TITLE: Comparison of Quidel Sofia SARS FIA Test to Hologic Aptima SARS-CoV-2 TMA Test for Diagnosis of COVID-19 in Symptomatic Outpatients

RUNNING TITLE: Sofia FIA vs. Aptima TMA for Diagnosis of COVID-19

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Abstract:
The Quidel Sofia SARS FIA test (SOFIA) is a rapid antigen immunoassay for detection of SARS-CoV-2 viral proteins from nasal or nasopharyngeal swab specimens. The purpose of this study was to compare the results of the SOFIA test to the Hologic Aptima SARS-CoV-2 TMA test (APTIMA TMA), a high-throughput molecular diagnostic test that uses transcription mediated amplification for detection of SARS-CoV-2 nucleic acid from upper respiratory specimens. Three hundred and forty-seven symptomatic patients, from an urgent care center in an area with a high prevalence of SARS-CoV-2 infections, were tested in parallel using nasal swabs on the SOFIA test and nasopharyngeal swabs on the APTIMA TMA test. The SOFIA test demonstrated an 82.0% positive percent agreement (PPA) compared to the APTIMA TMA test for symptomatic patients tested ≤ 5 days from symptom onset and a 54.5% PPA for symptomatic patients > 5 days from symptom onset. The Cepheid Xpert Xpress SARS-CoV-2 RT-PCR test was used to determine the cycle threshold (Ct) value from any specimens that were discrepant between the SOFIA and APTIMA TMA tests. Using a Ct value of ≤ 35 as a surrogate for SARS-CoV-2 culture positivity, we estimate that the SOFIA test detected 87.2% of symptomatic patients tested ≤ 5 days from symptom onset that were likely to be culture positive.

Introduction:
At present, diagnosis of active SARS-CoV-2 infection primarily relies on the use of molecular diagnostic testing. In addition to molecular diagnostic tests, seven rapid antigen tests have received Emergency Use Authorization (EUA) for use in the diagnosis of SARS-CoV-2 infection. The first of these tests to receive EUA status was the Quidel Sofia SARS Antigen FIA test (SOFIA) on July 17th, 2020 (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas, accessed 10/27/2020). This test is performed using either nasal or nasopharyngeal swabs and can be completed in approximately 15 minutes. Specimens are collected and placed directly into a
reaction tube containing a reaction solution. The test is a sandwich style lateral flow immunoassay that is used to detect the nucleocapsid protein of SARS-CoV-2. If the viral proteins are present in the test specimen, a fluorescent band will be present at a specific location on the test strip. The fluorescence is measured using either a Quidel Sofia or Quidel Sofia 2 test device. If viral proteins are not present above a specific concentration then no fluorescence will be detected and the test will be negative.

Recently, Advocate Aurora Health (and ACL Laboratories, which is owned and operated by Advocate Aurora Health) implemented the SOFIA test in several urgent care centers for diagnosis of coronavirus disease from 2019 (COVID-19) on patients experiencing signs and symptoms of upper respiratory infection. This data was collected at an urgent care center servicing patients in an area with a high prevalence of SARS-CoV-2. In the month prior to implementation of antigen testing, this site saw approximately 20 patients with signs and symptoms of COVID-19 each day. During this time period, specimens from these patients were sent to a reference lab for molecular testing with a positivity rate of approximately 18%. Due to limited data on the accuracy of the SOFIA test and reports of false-positive results (https://www.manchesterjournal.com/news/local/health-commissioner-takes-issue-with-covid-19-claims/article_b2718273-9089-5da2-a994-2a0476f9c89a.html, accessed 10/27/2020) and historical data showing decreased sensitivity of rapid antigen tests compared to molecular tests, this site collected a second specimen, during the same visit, from all patients for confirmatory testing with the Hologic Aptima SARS-CoV-2 TMA test (APTIMA TMA) in the ACL Laboratories central laboratory (1-4).

