Clinical comparison and agreement of PCR, antigen, and viral culture for the diagnosis of COVID-19
Clinical Agreement Between Diagnostics for COVID19

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ABSTRACT

The aim of this study is to compare the COVID-19 nasopharyngeal PCR (NP PCR) to antigen, nasal PCR, and viral culture. One-hundred-and-fourteen risk-stratified patients were tested by culture, nasal PCR, NP PCR, and Ag testing. Twenty (48%) of the high risk and 23 (32%) of the low risk were NP PCR positive. Compared with NP PCR, the sensitivity of nasal PCR, Sofia Ag, BinaxNOW Ag, and culture were 44%, 31%, 37%, and 15%. In the high risk group, the sensitivity of these tests improved to 71%, 37%, 50%, and 22%. Agreement between tests was highest between nasal PCR and both antigen tests. Patients who were NP PCR positive but antigen negative were more likely to have remote prior COVID-19 infection (p<0.01). Nasal PCR and antigen positive patients were more likely to have symptoms (p = 0.01).

1. Introduction

From the start of the SARS-CoV-2 pandemic, nasopharyngeal swab specimens obtained for reverse-transcriptase polymerase chain reaction (NP PCR) have been considered the most accurate method for diagnosing COVID-19. This is primarily due to its high analytical sensitivity and ability to detect low viral titers [1]. Despite laboratories having the ability to analyze thousands of specimens per day, peaks in cases lead to strains on PCR reagent and consumable availability and laboratory testing capacity resulting in delays in testing turnaround. Additionally, PCR cannot readily distinguish between active infection on persistent non-viable virus shedding [2,3]. Several documented cases of persistent PCR positivity despite clinical improvement, antibody development, and culture negativity exist [4,5]. The standard nasopharyngeal swabs used for collection are uncomfortable to patients and require trained healthcare professionals to collect [6,7].

For these reasons, alternative options for diagnosis would be worthwhile. Most commonly, antigen (Ag) detection-based rapid diagnostic tests are being utilized as a screening method in low pretest probability situations, United States universities, and other congregate settings such as nursing homes and businesses [8]. These tests require no special training to collect, generate results within 30 minutes, and are inexpensive. Additionally, antigen testing may better correlate with viral culture and higher viral loads which may correlate with infectivity [9]. Despite these benefits, Ag performance is variable. Sensitivity ranges from 22.9%-93.9% when compared to PCR with better concordance in symptomatic patients [10–13]. This wide range may be because it is difficult to interpret these comparisons due to difference in testing method sensitivity, lower clinical specificity of the PCR in the setting of prior infection [4], and the absence of a true clinical gold standard for diagnosis. Viral culture has been suggested to be a potential tool for clearly defining true disease, and not just RNA shedding. Positive cultures are often viewed as correlates of contagiousness; however low sensitivity and logistical requirements for specialized expertise and significant laboratory time limit its use outside of research purposes [14].

Based on the hypothesis that test performance likely changes based on acuity of the infection, this study evaluates the inter-modality agreement between PCR, antigen-based testing, and viral culture in patients with high and low pretest probability of true disease. Clinical presentations were analyzed to help delineate the strengths and weaknesses of the various tests in different patient settings to add to the growing literature on this subject [13,14]. The ultimate goal was to be able to suggest scenarios where less invasive or faster tests could be used instead of the NP PCR based on clinical presentation or indication for testing.

2. Materials and methods

2.1. Patient enrollment and study population

Patients were enrolled from February 9th to May 10th, 2021. All adult patients (age ≥ 18 years) who were hospitalized at IU Health University Hospital or Methodist Hospital in Indianapolis, Indiana and who were tested for SARS-CoV-2 using NP PCR (the current diagnostic standard of care) ordered by their provider were eligible. Patients with a pending test were identified using the electronic medical record. Study purpose was discussed with all eligible patients and those who agreed via written consent were enrolled. Patients were excluded if >24 hours had passed from NP PCR collection by nursing, they refused consent, were not eligible to provide consent, discharged prior to discussion, or deceased prior to discussion. All patient activities were performed according to protocols approved by the Indiana University Institutional Review Board (IU IRB# 2003718653).

