COVID-19 testing – Impact of Prevalence, Sensitivity, and Specificity on Patient Risk and Cost.

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Brief Title: Covid19 - risk as false results and cost

Key points:

1. Measuring risk metrics as the number and cost of false positive and negative results adds a great deal of knowledge that is masked by the usual statistical metrics of PPA, PNA, PPV and NPV.

2. The number and cost of false positive and negative test results are driven by prevalence, percent positive agreement (PPA/sensitivity), and percent negative agreement (PNA/specificity.)

3. The clinical implications and cost of false positive and negative test results can guide test selection and decisions about repeating test results for confirmation.

Key words: Covid19, risk, clinical, metrics, cost, false-positive, false-negative, prevalence, sensitivity, , specificity

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COVID-19 testing – Impact of Prevalence, Sensitivity, and Specificity on Patient Risk and Cost.

Abstract: Since the beginning of the year 2020, the global healthcare system has been challenged by the threat of the SARS-COV 2 virus. Molecular, antigen, and antibody testing are the mainstay to identify infected patients and fight the virus. Molecular and antigen tests that detect the presence of the virus are relevant in the acute phase only. Serological assays detect antibodies to the Sars-CoV-2 virus in the recovering and recovered phase. Each testing methodology has its advantages and disadvantages. To evaluate the test methods, sensitivity (percent positive agreement - PPA) and specificity (percent negative agreement – PNA) are the most common metrics utilized, followed by the positive and negative predictive value (PPV and NPV), the probability that a positive or negative test result represents a true positive or negative patient. In this paper, we illustrate how patient risk and clinical costs are driven by false-positive and false-negative results. We demonstrate the value of reporting PFP (probability of false positive results), PFN (probability of false negative results), and costs to patients and healthcare. These risk metrics can be calculated from the risk drivers of PPA and PNA combined with estimates of prevalence, cost, and Reff number (people infected by one positive SARS COV-2).

Introduction:

In early December 2019, a pneumonia of unknown cause was detected in Wuhan, China, and was reported to the World Health Organization (WHO). (1) On March 11th, 2020 WHO declared the virus a pandemic. (2) The novel virus, previously named the 2019-novel coronavirus (2019-nCoV), is currently designated as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2.) (3) According to the recent statistics of the World Health Organization (WHO), the disease COVID-19 has spread across continents, with 6,302,318 diagnosed cases and 376,210 deaths globally, and 1,811,277 cases 105,147 deaths in the USA until June 2, 2020. (4) Laboratory diagnosis and management of COVID-19 has

been instrumental in combating the spread of SARS-CoV-2. Clinical decisions rely on accurate molecular, antigen and antibody tests that correctly classify patients as positive or negative for presence of the SARS-CoV-2 virus, or for antibodies to that specific virus. The risk metrics of number and cost of false-positive and negative test results are driven by risk drivers of [A] prevalence of SARS-CoV-2 virus, or for antibodies in the test population, [B] Percent Positive Agreement (PPA/sensitivity), and [C] Percent Negative Agreement (PNA/specificity) of each test process.

Three test types

The gold standard at present for diagnosing suspected cases of COVID-19 is molecular testing, such as Real-time reverse transcription-polymerase chain reaction (rRT-PCR) which is a nucleic acid amplification test (NAAT) that detects unique sequences of the virus that causes COVID-19 (SARS-CoV-2). (5) Antigen tests, that also detect the presence of the SARS-CoV-2 virus, do not amplify viral components and are less sensitive (more likely to produce a false-negative result) than molecular tests. Negative antigen tests should be confirmed with a molecular test before considering a person negative for Covid-19. Molecular and antigen tests detect patients in the acute phase only.

A study by Yong et al (6) illustrates the shortcomings of RT-PCR as the only diagnostic method in surveillance, because of its inability to detect past infection, and the added value of serological testing. Serology tests can detect both active and past infections if the antibodies are captured within the relevant timeframe after the onset of the disease. (7) Serological assays detect IgG and IgM antibodies to the Sars-CoV-2 virus which could develop about 1-3 weeks after infection. Testing for IgG may be a superior marker of sustained immunity to SARS-CoV-2.(8) More scientific data on the immune response to SARS-CoV-2 is required to

design evidence-based recommendations for all testing scenarios and interpretation guidelines. (9)

On May 27, 2020, CDC issued Interim Guidelines for COVID-19 Antibody Testing stating "Although serologic tests should not be used at this time to determine if an individual is immune, these tests can help determine the proportion of a population previously infected with SARS-CoV-2 and provide information about populations that may be immune and potentially protected. Serologic test results may assist with identifying persons who may qualify to donate blood that can be used to manufacture convalescent as a possible treatment for those who are seriously ill from COVID-19." Contrary to early hopes to use serological testing to issue "immunity passports" to return to work and society, CDC now states clearly that "Serologic test results should not be used to make decisions about returning persons to the workplace." (9)

Table 1 describes the purpose of three types of tests with advantages, disadvantages and risks.

Table 1: Overview of Three Test Types

	Molecular Test	**Antigen Test**	**Antibody Test**
What does it detect?	This test detects the viral genome using a lab technique called polymerase chain reaction (PCR).	This test detects certain proteins from the surface of the virus.	Antibody testing detects IgG and IgM antibodies produced by the immune system in response to infection by the virus.
Sample type	A health care worker collects fluid from a nasal or throat swab or from saliva.	A nasal or throat swab is collected by the healthcare worker to get a fluid sample, antigen tests can produce results in minutes.	A health care professional takes a blood sample, usually by a finger prick or by drawing blood from a vein in the arm.

