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VIROLOGY

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

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ABSTRACT The Quidel Sofia severe acute respiratory syndrome (SARS) fluorescent immunoassay (FIA) test (SOFIA) is a rapid antigen immunoassay for the detection of SARS coronavirus 2 (SARS-CoV-2) proteins from nasal or nasopharyngeal swab specimens. The purpose of this study was to compare the results of the SOFIA test to those of the Hologic Aptima SARS-CoV-2 TMA test (APTIMA TMA), a high-throughput molecular diagnostic test that uses transcription-mediated amplification (TMA) for the detection of SARS-CoV-2 nucleic acid from upper respiratory tract specimens. Three hundred 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 for the SOFIA test and nasopharyngeal swabs for the APTIMA TMA test. The SOFIA test demonstrated a positive percent agreement (PPA) of 82.0% with the APTIMA TMA test for symptomatic patients tested ≤5 days from symptom onset and a PPA of 54.5% for symptomatic patients >5 days from symptom onset. The Cepheid Xpert Xpress SARS-CoV-2 reverse transcription-PCR (RT-PCR) test was used to determine the cycle threshold (C_T) value for any specimens that were discrepant between the SOFIA and APTIMA TMA tests. Using a C_T 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 who were likely to be culture positive.

KEYWORDS COVID-19, SARS-CoV-2, rapid antigen, Sofia

At present, diagnosis of active severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection relies primarily on the use of molecular diagnostic testing. In addition to molecular diagnostic tests, seven rapid antigen tests have received Emergency Use Authorization (EUA) for the diagnosis of SARS-CoV-2 infection. The first of these tests to receive EUA status was the Quidel Sofia SARS Antigen FIA (fluorescent immunoassay) test (SOFIA), on 17 July 2020 (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas; accessed 27 October 2020). This test is performed using either nasal or nasopharyngeal swabs and can be completed in approximately 15 min. 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 a 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.

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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 the diagnosis of coronavirus disease from 2019 (COVID-19) on patients experiencing signs and symptoms of upper respiratory tract infection. These data were collected at an urgent care center serving patients in an area with a high prevalence of SARS-CoV-2. In the month prior to the implementation of antigen testing, this site saw approximately 20 patients with signs and symptoms of COVID-19 each day. During that 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 27 October 2020), as well as historical data showing lower sensitivity of rapid antigen tests than of 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 transcription-mediated amplification (TMA) test (APTIMA TMA) in the central laboratory of ACL Laboratories (1–4).

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

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, WI, 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 in order 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 to 90 years old, and patients ≤18, 19 to 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 by 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 in which they came and were placed in a sealed plastic specimen transport bag. Specimens were delivered to the laboratory (located within the same building) within 10 min 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 minitip nylon flocked swab and were 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 central laboratory of ACL Laboratories. The specimens were tested with the Hologic Aptima SARS-CoV-2 transcription-mediated amplification test (Hologic, Marlborough, MA) on a Hologic Panther system (Hologic) according to the manufacturer's package insert.

Cepheid Xpert Xpress SARS-CoV-2 RT-PCR test. Any patients who 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 by the APTIMA TMA test were frozen at −70°C for approximately 3 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 by 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

TABLE 1 Comparison of results from the Quidel Sofia SARS FIA test with those from the Hologic Aptima SARS-CoV-2 TMA test by days from symptom onset

	No. of patients					Agreement (%)			Predictive value (%)	
Patient group	Total	SOFIA positive, APTIMA positive	SOFIA positive, APTIMA negative	SOFIA negative, APTIMA positive	SOFIA negative, APTIMA negative	Positive	Negative	Total	Negative	Positive
≤5 days post–symptom onset	298	41	0	9	248	82.0	100.0	97.0	96.4	100.0
>5 days post–symptom onset	48	6	1	5	36	54.5	97.3	87.5	87.8	85.7
Total	346	47	1	14	284	77.0	99.6	95.7	95.3	97.9

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 with the APTIMA TMA test were 72.7%, 81.5%, and 73.9% for patients ≤18 years of age, 19 to 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 with 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 from symptomatic outpatients presenting to an urgent care center. These data show that the SOFIA test has 82.0% or 54.5% PPA with the APTIMA TMA test for 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 patients in the former group.