Patients were assigned into a " high risk group" if they were symptomatic at the time of testing with abnormal chest radiographs within the last 48 hours. The "low risk group" included patients that were asymptomatic at the time of testing with normal chest radiographs. Chest radiographs have been cited to have a sensitivity for COVID-19 of up to 55-79% depending how late in the course of illness it is obtained. Specificities have also been cited to be high (70-83%) but depend on characteristic findings and evolution through time [15]. At IU Health, COVD-19 testing orders require an indication to be selected by ordering providers in order to stratify samples for rapid versus standard testing. These selections were used for initial stratification. If the provider selected asymptomatic screening, the chart was reviewed to confirm absence of symptoms or abnormal chest radiographs (if present) and the patient was enrolled into the low risk group after consent. If the provider selected any other indication, the chart was reviewed for respiratory symptoms and abnormal chest radiography. If both were present, the patient was enrolled in the high risk group after consent. Three trained individuals collected samples over the listed time interval, but enrollment had to be stopped shorter than expected due to improvement in the pandemic in Indiana in the Spring of 2021 resulting in fewer inpatients to enroll.

2.2. Patient characteristics

Information was collected from all participants by chart review. Demographics for analysis were age and gender. Clinical manifestations were evaluated by presence of fever, dyspnea, cough, anosmia, or ageusia at time of admission or at any point during hospitalization. Presence and type of chest imaging findings within 48 hours of PCR testing was recorded. History of prior COVID positivity in the last year was collected. Hospital course was evaluated for receipt of COVID-19 specific therapies (Remdesivir, Dexamethasone, or Tocilizumab), need for intensive care unit (ICU) admission, maximum oxygen requirement, need for mechanical ventilation, need for intravenous vasopressor therapy, occurrence of a thrombotic event, occurrence of a secondary infection, receipt of antibiotics during hospitalization, length of stay, 30-day all-cause readmission, and 30-day all-cause mortality.

2.3. Specimen collection

One "wet" nasal swab (flocked swab placed in viral transport medium [VTM] for PCR and viral culture) and two "dry" nasal swabs (manufacturer supplied Sofia (Quidel, San Diego, CA) and Binax (BinaxNOW, Abbott, Abbott Park, IL) swabs, not placed in VTM for antigen testing) were collected from all enrolled participants. Specimens were collected following CDC recommended collection techniques from both nares.

BinaxNOW testing was performed at the bedside within 1 hour of according to manufacturer's instructions. The remaining wet and dry swab were labeled and sent to the IU Health Pathology Laboratory (IUHPL) for PCR testing and the IU School of Medicine Laboratory Animal Resource Center (Animal) Biosafety Level-3 (LARC [A]BSL-3) Resource Laboratory for viral culture.

2.4. Sample receipt, standard of care PCR testing, and storage

Following collection and prior to all diagnostic testing, anterior nares swabs for Sofia SARS Antigen FIA testing and PCR were stored at 4°C for less than 48 hours and NP swabs were stored at 4°C for less than 72 hours. NP swabs submitted for SARS-CoV-2 PCR testing as part of standard of care procedures were assayed either by the cobas Liat SARS-CoV-2 (Roche Diagnostics, Basel, Switzerland) or Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA) assays in accordance with the respective manufacturer's instructions. Following selection of patient samples for the study, residual NP swabs were retrieved from 4°C storage and transferred to 80°C until culture was performed.

2.5. Nasal swab PCR

Anterior nares swabs collected for PCR testing were assayed via the cobas SARS-CoV-2 (Roche) assay performed on the cobas 8800 system, with a small subset (indicated on master datasheet) assayed by the Nx-TAG CoV Extended Panel (Luminex, DiaSorin, Salugia VC).

2.6. SARS-CoV-2 culture

Frozen swab specimens in VTM were thawed, centrifuged at 1,500 g for 10 minutes at 4°C, and supernatants were used to inoculate monolayers of Vero E6 cells grown in 6-well tissue culture plates. Following adsorption, cells were overlaid with 2 ml of DMEM containing 2% heat-inactivated fetal bovine serum and 1X antibiotic-antimycotic solution. A 200-μl aliquot of the medium was removed for SARS-CoV-2 real-time PCR analysis and cultures and incubated for 7 days at 37°C in 5% CO_2. On the final day of incubation, a 200-μl aliquot of medium was removed for real-time PCR analysis, and cell monolayers were harvested by scraping into the medium with a pipette tip followed by centrifugation at 600 g for 5 min at room temperature. Cell pellets were spotted onto PTFE-coated microscope slides and fixed with methanol-acetone for indirect immunofluorescence-based detection of SARS-CoV-2 infection using the S protein antibody (1A9) (Genetex [17]).