Advantages	Molecular tests are considered very accurate. Molecular tests are useful to track the spread of disease, identifying strains and mutations.	These tests are faster and less expensive than molecular tests. Antigen tests may be more practical to use for large population.	Accurate antibody testing can identify convalescent plasma donors and identify people who may have immunity.
Disadvantages	Molecular tests do not quantify viral load which becomes undetectable at the end of the disease course. A molecular test will not detect a prior infection, even one that has recently resolved.	Antigen tests are less sensitive than molecular tests. A molecular test may be recommended to confirm a negative antigen test result.	Positive antibody tests indicate that you were likely infected with SARS-CoV-2 at some time in the past and may have some immunity. The timing and type of antibody test affects accuracy. FDA advises that, if prevalence is low, as it usually is, laboratories should confirm positive tests using "an orthogonal testing algorithm (i.e., employing two independent tests in sequence.") (10)
Risk of False Positive Test	The patient would falsely believe they are infected and self-isolate. There would be unnecessary contact tracing.	The patient would falsely believe they are infected and self-isolate. There would be unnecessary contact tracing.	The patient would falsely believe they have antibodies, not practice physical distancing, and be at risk of infection and infecting others. Contacts may be traced.
Risk of False Negative Test	The patient would falsely believe they are virus-free, not self-isolate and infect Reff number of others.	The patient would falsely believe they are virus-free, not self-isolate and infect Reff number of others. FDA advises that negative antigen tests may need to be confirmed with PCR tests.	The patient would falsely believe they do not have antibodies, continue to practice physical distancing and fail to return to work and society.

Risk is the combination of the probability and severity of harm.

ISO/IEC Guide 51defines risk as "the combination of the probability of occurrence of harm and the severity of that harm." To evaluate/select test methods, laboratory professionals usually compare sensitivity (percent positive agreement - PPA) and specificity (percent negative agreement – PNA), followed by positive and negative predictive value (PPV and NPV) - the probability that a positive or negative test result represents a true positive or negative patient in the population tested. These metrics alone do not adequately or easily project the levels of patient risk or clinical costs associated with each test method. To estimate the probability of harm, the authors calculated the probability that a positive result is a false positive (PFP) and probability that a negative result is a false-negative (PFN). Probability of false-positive test results (PFP) is the number of false-positive results as a percent of all positive results. PFP is the remainder of positive predictive value (PPV), the probability that a positive result is a true positive. PFP = 1-PPV. Probability of false-negative test results (PFN) is the number of false-negative results as a percent of all negative results. PFP is the remainder of negative predictive value (NPV). PFN = 1-NPV. The authors roughly estimated cost of false results and from those we projected severity of harm as the costs incurred by patients and healthcare institutions.

PPA (sensitivity) and PNA (specificity) are inherent to the test method. Probabilities of true and false results in clinical settings change with prevalence of the virus or antibody in the population tested. "In a population where the prevalence is 5%, a test with 90% sensitivity and 95% specificity will yield a positive predictive value of 49%. In other words, less than half of those testing positive will truly have antibodies. Alternatively, the same test in a population with an antibody prevalence exceeding 52% will yield a positive predictive

greater than 95%, meaning that less than one in 20 people testing positive will have a false-positive test result." (10)

As of May 4, 2020, FDA requires that clinical agreement data should demonstrate a minimum overall 90.0% positive percent agreement (sensitivity) and 95.0% negative percent agreement (specificity.) (10) Most, but not all, values for sensitivity and specificity reported by FDA May 21, 2020 meet their goals. In the UK, recommended standards are set higher, at 98% PPA and 98% PNA (11). Recommendations are theoretical goals, and manufacturers' test results are created under controlled ideal conditions. The Foundation for Innovative New Diagnostics, FIND, working in partnership with WHO, maintains a diagnostics resource center that includes an interactive dashboard showing SARS-CoV-2 sensitivity and specificity, as assessed in laboratory on-site evaluation studies (12). We chose to model their meta-analysis results as the baseline in simulations as we felt these are more representative of current test performance in use in testing laboratories. **Table 2** shows baseline FIND PPA and PNA values for each test type, plus the number of different sample types, companies, individual test names, test formats or targets detected. Index sample types include nasopharyngeal swab, lower respiratory system, sputum, tracheal aspirate, capillary blood, serum, and plasma. Test formats include integrated systems, manual isothermal amplification, manual PCR, rapid diagnostic tests – with and without reader, chemiluminescence immunoassay, enzyme-linked immunosorbent assay (ELISA), and more. Notice the large number of companies and test names. Targets include RNA with and without extraction, nucleocapsid protein, nucleoprotein antigens, IgG, IgM and IgA.

Table 2. Baseline PPA and PNA (FIND)	Molecular	Antigen	Antibody
PPA Percent Positive Agreement (sensitivity)	86.14%	61.70%	68.44%
PNA Percent Negative Agreement (specificity)	95.84%	98.26%	95.6%
Index Sample Type	10	4	6
Company Names	33	3	54
Test Names	35	4	74
Test Formats	3	2	6
Targets	4	4	5

https://finddx.shinyapps.io/COVID19DxData/

The authors modeled the impact of +/-10% in PPA (sensitivity) from baseline. We modeled up to 100% PNA (specificity), with a lower limit of -10% from baseline. Prevalence of the SARS-CoV-2 virus and antibody is unknown and may vary widely between locations. Estimating prevalence is complicated by the existence of false-positive and false-negative tests. We modeled changes in prevalence for all tests from 2% to 20%, with an estimated baseline of 11%. The impact of the 'risk drivers' of prevalence, PPA (sensitivity) and PNA (specificity) on the 'risk metrics' of probability of false-positive and false-negative test results are shown in ~~Tables 3, 4 and 5.~~ Figures 1, 2 and 3.

