid result is defined by all included controls having their intended results. The DiaSorin results are valid as long as the daily quality controls (positive and no template control(NTC)) passed and the internal control supplied in the reagent is positive for each sample. A positive control and NTC were included in each CDC assay performed. The RP gene acts as an internal control for each sample. Five of the twelve samples were invalid when tested using the CDC assay based on the RP gene not being amplified.

Table 1

Sample	DiaSorin Molecular Simplexa COVID-19 Direct real time RT-PCR assay Result	Modified CDC 2019-nCoV real time RT-PCR RNase P Gene Ct Values	Modified CDC 2019-nCoV real time RT-PCR Result
2-1	Negative and Valid	25.0	Negative and Valid
2-2	Negative and Valid	25.4	Negative and Valid
2-3	Negative and Valid	30.6	Negative and Valid
2-4	Negative and Valid	24.8	Negative and Valid
2-5	Negative and Valid	Not Detected	Invalid
2-6	Negative and Valid	Not Detected	Invalid
2-7	Negative and Valid	Not Detected	Invalid
2-8	Negative and Valid	Not Detected	Invalid
2-9	Negative and Valid	27.1	Negative and Valid
2-10	Negative and Valid	Not Detected	Invalid
2-11	Negative and Valid	28.6	Negative and Valid
2-12	Negative and Valid	26.3	Negative and Valid

Conclusions

This study suggests that some samples tested for SARS-CoV-2 and resulted as negative using the DiaSorin Molecular Simplexa COVID-19 Direct real time RT-PCR are possibly falsely negative due to lack of enough human cells. Those PCR tests that do not control for the presence of human cells in the clinical specimen are not able to accurately determine false negatives resulting from a lack of cells that carry the virus

Discussion / Future Directions

This study is limited by the small number of clinical samples that were included in the study. Having more clinical samples would better represent the actual percentage of specimen that are invalid due to not enough human cells being present. The extraction performed in this study did not include any positive samples to act as an extraction control. However, amplification of the RP gene did occur in 7 of the 12 samples. The RP gene amplification is the expected result of the extraction control recommended by the CDC. Including an equal amount of positives and negatives in the extraction would show a better comparison of the RP gene detection seen in the two different result populations and would better show the importance of having a cellularity control. Future studies could be conducted on a larger scale and performed on multiple extractions of the same sample to rule out the possibility of error during that step.

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