For real-time RT-PCR-based detection of SARS-CoV-2 RNA in cultured samples, the method described by Corman et al. was used [16]. A decrease in cycle threshold (Ct) value between PCR-1 and PCR-2 was used as an indicator of virus replication. All work with infectious SARS-CoV-2 cultures was performed in the LARC (A)BSL-3 Resource Laboratory at the Indiana University School of Medicine according to the Institutional Biosafety Committee-approved protocols.

2.7. Statistical analysis

Agreement between tests was evaluated using Krippendorff's alpha and Cohen's kappa. Both values were used because Krippendorff's alpha handles fields with missing data since not all patients had every sample

type collected or available for analysis. Krippendorff's alpha was the primary method of comparing overall testing agreement. In our analysis, the Krippendorff's results matched very closely with the Cohen's results for paired test comparisons and only the Cohen's kappas were included in the tables. Chi squared and Fisher exact test were used for comparing dichotomous variables. Continuous variables were evaluated using Wilcoxon Rank Sum tests and length of stay was evaluated using log rank tests due to some inpatient deaths. All statistics were run using R version 4.1.1 (R Core Team, 2021).

3. Results

Samples were collected from 119 consented patients with 5 patients being excluded because original PCR samples were discarded prior to additional testing being performed on the remnant samples. Of the remaining 114 patients, 61% were female, the average age was 56, and 43 (38%) were NP PCR positive. Five individuals had invalid controls for the Sophia antigen test (of these, only one had a positive NP PCR), 9 had missing nasal PCR tests (of these, 8 had a positive NP PCR), and 15 had missing culture data (of these 3 had a positive NP PCR). All patients with missing data were included in the final analysis. Among 114 participants, 42 were high risk and 72 were low risk. Of the high risk, 20 were NP PCR positive, and 22 were NP PCR negative. Of the low risk, 23 were NP PCR positive and 49 were NP PCR negative. All NP PCR positive patients were defined as COVID positive.

When comparing antigen, culture, and nasal PCR to the current gold standard NP PCR (Table 1), specificity and positive predictive value were always 100% even when stratifying patients by high and low risk. Negative predictive value was generally lower in low risk than in high risk patients across modalities (with the exception of nasal PCR). Sensitivity was generally higher in high risk patients across modalities. In both high risk and low risk groups, the nasal PCR had the best negative predictive value. In the high risk group, sensitivity was best with the nasal PCR. In the low risk group, sensitivity was fairly equivalent between groups (with the exception of culture being lower).

Agreement between tests was highly variable (Table 2). Moderate to strong agreement was noted between the two antigen tests and the nasal PCR. Absent to weak agreement was noted between culture and all other tests (though the Sofia SARS Antigen FIA had moderate correlation) as well as NP PCR and all other tests. When stratifying by high risk, NP

PCR correlated better with the nasal PCR. In the low risk group, most tests had less correlation when compared to high risk patients.

We attempted to understand if modality test positivity was associated with certain symptom patterns (Table 3). Of note, nasal PCR was the only test associated with positive imaging findings (67% vs. 33%, p = 0.014), though any positive test was associated with an order for chest imaging from a provider. Positive BinaxNOW COVID-19 Ag Card and Sofia Antigen FIA tests were both associated with the presence of fever (38% vs. 12%, p=0.022 and 38% vs. 15%, p=0.049 respectively). Any positive test (except culture) was associated with receipt of COVID-specific treatment and with the presence of thrombosis. No tests were associated with ICU stay or ventilator requirement (26 total patients and 7 total patients respectively). NP PCR positive patients were more likely to have a previous positive COVID test (23% vs. 4.3%, p=0.004) where the association with a previous positive test was not found with the other testing modalities. NP and nasal PCR-negative patients were more likely to receive antibiotics than PCR-positive patients (68% vs. 37%, p=0.002 and 62% vs. 33%, p=0.036 respectively).