Figure 1. Impact of Prevalence on False Results

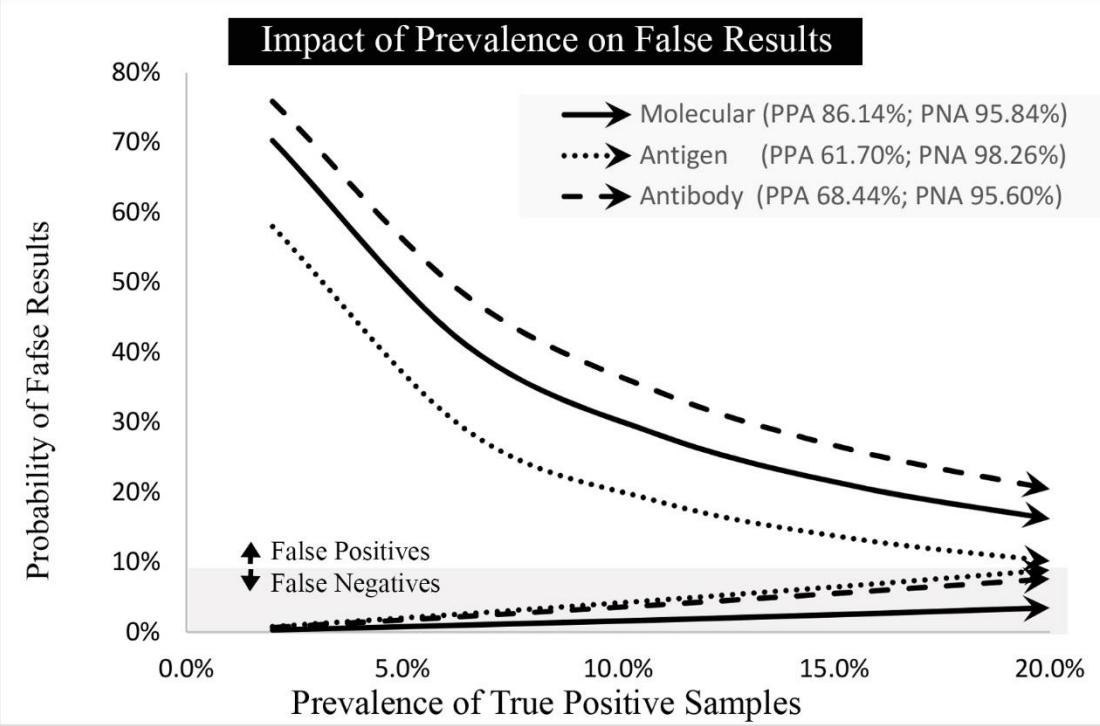

Figure 1 illustrates how increasing prevalence of true-positive samples impacts the probability that a positive test is false (PFP) and the probability of false-negative results (PFN). The number of patients who are positive for the SARS-CoV-2 virus or antibody increases with prevalence. Prevalence is governed by the spread of COVID-19 in the population tested and is beyond control of test selection and quality. The number of true positive samples increases with prevalence and true negative samples decrease. False-positive tests are a portion of true-negative samples, so they also decrease. The patterns for all tests are similar, but not identical because the baseline PPA and PNA values differ between test types. As prevalence increases from 2% to 20%, with PPA and PNA constant at baseline, the probability that a positive test result is a false-positive (PFP) decreases significantly from 70.3% to 16.2% for molecular tests, 58.0% to 10.1% for antigen tests and 75.9% to 20.5% for antibody tests. Unlike false positives that decrease with prevalence, false negatives increase. False-negative test results are a portion of true positive samples, so

they increase over tenfold in proportion to prevalence - from 0.3% to 3.5% for molecular tests, 0.8% to 8.9% for antigen and 0.7% to 7.6% for antibody tests. This dramatic increase may be masked by examining only negative predictive value (NPV) which decreases slightly from 99.7% to 96.5% overall.