Several recent studies have correlated the C_T value of SARS-CoV-2 PCR tests with the ability to culture live SARS-CoV-2. Most of these studies have shown that it is very rare to culture infectious virus from samples with C_T values above 34 or 35 (5–7). The XPERT test was performed on any samples that were APTIMA TMA test positive and SOFIA negative in order to determine the C_T value and better understand the clinical significance of this discrepancy. Two of these 14 specimens were negative by the XPERT test, and another had a C_T value that was >35 for both targets in the test (Table 3). If the assumptions are made that (i) all samples testing positive by the APTIMA TMA

TABLE 2 Comparison of results from the Quidel Sofia SARS FIA test with those from the Hologic Aptima SARS-CoV-2 TMA test by patient age

	No. of patients					Agreement (%)			Predictive value (%)	
Patient group	Total	SOFIA positive, APTIMA positive	SOFIA positive, APTIMA negative	SOFIA negative, APTIMA positive	SOFIA negative, APTIMA negative	Positive	Negative	Total	Negative	Positive
≤18 yr	122	8	0	3	111	72.7	100.0	97.5	97.4	100.0
19–50 yr	134	22	0	5	107	81.5	100.0	96.3	95.5	100.0
>50 yr	90	17	1	6	66	73.9	98.5	92.2	91.7	94.4
Total	346	47	1	14	284	77.0	99.6	95.7	95.3	97.9

TABLE 3 XPERT RT-PCR cycle threshold values from patients with SOFIA antigen-negative and APTIMA TMA-positive results

		Result of:		XPERT RT-PCR test		
Sample ID	Days post–symptom onset	SOFIA antigen test	APTIMA TMA test	Result	Cepheid E C_T value	Cepheid N2 C_T value
248	4	Negative	Positive	Positive	21.7	23.6
216	1	Negative	Positive	Positive	23.9	26.2
277	7	Negative	Positive	Positive	24.9	26.5
253	2	Negative	Positive	Positive	25.2	27
232	3	Negative	Positive	Positive	26.2	28.1
244	4	Negative	Positive	Positive	26.3	28.1
231	7	Negative	Positive	Positive	28.6	30.5
209	2	Negative	Positive	Positive	29.8	31.9
200	14	Negative	Positive	Positive	30.6	33.6
8	6	Negative	Positive	Positive	31.6	32.1
11	10	Negative	Positive	Positive	34.9	33.5
68	3	Negative	Positive	Positive	0	42.8
50	1	Negative	Positive	Negative	0	0
152	1	Negative	Positive	Negative	0	0
299	7	Positive	Negative	Negative	0	0

test are true positives and (ii) only those patients who either have an XPERT C_T value under 35 or tested positive on both the SOFIA and APTIMA TMA tests would be SARS-CoV-2 culture positive, then the PPA of the SOFIA test with the APTIMA TMA test for patients likely to be culture positive is 87.2% for symptomatic patients tested $\leq$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 by both tests, only one was positive by the SOFIA test and negative by the APTIMA TMA test. The residual nasopharyngeal specimen that was tested by the APTIMA TMA test was also negative by the XPERT test. It is most likely that this represents a false-positive SOFIA test result. 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 between the SOFIA and APTIMA TMA tests for symptomatic patients. It should be noted that these data were collected in an area with a high prevalence of SARS-CoV-2 and that the percentage of false-positive results 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 included only 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 C_T 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 $\leq$5 days from symptom onset) relative 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 believe that the PPA and NPV of the SOFIA test would be even greater if the test were compared 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 can 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 high speed and moderate sensitivity of such tests can be beneficial in at least some of their patient settings.

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