Combination of testing results were also assessed for association with symptoms (Table 4). For the purpose of this analysis, antigen negativity meant that both Sofia SARS Antigen FIA and BinaxNOW COVID-19 Ag Card testing were negative and antigen positivity meant that either or both antigen tests were positive. We compared the antigen coupled with NP PCR results for further analysis. PCR+/Ag+ patients were more likely to have an order for chest imaging, be symptomatic (specifically with fever), receive COVID therapeutics, develop a thrombosis during their encounter, stay in the hospital longer and die compared to the other groups . PCR+/Ag- patients (25 patients) were more likely to have a previously positive test for COVID and the average timing between tests was 72 days compared to 10 days in the 17 patients in the PCR+/Ag+ group (p=0.09). PCR-/Ag- patients were more likely to get antibiotics for treatment of suspected or confirmed bacterial infection (p=0.01).

4. Discussion

Unfortunately, the diagnosis of COVID-19 still lacks a true gold standard. The persistent positivity of NP PCRs in some patients (likely due to persistent non-viable RNA shedding [18]) creates difficult decisions for treatment of patients with relapsed infections, recurrent disease, secondary infections, and in the approach to infection prevention [5].

Table 1
Comparison of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) across testing modalities using the NP PCR as the gold standard. Each category is then analyzed in high and low risk populations in parentheses below the combined value (Ag = Antigen, NP = nasopharyngeal, PCR = polymerase chain reaction).

	Sensitivity (High-risk / Low-risk)	Specificity (%) (High-risk / Low-risk)	PPV (%) (High-risk / Low-risk)	NPV (%) (High-risk / Low-risk)
BINAX Ag	39 (50 / 24)	100 (100 / 100)	100 (100 / 100)	74 (67 / 75)
Sofia Ag	31 (37 / 26)	100 (100 / 100)	100 (100 / 100)	70 (65 / 73)
Nasal PCR	43 (71 / 22)	100 (100 / 100)	100 (100 / 100)	78 (83 / 78)
NP Culture	15 (22 / 9)	100 (100 / 100)	100 (100 / 100)	63 (50 / 69)

Table 2
Cohen Kappa agreement values between each testing modality. High and low risk populations separately analyzed in the parentheses below the combined value. (Ag = antigen, NP = nasopharyngeal, PCR = polymerase chain reaction). Krippendorff Alphas were also calculated and were almost identical to the Cohen Kappa assessments. Cohen is presented here for organization.

	NP PCR (High-risk / Low-risk)	BinaxNOW Ag (High-risk / Low-risk)	Sofia Ag (High-risk / Low-risk)	Nasal PCR (High-risk / Low-risk)	NP Culture (High-risk / Low-risk)
NP PCR					
BINAX Ag	0.45 (0.56 / 0.30)				
Sofia Ag	0.36 (0.39 / 0.32)	0.75 (0.72 / 0.78)			
Nasal PCR	0.50 (0.70 / 0.32)	0.96 (0.93 / 1.00)	0.77 (0.68 / 0.88)		
NP Culture	0.17 (0.20 / 0.12)	0.43 (0.43 / 0.32)	0.60 (0.68 / 0.48)	0.53 (0.47 / 0.55)	