Figure 2 Impact of increased PPA (sensitivity) on false results

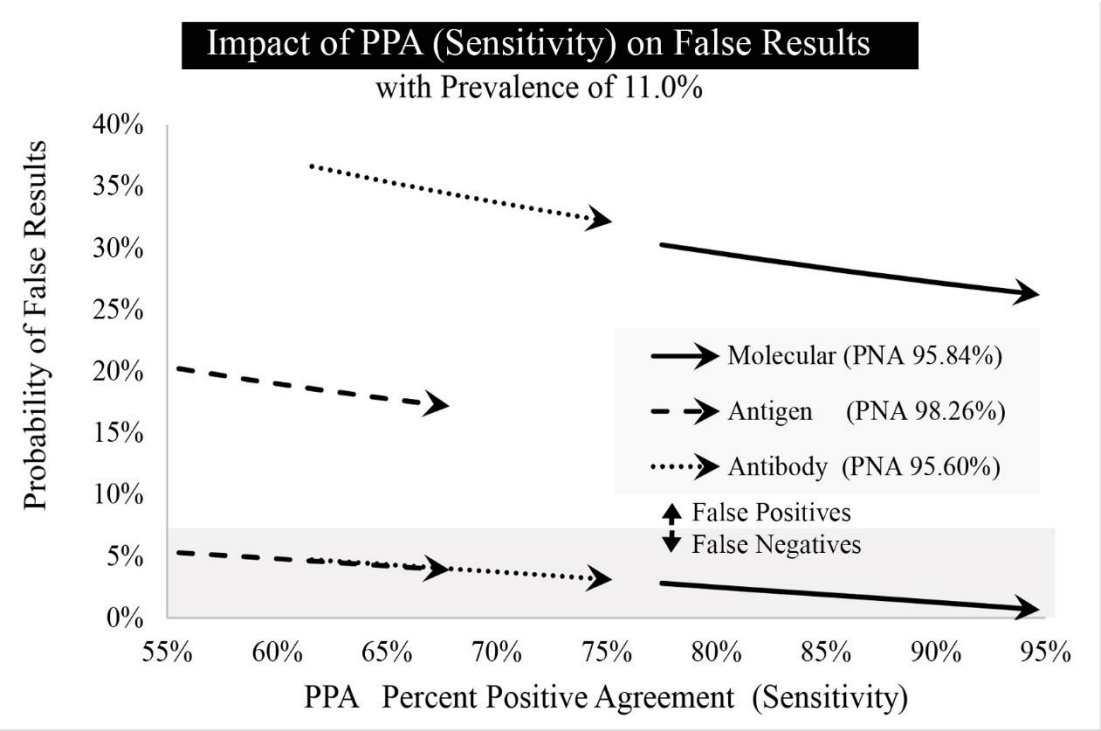

Figure 2 shows the impact of modeled changes of +/- 10% from baseline percent positive agreement, PPA (sensitivity for each test type, with prevalence and PNA constant at baseline. Higher PPA indicates a larger percent of positive test results in true positive samples. True-positive test results increase, but the number of false positives is not affected by PPA. As true-positive tests increase with PPA, the constant number of false-positive tests (that are driven by PNA) form a smaller portion of all positive results, decreasing PFP (probability of false positive) from 30.3% to 26.2% for molecular tests. Antigen tests have a lower range of PPA and a higher PNA, causing a smaller change in probability of false positive tests from 20.2% to 17.2%. As PPA increases for antibody tests, the range of PFP

decreasing from 36.6% to 32.1%. As sensitivity (PPA) increases, the number of true positive test results increases, and false negatives decrease.

Figure 3 Impact of changes in PNA (specificity) on false results

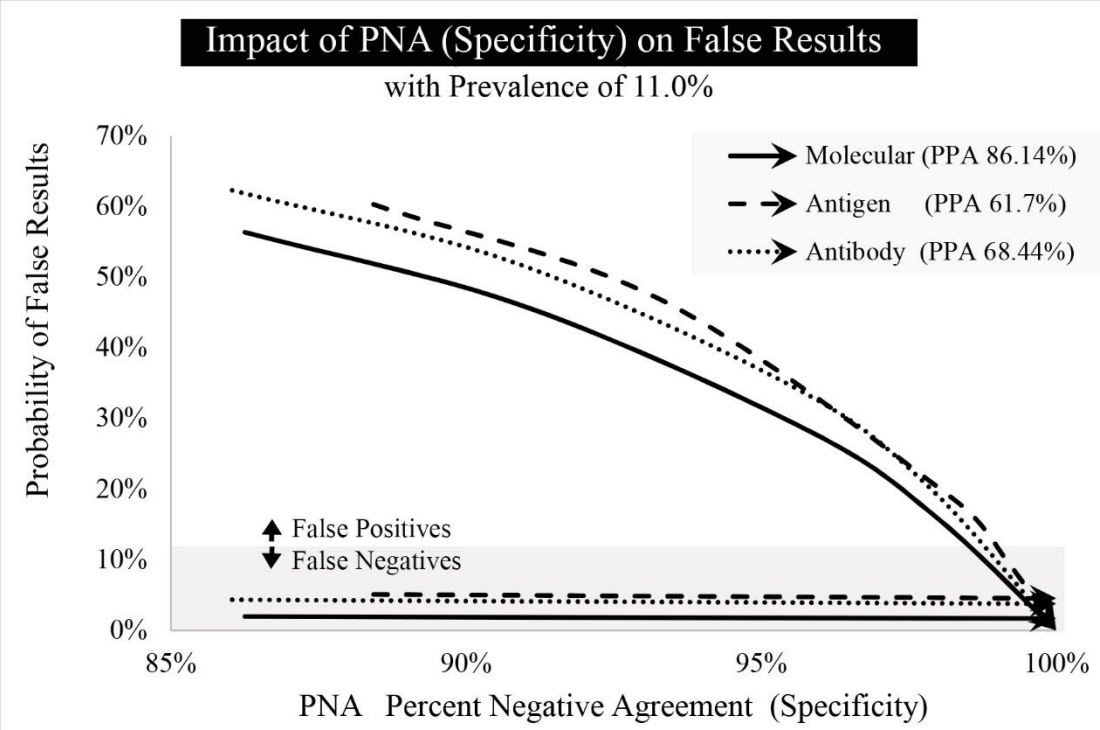

Figure 3 shows the impact of modeled changes in PNA (specificity) on false test results for each test type. When percent negative agreement (PNA) reaches 100%, all negative results are true negatives and probability of false positives decrease to zero. As PNA increases from 86.3% to 100%, PFP decreases from 56.3% to 0% for molecular tests. Antigen tests have a higher range of PNA (88.4% to 100%) with a resultant change in PFP from 60.3%% to 0%. Antibody tests, with a range of PNA from 86.0% to 100%, show a range of PFP decreasing from 62.3%% to 0%. Notice that Percent Positive Agreement (sensitivity) had less impact than prevalence or PNA (specificity) on probability of false-positive tests.

As specificity (PNA) increases, the number of true negative results increases; false negatives are unchanged in number but form a smaller portion of all negatives, driving the probability

of false negatives (PFN) down. PFN decreases from 2.0% to 1.7% for molecular, 5.1% to 4.5% for antigen and 4.3% to 3.8% for antibody tests.

Implications of False Results for Patient and Clinical Cost

Laboratories invest a great deal of effort in test selection to minimize patient risk and clinical cost caused by false results. Table 1 presented the different clinical interpretation of each type of test. False positive and false-negative results drive patient risk and clinical care costs. The authors estimated costs for the USA in May 2020 as shown in Table 6a and 6b, with the understanding that these are rough estimates.

The potential harm of false-positive and false-negative results (13) as discussed in Table 1 is applied in **Figures 4, 5, 6 and 7** to create a rough estimate of patient and clinical care costs for the USA. These costs are used as a model to illustrate the process of converting risk drivers of prevalence plus method PPA (sensitivity) and PNA (specificity) to risk metrics of the number and cost of erroneous results.