This report compares the results of the SOFIA test to the APTIMA TMA test on patients presenting to the urgent care department with signs and symptoms of COVID-19. The accuracy of the test was further stratified by the days post symptom onset and patient age. Discrepant
specimens were run in a second molecular test, the Cepheid Xpert Xpress SARS-CoV-2 RT-PCR test (XPERT), to determine the cycle threshold (Ct) value and better assess the clinical significance of the false negative SOFIA tests.

Materials and Methods:

Patient Selection and Collection:
All patients with signs and symptoms of COVID-19 presenting to the Advocate Aurora Health Urgent Care Center located in West Bend, Wisconsin were tested in parallel using the SOFIA test and the APTIMA TMA test. This testing strategy was utilized as the standard diagnostic algorithm by providers at this clinic to obtain a better understanding of the performance of the SOFIA test compared to molecular testing. At the time of presentation, a nasopharyngeal swab was collected for molecular testing followed by a nasal swab for antigen testing and both specimens were sent to the laboratory for testing. Patients ranged from 1 – 90 years old and patients ≤18, 19 – 50, and > 50 years of age accounted for 35.4%, 38.3%, and 26.2% of the subjects tested, respectively.

Quidel Sofia SARS FIA Antigen Test:
Nasal specimens were collected in the patient room using the swabs provided in the Quidel Sofia SARS FIA (Quidel, San Diego, CA) test kit. After collection, the swabs were carefully returned to the paper envelope that they came in and placed in a sealed plastic specimen transport bag. Specimens were delivered to the laboratory (located within the same building), within 10 minutes of collection. Upon receipt in the laboratory, specimens were tested with the SOFIA test according to the manufacturer’s package insert.

Hologic Aptima Panther SARS-CoV-2 TMA Test:
Nasopharyngeal swab specimens were collected in the patient room using a mini-tip nylon flocked swab and placed into a transport tube containing approximately 1 mL of Liquid Amies bacterial transport medium (Copan, Brescia, Italy). Following collection, specimens were refrigerated and sent via courier to the ACL Laboratories central laboratory. The specimens were tested with the Hologic Aptima SARS-CoV-2 transcription mediated amplification test (Hologic, Marlborough, MA) on a Hologic Panther instrument system (Hologic) according to the manufacturer’s package insert.

Cepheid Xpert Xpress SARS-CoV-2 RT-PCR Test:

Any patients that had discrepant results on the SOFIA and APTIMA TMA tests were also tested with the Cepheid Xpert Xpress SARS-CoV-2 test (Cepheid, Sunnyvale, CA). Residual nasopharyngeal swab specimens submitted for testing on the APTIMA TMA test were frozen at -70°C for approximately three weeks prior to testing on a Cepheid GeneXpert DX instrument (Cepheid) with the XPERT test according to the manufacturer’s package insert.

Ethics:

This work was reviewed and determined not to be human subject research by the Advocate Aurora Health Institutional Review Board and Human Research Subject Protection Program (Determination HSR 2020-173).

Results:

In total 347 patients were tested on both the SOFIA and APTIMA TMA tests. One specimen was invalid on the SOFIA test and was not further evaluated in this study yielding a total of 346 paired patient specimens. The overall positive percent agreement (PPA), negative percent agreement (NPA), and total agreement (TA) of the SOFIA test compared to the APTIMA TMA test were 77.0% (47/61), 99.6% (284/285), and 95.7% (331/346), respectively (Tables 1 and 2).
The PPA of the SOFIA test compared to the APTIMA TMA test was 72.7%, 81.5%, and 73.9% for patients ≤ 18 years of age, 19–50 years of age, and > 50 years of age, respectively (Table 2). The current version of the SOFIA package insert indicates that the test should be used on symptomatic patients who are ≤ 5 days from the onset of COVID-19 symptoms. In this study, the PPA of the SOFIA test compared to the APTIMA TMA test was 82.0% (41/50) for patients tested ≤ 5 days from symptom onset and 54.5% (6/11) for patients tested > 5 days from symptom onset (Table 1).