Table 3
Clinical Characteristics of Tested Patients by Result

Characteristic	NP PCR			Binax			Sofia			Nasal PCR			Culture		
	Positive, N = 43[1]	Negative, N = 71[1]	p-value[2]	Positive, N = 16[1]	Negative, N = 96[1]	p-value[2]	Positive, N = 13[1]	Negative, N = 96[1]	p-value[3]	Positive, N = 15[1]	Negative, N = 90[1]	p-value[2]	Positive, N = 6[1]	Negative, N = 93[1]	p-value[4]
Gender			0.2			0.9			0.8			0.3			>0.9
Female	30 (70%)	40 (56%)		10 (62%)	58 (60%)		9 (69%)	59 (61%)		11 (73%)	52 (58%)		4 (67%)	58 (62%)	
Male	13 (30%)	31 (44%)		6 (38%)	38 (40%)		4 (31%)	37 (39%)		4 (27%)	38 (42%)		2 (33%)	35 (38%)	
Age	63 (52, 74)	56 (33, 67)	0.023	66 (59, 76)	58 (37, 68)	0.041	60 (52, 76)	60 (40, 68)	0.3	68 (58, 80)	56 (34, 67)	0.029	50 (41, 71)	60 (41, 69)	>0.9
Positive Image Finding	20 (47%)	26 (37%)	0.3	10 (62%)	36 (38%)	0.060	8 (62%)	37 (39%)	0.11	10 (67%)	30 (33%)	0.014	4 (67%)	32 (34%)	0.2
Fever	9 (21%)	10 (14%)	0.3	6 (38%)	12 (12%)	0.022	5 (38%)	14 (15%)	0.049	5 (33%)	12 (13%)	0.065	3 (50%)	13 (14%)	0.052
Dyspnea	17 (40%)	18 (25%)	0.11	8 (50%)	27 (28%)	0.090	4 (31%)	30 (31%)	>0.9	7 (47%)	23 (26%)	0.12	2 (33%)	24 (26%)	0.7
Cough	11 (26%)	13 (18%)	0.4	7 (44%)	17 (18%)	0.042	4 (31%)	19 (20%)	0.5	5 (33%)	14 (16%)	0.14	3 (50%)	14 (15%)	0.061
Treatment	15 (35%)	0 (0%)	<0.001	9 (56%)	6 (6.2%)	<0.001	4 (31%)	10 (10%)	0.062	9 (60%)	2 (2.2%)	<0.001	2 (33%)	10 (11%)	0.2
Thrombosis	6 (14%)	1 (1.4%)	0.011	4 (25%)	3 (3.1%)	0.008	3 (23%)	3 (3.1%)	0.022	3 (20%)	2 (2.2%)	0.020	0 (0%)	6 (6.5%)	>0.9
ICU Stay	9 (21%)	17 (24%)	0.7	4 (25%)	22 (23%)	>0.9	3 (23%)	23 (24%)	>0.9	3 (20%)	22 (24%)	>0.9	0 (0%)	24 (26%)	0.3
Vent	2 (4.7%)	2 (2.8%)	0.6	2 (12%)	2 (2.1%)	0.10	2 (15%)	2 (2.1%)	0.069	2 (13%)	2 (2.2%)	0.10	0 (0%)	3 (3.2%)	>0.9
Pressors	0 (0%)	2 (2.8%)	0.5	0 (0%)	2 (2.1%)	>0.9	0 (0%)	2 (2.1%)	>0.9	0 (0%)	2 (2.2%)	>0.9	0 (0%)	2 (2.2%)	>0.9
Previous Positive	10 (23%)	3 (4.2%)	0.004	2 (12%)	11 (11%)	>0.9	1 (7.7%)	12 (12%)	>0.9	2 (13%)	10 (11%)	0.7	1 (17%)	11 (12%)	0.5
Antibiotics	16 (37%)	48 (68%)	0.002	6 (38%)	58 (60%)	0.086	5 (38%)	55 (57%)	0.2	5 (33%)	56 (62%)	0.036	1 (17%)	51 (55%)	0.10
Secondary Infection	10 (23%)	11 (15%)	0.3	4 (25%)	17 (18%)	0.5	3 (23%)	18 (19%)	0.7	3 (20%)	15 (17%)	0.7	1 (17%)	14 (15%)	>0.9
Imaging Ordered by Provider	29 (67%)	30 (42%)	0.009	14 (88%)	45 (47%)	0.003	10 (77%)	47 (49%)	0.058	12 (80%)	38 (42%)	0.007	5 (83%)	43 (46%)	0.10
30 Day Readmission	12 (28%)	11 (15%)	0.11	2 (12%)	19 (20%)	0.7	4 (31%)	19 (20%)	0.5	3 (20%)	15 (17%)	0.7	1 (17%)	20 (22%)	>0.9
30 Day Death	5 (12%)	3 (4.2%)	0.2	4 (25%)	4 (4.2%)	0.014	3 (23%)	5 (5.2%)	0.053	3 (20%)	4 (4.4%)	0.059	0 (0%)	7 (7.5%)	>0.9

[1] n (%); Median (IQR)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test

[3] Fisher's exact test; Wilcoxon rank sum test; Pearson's Chi-squared test

[4] Fisher's exact test; Wilcoxon rank sum test

Very frequently, clinical presentation has to be factored into the testing in order to decide on a treatment or infection control approach [19,20].