Figure 4 shows how costs are applied to true and false positive patient samples. Individual costs (A to F) were roughly estimated by authors based on research and opinion. The total cost for each sample is calculated by adding all the checked costs and multiplying by Reff where indicated. An online calculator is available at https://awesome-numbers.com/risk-calculator/ for readers to modify costs and model various scenarios with user-input variables of prevalence, PPA PNA and Reff. Current Reff values by US state can be found at

Figure 4 Clinical and patient costs per sample by test type

Clinical & patient costs of each Covid-19 sample/patient for true and false test results		☑ = Costs of true and false test results											
	Sample is:	Molecular				Antigen				Antibody			
		Pos		Neg		Pos		Neg		Pos		Neg	
	Test is:	TP	FN	TN	FP	TP	FN	TN	FP	TP	FN	TN	FP
A. Obtain, perform and report test	$200	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
B. Covid-19 clinical treatment	$3,045	☑	☑			☑							
C. Contact tracing	$1,000	☑	☑		☑	☑			☑	☑			☑
D. Patient cost for self isolation	$1,400	☑	☑		☑	☑			☑		☑	☑	
E. Confirm with orthologous test	$50-$200						☑	☑		☑			☑
F. Cost is impacted (multiplied) by Reff (R_0)			☑										☑
G. Probability of cost varies with prevalence													☑
Estimated total cost of each true and false test result reported													
H. Clinical & patient cost per True-Positive test		**$5,645**				**$5,645**				**$1,250**			
I. Clinical & patient cost per True-Negative test		**$200**				**$400**				**$1,600**			
J. Clinical cost per False-Positive test		**$2,600**				**$2,600**				**$522 to $1618**			
K. Clinical cost per False-Negative test		**$11,290**				**$400**				**$1,600**			

A. Healthcare system costs to obtain, perform and report the test were roughly estimated by authors at $200.

B. Although costs are much higher for hospitalized patients, “A single symptomatic COVID-19 case could incur a median direct medical cost of $3,045 during the course of the infection alone.” (16)

C. A report from Johns Hopkins University put the cost of hiring 100,000 new community health workers for contact tracing at an estimated $3.6 billion and the Association of State and Territorial Health Officials has echoed that estimate as the minimum requirement in a memo to Congress.” (17) The authors roughly projected cases at 3,600,000 based on the 2,157,768 cases as of June 16, 2020 (18) to estimate cost at $1,000 per patient.

D. Patient cost for self isolation was estimated by authors at $100 per day.

E. The FDA advises that "antigen tests may not detect all active infections, as they do not work the same way as a PCR test. … negative results from an antigen test may need to be confirmed with a PCR test prior to making treatment decisions or to prevent the possible spread of the virus due to a false negative." (19) The authors set cost to confirm positive

antigen tests at $200, as that includes collecting a new sample and PCR testing. CDC advises that "Three strategies can be used to improve positive predictive value: 1. Choose a test with a very high specificity, perhaps 99.5% or greater. 2. Focus testing on persons with a high pre-test probability of having SARS-CoV-2 antibodies, such as persons with a history of COVID-19-like illness or 3. Employ an orthogonal testing algorithm in which persons who initially test positive are tested with a second test."(20) The authors set cost to confirm positive antibody tests at $50, as a new sample is not required.

F. Reff or R_0 is the effective number of people infected by one positive Covid19 case. (19, 20). False-negative molecular tests in true-positive samples, and false-positive antibody tests in true-negative samples, may mislead patients to move freely in society, and infect #Reff others. These people are, or may become, infected, incur the same costs as the true positive patients and will infect Reff others. Other checked costs are multiplied by (1+Reff) where indicated.

G. False-positive antibody tests may mislead patients to move freely in society become infected at the rate of prevalence. Other costs for are multiplied by prevalence.

In the following section D=Disease; T = Test; + = Positive; - = Negative

H. True- positive tests (D+/T+) include costs of all checked items. Molecular and antigen positives indicate current infection with associated clinical costs ($5645.) True-positive antibody tests were assumed to protect patients from infection; costs include sample testing, contact tracing and confirmation with an orthogonal test ($1200.)

I. True-negative tests (D-/T-) include only testing for molecular tests ($200); testing and confirmation for antigen tests ($400); and testing plus self-isolation for antibody tests ($1600.)

J. False-positive tests (D-/T+) incur costs of testing plus self-isolation and contact tracing for molecular and antigen tests. Costs of false positive antibody tests include the risk that the patient s receiving these results may become infected and infect other and are calculated as ((Cost of treatment x prevalence) x (1+Reff)) x ((1+Reff) x Testing)).

K. False-negative molecular tests (D+/T-) occur in people who are actually infected, will incur costs of the true positive result, multiplied by (1 + Reff) to account for other people infected. With Reff set at 1.0, false-negative molecular tests cost $11,290. False-negative antigen tests are confirmed with an orthogonal test to incur total costs of $400. False-negative antibody tests incur the same costs as true negatives for testing plus self-isolation for antibody tests ($1600.)

Figure 5 presents the impact of increased prevalence on cost of false results. The x-axis represents the modeled value of prevalence; the y-axis shows patient and clinical cost of error per one thousand samples tested. Cost of false-positive results decreases slightly as prevalence increases because the number of true-negative samples decrease from 980 to 800 per 1,000 samples. False-positive tests are a fraction of true-negative samples which is driven by PNA (percent negative agreement/specificity.) The number of true positive samples increases from 20 to 200 per 1,000 samples as prevalence increases from 2% to 20%, driving up true-positive and false-negative test results and costs. False-negative tests are a fraction of true-positive samples which is driven by PPA (percent positive agreement/sensitivity.)

Costs vary between test types due to variation in baseline PPA (sensitivity) and PNA (specificity.) Costs are based on patient and clinical cost in Figure 4. Costs of each false negative molecular test result is much higher than other tests.