Discussion:
Here, we report the accuracy of nasal swabs tested with the Quidel Sofia SARS FIA antigen test compared to nasopharyngeal swab specimens submitted for molecular testing on the Hologic Aptima SARS-CoV-2 TMA molecular test in symptomatic outpatients presenting to an urgent care center. This data shows that the SOFIA test has an 82.0% and 54.5% PPA compared to APTIMA TMA in symptomatic patients who were tested ≤ 5 days from symptom onset or > 5 days from symptom onset, respectively (Table 1). The notable difference observed between these two groups of patients validates the manufacturer’s recommendation to use the SOFIA test for those patients in the former group.

There have been several recent studies correlating the Ct value of SARS-CoV-2 PCR tests with the ability to culture live SARS-CoV-2 virus. Most of these studies have shown that it is very rare to culture infectious virus from samples with Ct values above 34 or 35 (5–7). The XPERT test was performed on any samples that were APTIMA TMA positive, SOFIA negative to determine the Ct value and better understand the clinical significance of this discrepancy. Two of the fourteen specimens were negative on the XPERT test and another had a Ct value that was > 35 for both targets in the test (Table 3). If the following two assumptions are made: 1) all
samples testing positive in the APTIMA TMA test are true positives and 2) only those patients that either have an XPERT Ct value under 35 or that tested positive in both the SOFIA and APTIMA TMA tests would be SARS-CoV-2 culture positive, then the PPA of the SOFIA test compared to the APTIMA TMA test in patients likely to be culture positive is 87.2% for symptomatic patients tested ≤ 5 days from symptom onset and 54.5% for patients tested > 5 days from symptom onset.

Of the 346 patients for whom we obtained valid results in both tests, only one was positive in the SOFIA test and negative in the APTIMA TMA test. The residual nasopharyngeal specimen that was tested on the APTIMA TMA test was also negative with the XPERT test. It is most likely that this represents a false positive SOFIA test. There have been several news reports of significant false positive rates in SARS-CoV-2 rapid antigen tests, however, that was not observed in this set of patients. In this environment we observed an overall 99.6% NPA when comparing the SOFIA and APTIMA TMA tests in symptomatic patients. It should be noted that this data was collected in an area with a high prevalence of SARS-CoV-2 and that the percent of false positives may increase in lower prevalence settings.

This study has several limitations. The first is that nasal swabs were utilized with the SOFIA test while nasopharyngeal swabs were utilized with the APTIMA TMA test. It is possible that collection of a nasopharyngeal swab for use in the antigen test would have further increased the agreement between the SOFIA and APTIMA TMA tests. Another limitation of this study is the sample size, particularly for those patients whose specimens were collected > 5 days post symptom onset. This subset of patients made up 14% of the study and only included 11 positive specimens. Finally, a significant limitation of this study is that, due to a lack of resources, positive specimens could not be cultured. It would have been beneficial to truly
understand how the SOFIA test performed in culture positive patients rather than estimating that
result based on the Ct values from the patient specimens.

In this study, we observed an 82% PPA, a 96.4% negative predictive value (NPV), and a 100%
positive predictive value (PPV) with the SOFIA test (in symptomatic patients ≤ 5 days from
symptom onset) compared to molecular testing. While the PPA is lower than what is reported in
the manufacturer’s package insert, the SOFIA test allowed providers to very quickly identify the
majority of SARS-CoV-2 positive patients presenting to our urgent care center. We estimate the
PPA and NPV of the SOFIA test would be even greater when comparing the test to virus
culture (estimated at 87.2% and 97.7%, respectively in this study).

For those facilities with limited access to molecular testing (or slow turnaround times due to high
volumes or transport to off-site labs) and a high prevalence of SARS-CoV-2, rapid antigen tests
may provide a rapid and accessible method for diagnosing SARS-CoV-2 infection in
symptomatic patients. The NPV of these tests may not be sufficient to completely rule out
SARS-CoV-2 in symptomatic patients, but the ability to rapidly identify most positive patients
can significantly decrease the efforts required by public health officials to perform contact
tracing and save precious molecular testing materials for those patients most in need (8).
Ultimately, facilities will need to decide if/how SARS-CoV-2 antigen testing can help them
combat the current pandemic and whether the rapid speed and moderate sensitivity can be
beneficial in at least some of their patient settings.