Since the NP PCR is the current test of choice for COVID-19 diagnosis at our institution, we first compared the other testing modalities to this as the gold standard (Table 1). We found high specificity and PPV in all tests when compared to the NP PCR which fits with results from other studies [21,22]. This suggests that all of the analyzed testing modalities are reliable when they result positive. Also in alignment with other studies, decreased sensitivity was observed when comparing the other modalities to the NP PCR [15,21]. Nasal PCR offered the best sensitivity (44%) while the antigen tests were a bit lower (BinaxNOW COVID-19 Ag Card 37%, Sofia SARS Antigen FIA 31%), and culture was the worst (15%). Unfortunately, this raises questions about the adequacy of antigen or nasal PCR for hospital-based diagnosis of COVID-19 given that under-isolation could lead to outbreaks. In the hospital environment, false negatives are of higher consequence to healthcare workers and other patients as it relates to infection control and higher consequence to patients as it relates to access to treatment options [23].

In order to understand how these tests interacted, agreement statistics were calculated (Table 2) and showed generally stronger agreement between antigen tests and nasal PCR compared to other modalities. It has been suggested in the available literature that these less invasive collection modalities may be better predictors of infectivity than the NP PCR [24]. They are certainly more comfortable for patient collection [25]. Despite this, NP PCR remains the gold standard for diagnostics in a hospital setting given the highest sensitivity and the ability to detect prolonged low-level shedding.

Culture was included in the modalities for comparison given that a positive test is likely the most absolute predictor of *infectious* disease and not just non-viable shedding. Other studies also support the low sensitivity of culture-based techniques [26]. However, more sensitive cell lines, such as Ver E6 cells that overexpress the cell membrane protease TMPRSS2, have since become available, so it's possible that a modest increase in culture sensitivity would be obtained if study samples could be re-cultured using such a cell line. Antigen and nasal PCR testing had higher agreement with culture positivity than NP PCR which is likely related to the high prevalence of prolonged NP shedding after remote infection [18] and may be related to different rates of shedding by location over time after onset of infection [27].

We sought to understand if the less invasive collection modalities were related to certain patient presentations such that test performance would be improved by various clinical factors that could be used to predict improved sensitivity and adequate NPV. Symptoms and hospital course were assessed for all enrolled patients (Table 3). Low power limited the analysis of the most severe outcomes (ICU stay, ventilator requirement, pressor requirement, death). Generally, symptoms (specifically fever and cough) were more associated with antigen and nasal PCR positivity than with NP PCR or culture positivity. This is likely because of persistent RNA shedding after remote infection and may indicate that antigen or nasal PCR positivity is more indicative of acute disease and could be used as a surrogate for infectivity as suggested by other studies [24].

When analyzing patients who were NP PCR positive but antigen negative (Table 4), they were more likely to have a remote positive test compared to NP PCR positive and antigen positive patients. They were also less likely to be symptomatic, less likely to be treated with COVID therapeutics, less likely to have an acute clot, less likely to die, and more likely to be treated with antimicrobials for other infections. All of these findings seem to group patients into two separate phenotypes, the acutely ill, likely infectious (PCR+/Ag+ group) and the incidentally NP PCR positive, antigen negative group who is in the hospital for other issues. It should be noted that these are not completely exclusive categories, and some patients did not fit perfectly in either category.

Antigen and nasal PCR testing may be an easier way to determine if someone is infectious with SARS-CoV-2. Minimization of patient discomfort and faster turnaround time facilitate patient placement, triage, treatment, and infection prevention decisions. This study may support the use of antigen and nasal PCR testing to exclude infectivity in a patient with persistent NP positivity, but it must be balanced against the risk for false negatives which is a hard metric to define given lack of a true diagnostic gold standard. There were no positive cultures in antigen or nasal PCR negative patients. Additionally, antigen-negative and nasal PCR negative patients tended to be asymptomatic.

Caution must be advised if using antigen or nasal PCR testing for inpatient or outpatient diagnostics given the potential for false negatives. Given the possibility of NP PCR false positives after previous infection and culture false negatives due to poor sensitivity, there remains uncertainty as to the true sensitivity of the antigen and nasal PCR in the clinical environment. These tests perform better in symptomatic patients (Table 1), but the number of false negatives compared to NP PCR may

Table 4

Testing Combinations associated with clinical factors. (PCR = nasopharyngeal PCR, Ag+ = either Sofia SARS Antigen FIA or BinaxNOW COVID-19 Ag Card test positive, Ag- = both antigen tests negative).