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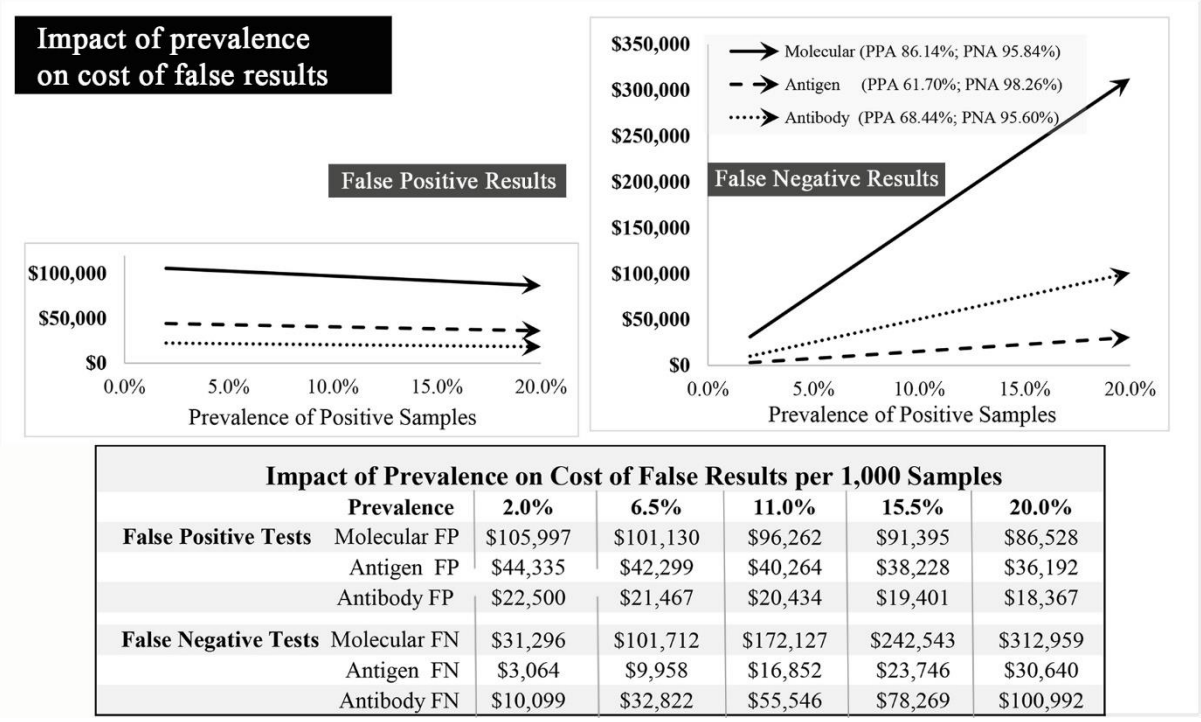
Figure 5 Impact of prevalence on cost of false results, with PPA and PNA at baseline.



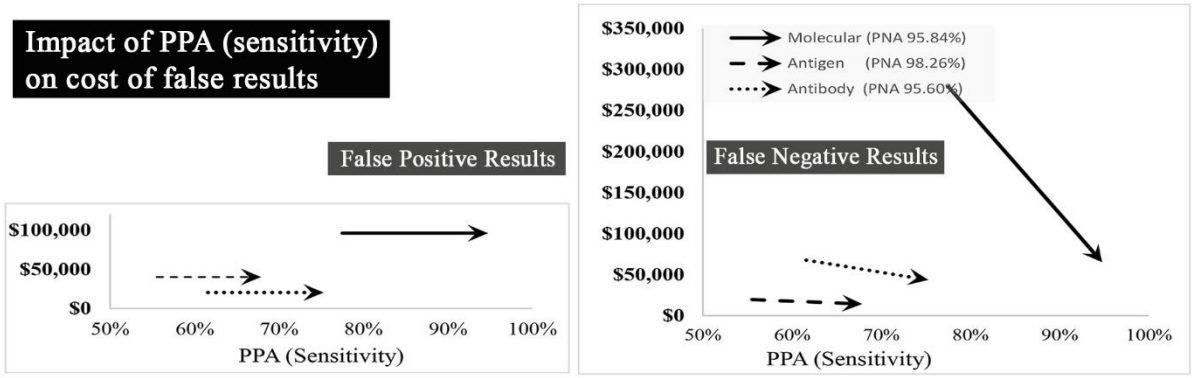
Impact of Prevalence on Cost of False Results per 1,000 Samples

	Prevalence	2.0%	6.5%	11.0%	15.5%	20.0%
False Positive Tests	Molecular FP	$105,997	$101,130	$96,262	$91,395	$86,528
	Antigen FP	$44,335	$42,299	$40,264	$38,228	$36,192
	Antibody FP	$22,500	$21,467	$20,434	$19,401	$18,367
False Negative Tests	Molecular FN	$31,296	$101,712	$172,127	$242,543	$312,959
	Antigen FN	$3,064	$9,958	$16,852	$23,746	$30,640
	Antibody FN	$10,099	$32,822	$55,546	$78,269	$100,992

Figure 6 shows the impact of PPA (sensitivity) on cost of false results, with prevalence and PNA at baseline. The x-axis shows the baseline PPA for each test type +/- 10%; the y-axis shows patient and clinical costs as shown in Figure 4. With baseline prevalence of 11%, the number of total positive samples is constant at 110 per 1,000. It is somewhat counterintuitive that percent positive agreement has no impact on false positives. False-positive tests are a fraction of true-negative samples (890 per 1,000 samples); that fraction is driven by PNA (percent negative agreement/specificity.)