Acknowledgements:

We would like to acknowledge the providers and laboratory staff in the Advocate Aurora Health
Care West Bend South Urgent Care Center and within ACL Laboratories who diligently treat
and perform testing for our patients. We would also like to acknowledge, Dr. Allen Bateman of
the Wisconsin State Laboratory of Hygiene for his critical review of this manuscript prior to submission.

**References:**


Table 1. Comparison of results from Quidel Sofia SARS FIA test to Hologic Aptima SARS-CoV-2 TMA test by days from symptom onset

<table>
<thead>
<tr>
<th>Patient Groups</th>
<th># Patients</th>
<th>SOFIA Positive / APTIMA Positive</th>
<th>SOFIA Positive / APTIMA Negative</th>
<th>SOFIA Negative / APTIMA Positive</th>
<th>SOFIA Negative / APTIMA Negative</th>
<th>Positive % Agreement</th>
<th>Negative % Agreement</th>
<th>Total % Agreement</th>
<th>Negative Predictive Value</th>
<th>Positive Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5 days post symptom onset</td>
<td>298</td>
<td>41</td>
<td>0</td>
<td>9</td>
<td>248</td>
<td>82.0%</td>
<td>100.0%</td>
<td>97.0%</td>
<td>96.4%</td>
<td>100.0%</td>
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<tr>
<td>&gt; 5 days post symptom onset</td>
<td>48</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>36</td>
<td>54.5%</td>
<td>97.3%</td>
<td>87.5%</td>
<td>87.8%</td>
<td>85.7%</td>
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<tr>
<td>Total</td>
<td>346</td>
<td>47</td>
<td>1</td>
<td>14</td>
<td>284</td>
<td>77.0%</td>
<td>99.6%</td>
<td>95.7%</td>
<td>95.3%</td>
<td>97.9%</td>
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Table 2. Comparison of results from Quidel Sofia SARS FIA test to Hologic Aptima SARS-CoV-2 TMA test by patient age

<table>
<thead>
<tr>
<th>Patient Groups</th>
<th># Patients</th>
<th>SOFIA Positive / APTIMA Positive</th>
<th>SOFIA Positive / APTIMA Negative</th>
<th>SOFIA Negative / APTIMA Positive</th>
<th>SOFIA Negative / APTIMA Negative</th>
<th>Positive % Agreement</th>
<th>Negative % Agreement</th>
<th>Total % Agreement</th>
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<tr>
<td>≤18 years</td>
<td>122</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>111</td>
<td>72.7%</td>
<td>100.0%</td>
<td>97.5%</td>
<td>97.4%</td>
<td>100.0%</td>
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<td>19–50 years</td>
<td>134</td>
<td>22</td>
<td>0</td>
<td>5</td>
<td>107</td>
<td>81.5%</td>
<td>100.0%</td>
<td>96.3%</td>
<td>95.5%</td>
<td>100.0%</td>
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<tr>
<td>&gt; 50 years</td>
<td>90</td>
<td>17</td>
<td>1</td>
<td>6</td>
<td>66</td>
<td>73.9%</td>
<td>98.5%</td>
<td>92.2%</td>
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<td>94.4%</td>
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<tr>
<td>Total</td>
<td>346</td>
<td>47</td>
<td>1</td>
<td>14</td>
<td>284</td>
<td>77.0%</td>
<td>99.6%</td>
<td>95.7%</td>
<td>95.3%</td>
<td>97.9%</td>
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Table 3. XPERT RT-PCR Cycle Threshold values from patients with SOFIA antigen negative, APTIMA TMA positive results

<table>
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<tr>
<th>Sample ID</th>
<th>Days Post Symptom Onset</th>
<th>SOFIA Antigen Test Result</th>
<th>APTIMA TMA Test Result</th>
<th>XPERT RT-PCR Test</th>
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<td></td>
<td></td>
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<td>Cepheid E Ct Value</td>
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<td>248</td>
<td>4</td>
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<td>216</td>
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<td>6</td>
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<td>Positive</td>
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<td>Positive</td>
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