	PCR- / Ag-N (%)	PCR+ / Ag-N (%)	PCR+ / Ag+N (%)	p-value
Imaging Ordered by Provider	30 (42)	14 (56)	15 (83)	0.01
Imaging Abnormal	26 (37)	9 (36)	11 (61)	0.16
Previous Positive Test	3 (4)	8 (32)	2 (11)	< 0.01
Average Time Between Tests	NA	72.32	10.03	0.09
Median Time Between Tests	NA	63.7	10.03	
Symptomatic	39 (55)	16 (64)	17 (94)	0.01
Average Time Between Symptom Onset and Test	NA	53.64	7.89	0.44
Median Time Between Symptom Onset and Test	NA	6.19	6.71	
Fever	10 (14)	2 (8)	7 (39)	0.02
Dyspnea	18 (25)	9 (36)	8 (44)	0.24
Cough	13 (18)	4 (16)	7 (39)	0.13
Receipt of COVID Therapeutic	0 (0)	6 (24)	9 (50)	<0.01
Thrombosis	1 (1)	2 (8)	4 (22)	<0.01
ICU Admission	17 (24)	5 (20)	4 (22)	0.92
Ventilator Requirement	2 (3)	0 (0)	2 (11)	0.21
Pressor Requirement	2 (3)	0 (0)	2 (11)	1
Secondary Infection	11 (15)	6 (24)	4 (22)	0.58
Antibiotics	48 (68)	10 (40)	6 (33)	0.01
Average Length of Stay	8.43	9.64	16.06	0.03
Median Length of Stay	4	7	11	
30-day Readmission	11 (15)	8 (32)	4 (22)	0.2
30-day Mortality	3 (4)	1 (4)	4 (22)	0.03

be unacceptably high in an inpatient, high-consequence environment. Additionally, antigen is becoming more widespread outpatient diagnosis and is even recommended for removal from isolation by the CDC creating the potential for early release of infectious individuals. Ultimately, further work should be done looking at these two groups (NP PCR positive, antigen negative vs. NP PCR positive, antigen positive) randomized to discontinuation of isolation and ongoing isolation with robust contact tracing and potentially environmental sampling and serial culturing using more sensitive cell lines to assure patients are being stratified appropriately.

The strengths of this study include the collection of multiple samples in close proximity to the patient presentation. Separation of samples in time, could result in lack of true correlation between tests given variance in viral shedding over time [28]. We were able to collect all tests within 24 hours of a PCR test being ordered by a provider. An additional strength is the addition of culture to the comparisons which has also been done in other studies [22] and adds a "bare minimum" sensitivity expectation for tests being compared (i.e. any test being considered should be able to define all positive cultures). Lastly, the correlation with clinical presentation and stratification by high and low risk groups adds to the growing literature on clinical phenotype associated with patients who are antigen positive vs. antigen negative and have a positive NP PCR.

This study has several weaknesses. We were not able to collect the number of samples we were initially budgeted to collect given improvement of the pandemic in the Spring of 2021 and graduation of our fellow workforce for sample collection. As a result, due to low sample size, we may have missed the ability to define significant associations especially as it relates to severe outcomes (death, ICU stay, etc.). We are uncertain how these results will generalize to new variants. Though samples were collected quickly after a NP sample was collected, transportation to the lab, sample freezing, and the performance of delayed culture using a less sensitive cell line compared to what is currently available may have affected culture sensitivity. Future studies in this area should attempt to duplicate at multiple institutions with a larger sample size. Temporal and clinical relationships in test performance and safety of removal from isolation based on these tests should continue to be explored.

COVID-19 will continue to be present in our clinical environment for the foreseeable years to come. Understanding the agreement between testing modalities when weighed against clinical inference can lead to better outcomes and a safer environment for both the patient and care team. Further work is needed to better understand this agreement.

Author contributions

- Cole Beeler developed the study, wrote the manuscript, collected samples, and organized submission.
- Amanda Agard and Omar Elsheikh developed the study, collected samples, and edited the manuscript.
- Drew Bell, Ryan Relich, Mariel Carozza, Guang-Shen Lei and Bryan Schmitt developed the study, performed microbiologic and diagnostic testing on samples, and edited the manuscript
- Josh Sadowski and William Fadel provided statistical support
- Douglas Webb, Lana Dbeibo, Kristen Kelley, and Paul Calkins supported the project from an IU Health system level, reviewed the data, and edited the manuscript

Declaration of Competing Interest

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