Increasing percent positive agreement drives the number/cost of true positive results up and number/cost of false negative results down. Again, because false negative molecular tests cost more than false negative antigen or antibody tests, their costs show the greatest impact. If one looks only at the statistical indicator of probability of false results, the impact on cost is not apparent.

Figure 6 Impact of PPA (sensitivity) on cost of false results, with prevalence and PNA at baseline.

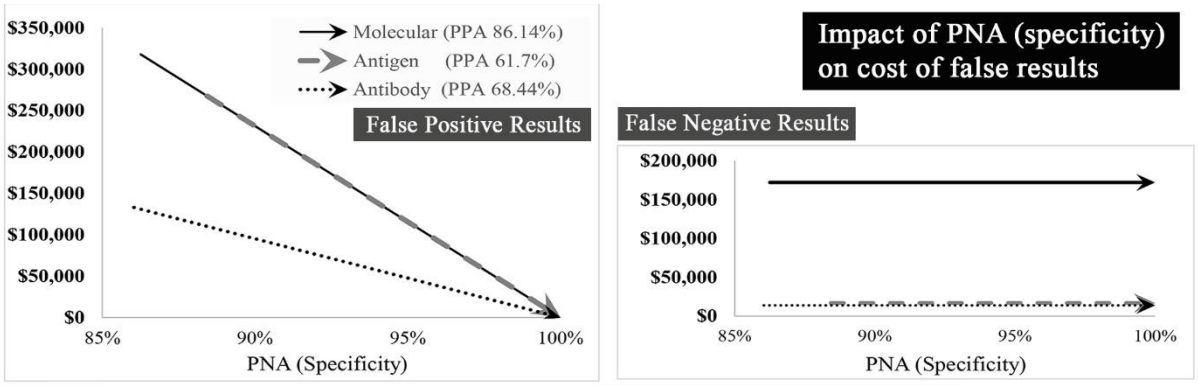


Modeled range of PPA		-10%		Baseline		+10%
	Molecular PPA	77.53%	81.83%	**86.14%**	90.45%	94.75%
	Antigen PPA	55.53%	58.62%	**61.70%**	64.79%	67.87%
	Antibody PPA	61.60%	65.02%	**68.44%**	71.86%	75.28%
Impact of PPA (sensitivity) on Cost of False Results						
False Positive Tests	Molecular FP	$96,262	$96,262	$96,262	$96,262	$96,262
	Antigen FP	$40,264	$40,264	$40,264	$40,264	$40,264
	Antibody FP	$20,434	$20,434	$20,434	$20,434	$20,434
False Negative Tests	Molecular FN	$279,105	$225,616	$172,127	$118,639	$65,150
	Antigen FN	$19,567	$18,209	$16,852	$15,495	$14,137
	Antibody FN	$67,591	$61,568	$55,546	$49,523	$43,500

Figure 7 shows the impact of PNA (specificity) on cost of false results, with prevalence and PPA at baseline. The x-axis shows the baseline PNA for each test type +/- 10% (to a maximum of 100%); the y-axis shows patient and clinical costs as shown in Figure 4. With baseline prevalence of 11%, the number of total positive and negative samples are constant at 110 and 890 per 1,000, respectively. Increasing percent negative agreement drives the number/cost of true negative results up and number/cost of false positive results down. False-positive tests are a fraction of true-negative samples (890 per 1,000 samples); false positives decrease as PNA (specificity) increases. Increased PNA (specificity), percent negative agreement, has the greatest impact on costs of false positive test results because of the vastly higher portion of negative patient samples.

Percent positive agreement has no impact on false negative test results. Again, because false negative molecular tests cost more than false negative antigen or antibody tests, their costs show the greatest impact.

Figure 7 Impact of PNA (specificity) on cost of false results, with prevalence and PPA at baseline.



Modeled range of PNA		-10%		Baseline		+10%
Molecular PNA		86.26%	91.05%	**95.84%**	97.92%	100.00%
Antigen PNA		88.43%	93.35%	**98.26%**	99.13%	100.00%
Antibody PNA		86.04%	90.82%	**95.60%**	97.80%	100.00%
Impact of PNA (specificity) on Cost of False Results						
False Positive Tests	Molecular FP	$318,036	$207,149	$96,262	$48,131	$0
	Antigen FP	$267,637	$153,950	$40,264	$20,132	$0
	Antibody FP	$132,929	$87,413	$41,897	$20,949	$0
False Negative Tests	Molecular FN	$172,127	$172,127	$172,127	$172,127	$172,127
	Antigen FN	$16,852	$16,852	$16,852	$16,852	$16,852
	Antibody FN	$13,886	$13,886	$13,886	$13,886	$13,886

Table 3 presents the total cost per 1,000 samples tested of false results for each test type, with modeled variations in risk drivers of prevalence, PPA (sensitivity) and PNA (specificity.) In each case, molecular tests carry the greatest risk of cost of false results due to the high cost of false-negative results. The specific numbers vary with baseline prevalence, PPA, PNA and costs of each test type.

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Table 3 Total cost of false results per 1,000 samples with variations in risk drivers

Impact of prevalence of cost of false results on patient and healthcare costs / 1,000 samples tested					
Prevalence	2.0%	6.5%	11%	15.5%	**20%**
Molecular	$137,293	$202,841	$268,390	$333,938	**$399,487**
Antigen	$47,399	$52,257	$57,116	$61,974	$66,832
Antibody	$32,599	$54,289	$75,979	$97,669	$119,359

Impact of PPA (sensitivity) of cost of false results on patient and healthcare costs / 1,000 samples tested					
Modeled range of PPA	**-10%**		**Baseline**		**+10%**
Molecular PPA	77.53%	81.83%	**86.14%**	90.45%	94.75%
Antigen PPA	55.53%	58.62%	**61.70%**	64.79%	67.87%
Antibody PPA	61.60%	65.02%	**68.44%**	71.86%	75.28%
Molecular	**$375,367**	$321,878	$268,390	$214,901	$161,412
Antigen	$59,830	$58,473	$57,116	$55,758	$54,401
Antibody	$88,025	$82,002	$75,979	$69,957	$63,934

