VALIDATION OF RAPID SARS-CoV-2 TESTING IN ASYMPOTOMATIC INDIVIDUALS

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ABSTRACT

Rapid antigen testing for SARS-CoV-2 has lowered the cost and increased turnaround times for clinics and labs around the United States. This widely used but developing method has only received Emergency Use Authorization (EUA) in symptomatic individuals from the Food and Drug Administration (FDA). Thorough validation for rapid antigen tests in both symptomatic and asymptomatic individuals is needed in order to reduce the spread of COVID-19. Validation was done by comparing the results of reverse transcription PCR (RT-qPCR) methodology to the gold standard method used antigen testing, specifically the BD Veritor testing system in asymptomatic patients. In this study, the additional nasal swab was collected from about 200 individuals at Weber State University’s main campus, those swabs were run in parallel on the Quantstudio 3 RT-qPCR analyzer with the BD antigen methodology. The comparison method utilized in this research will gauge the specificity and sensitivity for antigen testing juxtaposed against the gold standard RT-qPCR methodology.

BACKGROUND

Rapid antigen testing is approved for symptomatic patients, however, test data obtained from asymptomatic individuals is inadequate. This research will assess the data performance of symptomatic and asymptomatic individuals’ rapid antigen screen in asymptomatic patients, in order to reduce the spread of COVID-19. In a recent study patients with antigen-negative and RT-qPCR-positive specimens were assessed, more than half of the samples came from asymptomatic individuals where the viral components were undetected on rapid screen tests. Understanding the data presented from the method comparison will allow for further research and analysis of same testing strategies.

METHOD

NASAL swabs in TRIzol

1 mL of NF-H2O & TRIzol mixture → 1.5 mL microfuge tube

Add 200 µL Chloroform

Add 4 µL Glycerol

Incubate at RT for 3 minutes

12,000 g, 4°C, 15 minutes

Transfer ~600 µL aqueous layer

Add 0.5 mL isopropanol

Incubate at RT 10 minutes

12,000 g, 4°C, 10 minutes

Remove supernatant

Resuspend pellet in 1 mL of 75% ethanol

7,500 g, 4°C, 5 minutes

Discard supernatant

Air dry on ice

Add 25 µL of NF-H2O

Incubate in 55°C water bath, 10 minutes

Use 5 µL RNA per each reaction

(nCov-N1, nCov-N2, RP primer/probe, positive/negative control)

RESULTS

The 2x2 table below, shows that the BD Veritor antigen test provides a highly specific assay that performs well with an abundance of truly negative tests. Our negative predictive value (NPV) shows a 100% ability to test truly negative values, while the positive predictive value (PPV) resulted as zero. This shows that the BD Veritor is a n effective assay for SARS-CoV2 testing on the samples collected at Weber State.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Positive Test Results</th>
<th>Negative Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen Test</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RT-qPCR</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

DISCUSSION

- After collection, samples were extracted, stored, and tested using the gold standard test RT-qPCR.
- A simplified SARS-CoV-2 test protocol was used as a guide for the extraction and testing methods.
- Discrepancies were found between the article compared to standard RNA extraction protocols and RT-qPCR testing protocols.
- During the extraction process the nasal swabs collected did not hold enough sample to cause a separation of the TRIzol and chloroform mixture as stated in the SARS-CoV-2 protocol. Instead, 1 mL was added to the sample to create a definite separation of the sample.
- All centrifugation of the samples were conducted at 4°C and followed the times specified in the standard RNA extraction protocols. Once these differences were resolved, the extraction process was consistent, and the RNA purification ratio was acceptable within samples.
- Once extracted, the sample was either stored or tested using the Quantstudio 3 PCR analyzer.
- Three detection markers (N1, N2, RP) were used in order to detect results for SARS-CoV-2. Positive, Negative, and Non-template controls were tested and showed proper detection. RP markers should appear positive in all patient samples, if negative, the sample was considered inconclusive. Amplification of the RP, N1, and/or N2 would result in a positive SARS-CoV-2 sample.

LIMITATIONS

- The most prevalent limitation is the lack of positive samples that have been collected. This study cannot make adequate conclusions about sensitivity.
- The total number of samples collected could be considered a limitation, with vaccinations becoming apparent there has been less need for testing. Starting earlier in the pandemic would have resolved these limitations.

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