Impact of PNA (specificity) of cost of false results on patient and healthcare costs / 1,000 samples tested					
Modeled range of PNA	**-10%**		**Baseline**		**+10%**
Molecular PNA	86.26%	91.05%	**95.84%**	97.92%	100.00%
Antigen PNA	88.43%	93.35%	**98.26%**	99.13%	100.00%
Antibody PNA	86.04%	90.82%	**95.60%**	97.80%	100.00%
Molecular	**$490,164**	$379,277	$268,390	$220,259	$172,127
Antigen	$284,489	$170,802	$57,116	$36,984	$16,852
Antibody	$146,815	$101,299	$55,784	$34,835	$13,886

Discussion:

The authors combined PPA and PNA values from user evaluation studies with estimates of prevalence, cost, and Reff number to illustrate a model showing how patient risk and clinical cost are driven by test selection. Knowledge of the probability that a positive test result is false (PFP), and that a negative test result is false (PFN) add valuable information to method evaluation and review. Statistical indicators of PPA, PNA, PPV, NPV, PFP, PFN or even the number of false results alone, cannot evaluate risk as the patient risk and clinical cost of the

analytical method selected. It would be worthwhile repeating this exercise with locally verified costs, prevalence and Reff number. The authors have posted an online calculator at https://awesome-numbers.com/risk-calculator/ to allow readers to simulate changes with their projected variables and estimates of cost in local currency.

ISO/IEC Guide 51defines risk as "the combination of the probability of occurrence of harm and the severity of that harm." (21) Examination of only PPA (sensitivity) and PNA (specificity) does not give an indication of patient risk as the number and clinical cost of false results. Risk as the probability and severity of false positive and false negative results can be extrapolated from manufacturers' claims and/or user data for PPA (sensitivity) and PNA (specificity) plus estimates of prevalence, Reff number and cost for your healthcare setting. Reff values for each US state can be found at https://rt.live/ (22) The authors estimated costs roughly for the USA but did not enter a value for loss of life in our equations as human life is invaluable. It may be wise, if difficult, to factor that in when evaluating cost in your location and currency.

The relationships between the various acronyms are confusing. Increased PPA (sensitivity), percent positive agreement, drives the number and cost of false negative results down, but has no impact on false positives. Increased percent negative agreement, PNA (specificity), drives the probability of false positives (PFP) and the resultant patient risk and healthcare cost down. PNA (specificity), percent negative agreement, has no impact on false negatives.

The authors found it thought-provoking that, as prevalence increases from 2% to 20%, cost of false molecular test results increase by over $250,000 for every one-thousand molecular tests performed. This happens because the number of true-positive and very costly false-negative tests increase in proportion to prevalence. With the baseline PNA (specificity) of 95.8%,

there are few false-positive results (41 at prevalence of 2% and 33 with prevalence of 20%), the decrease in their cost make little difference to the total costs.

Selecting a molecular test with PPA (sensitivity) of 94.8% instead of 77.5% would save patients and the healthcare system over $200 thousand. A test with PNA (specificity) of 100.0% instead of 86.3% reduces patient and clinical cost by over $300,000.

Similar patterns were observed for antigen and antibody tests.

Acceptable risk is “a state achieved in a measuring system where all known potential events have a degree of likelihood for or a level of severity of an adverse outcome small enough such that, when balanced against all known benefits—perceived or real—patients, physicians, institutions, and society are willing to risk the consequences.” (23). The COVID-19 pandemic has brought “patients, physicians, institutions, and society” together as never before; ask them if they are willing to risk the consequences of your chosen method. What is their maximum acceptable risk level – as the number and cost of false results? Although methods report a qualitative result, these are typically based on quantitative measurements and cutoff levels. The same concept can be applied to risk-based standards through on-site method validation experiments and daily quality control to maintain risk within acceptable risk limits.

Conclusion:

Three types of laboratory tests play critical roles in the diagnosis and management of Covid 19. The existing practice of examining PPA (sensitivity) and PNA (specificity) fails to project risk as the probability and severity of harm. The probability and cost of false positive test results (PFP) decreases as prevalence and PNA increase. Probability of false negative test results (PFN) increases with prevalence and decreases with PNA. Measuring risk metrics

as the number and cost of false results adds a great deal of insight that is masked by the usual statistical metrics. Patient risk and clinical cost are governed by the number, clinical implications, and cost of false positive and false negative patient results for each test type. Small changes in statistical metrics can produce large changes in risk metrics. Knowledge of the clinical implications and cost of false positive and negative test results can add valuable insight to test selection and guides decisions of repeating test results for confirmation with an orthogonal method. The authors are providing an online calculator to encourage and enable future studies with localized statistical indicators and cost.

Glossary

WHO: World Health Organization
CDC: Centre for Disease Control
2019-nCoV : 2019-novel coronavirus
SARS-CoV-2- severe acute respiratory syndrome coronavirus-2
PPA: Percent Positive Agreement
PNA: Percent Negative Agreement
rRT-PCR: Real-time reverse transcription-polymerase chain reaction
NAAT: nucleic acid amplification test
PPV: Positive predictive value = Probability that a positive result is true
NPV: Negative predictive value = Probability that a negative result is true
PFP: Probability that a positive result is false (PFP = 1-PPV)
PFN: Probability that a negative result is false (PFP = (1-NPV)
FIND: Foundation for Innovative New Diagnostics
PCR: Polymerase Chain Reaction
ELISA: Enzyme Linked Immunosorbant Assay
PPA: Percent positive agreement
PNA: Percent negative agreement
IgG: Immunoglubulin G
IgM: Immunoglobulin M
IgA: Immunoglobulin A

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