A "Clinician's Probability Calculator" to convert pre-test to post-test probability of COVID-19 , based on method validation from each laboratory

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Abstract

Background

During coronavirus pandemic testing and identifying the virus has been a unique and constant challenge for the scientific community. In this paper, we discuss a practical solution to help guide clinicians and public health staff with the interpretation of the probability that a positive, or negative, COVID-19 test result indicates an infected person, based on their clinical estimate of pre-test probability of infection.

Methods

The authors postulated that the clinical pre-test probability of COVID-19 increases relative to local prevalence of disease plus patient age, known contact, and severity of symptoms. We conducted a small survey on LinkedIn to confirm that hypothesis. We examined results of PPA (Positive Percent Agreement, sensitivity) and NPA (Negative Percent Agreement, specificity) from 73 individual laboratory experiments for molecular tests for SARS-CoV-2as reported to the FIND database,(1) and for selected methods in FDA EUA submissions (2,3). We calculated likelihood ratios to convert pre-test to post-test probability of disease, then further calculated the

number of true and false results expected in every ten positive or negative test results, plus an estimate that one in 'x' test results is true. We designed an online calculator to create graphics and text to fulfill the objective.

Results

The LinkedIn survey confirmed that the pre-test probability of COVID-19 increases with patient age, known contact, and severity of symptoms, as well as prevalence of disease in the local population. PPA (Positive Percent Agreement, PPA) and NPA (Negative Percent Agreement, specificity), differ between individual methods. Results vary between laboratories and the manufacturer for the same method. The confidence intervals of results vary with the number of samples tested, often adding a large range of possibilities to the reported test result. The online calculator met the objective.

Conclusions

A positive or negative test result from one laboratory conveys a higher probability for the presence or absence of COVID-19 than the same result from another laboratory, depending on clinical pre-test probability of disease plus proven method PPA and NPA in each laboratory.   Likelihood ratios and confidence intervals provide valuable information but are seldom used in clinical settings. We recommend that testing laboratories verify PPA and NPA, and utilize a tool such as the "Clinician's Probability Calculator" to verify acceptable test performance and create reports to help guide clinicians and public health staff with estimation of post-test probability of COVID-19 .

Keywords: COVID-19; SARS-COV-2; False-Positive; False-Negative; Likelihood Ratio; Probability; Calculator; Interpretation

Introduction:

Since the beginning of the year 2020 the whole world has been struggling to control the coronavirus (COVID-19) outbreak. During this outbreak accurate testing has been a unique and constant challenge for the scientific community. Despite best efforts, false positive and false negative test results are unavoidable.(4,5) At no time in history has the medical community embarked on a diagnostic testing campaign that is not being pursued for clinical reasons, but instead for epidemiological reasons unrelated to the medical aspects of the illness. Never have so many patient decisions been made, not by the patient's personal clinician, but by public health employees who may not be clinically trained. With the importance of asymptomatic patients, understanding of

the probability of true and false results has never been so critical. This article explores a modified application of likelihood ratios to provide practical guidance to the relative probability of true and false results.

What are pre-test and post-test probability?

Pre-test probability and post-test probability are the probabilities of the presence of a disease (such as COVID-19) before (pre) and after (post) a diagnostic test. (6) In some scenarios a diagnostic test may not be of help and may lead to increased confusion especially when the pre-test probability of a disease is either very high or very low. With COVID-19 and the importance of pre-symptomatic or asymptomatic patients however, tests are often performed even with low pre-test probability Although sometimes confused with simple prevalence of disease (7), the clinical pre-test probability of disease can be more precisely estimated with clinical information on each patient. In a small survey with 16 respondents on LinkedIn, the authors asked healthcare professionals to "estimate the probability that a 20-to-30-year-old patient, and 60-to-70-year-old patient, actually has, or will soon develop COVID-19 infection?" Symptoms ranged from i) none, to ii) sore throat, and nasal stuffiness, to iii) sore throat, and nasal stuffiness, with reduced taste or smell to iv) sore throat, and nasal stuffiness, with reduced taste or smell, fever, and body ache. We found, as logic and clinical experience would dictate, that pre-test probability increases with local COVID-19 prevalence, patient age, SARS-COV-2 exposure history and clinical symptoms.

Post-test probability is driven by pre-test probability and the likelihood ratio of the test method – as that method was verified in each laboratory. The likelihood ratio is driven by test PPA and NPA. PPA and NPA vary between methods as reported by manufacturers to FDA for EUA evaluation, and between laboratories using the same method. Different labs choose different numbers and criteria for known positive and known negative samples.

Clinicians and public health professionals who interpret test results are not always provided with the Fact Sheet for Healthcare Providers provided by test manufacturers that describe the interpretation of positive and negative test results. Different tests are approved for different clinical situations; few are approved for asymptomatic patients.   The following is an excerpt from the "Fact Sheet for Healthcare Providers" for both Manufacturers 1 (8) and 2 (9):

Manufacturer 1 (8): "This test is to be performed only using respiratory specimens collected from individuals who are suspected of COVID-19 by their healthcare provider within the first seven days of the onset of symptoms."

Manufacturer 2 (9): "This test is to be performed only using respiratory specimens collected from individuals suspected of COVID-19 by their healthcare provider."

Both manufacturers:

"What does it mean if the specimen tests positive for the virus that causes COVID-19?

A positive test result for COVID-19 indicates that RNA from SARS-CoV-2was detected, and therefore the patient is infected with the virus and presumed to be contagious.   Laboratory test results should always be considered in the context of clinical observations and epidemiological data (such as local prevalence rates and current outbreak/epicenter locations) in making a final diagnosis and patient management decisions.

The SARS-CoV-2test has been designed to minimize the likelihood of false positive test results. However, it is still possible that this test can give a false positive result, even when used in locations where the prevalence is below 5%.

What does it mean if the specimen tests negative for the virus that causes COVID-19?

A negative test result for this test means that SARSCoV-2 RNA was not present in the specimen above the limit of detection. However, a negative result does not rule out COVID-19 and should not be used as the sole basis for treatment or patient management decisions. It is possible to test a person too early or too late during COVID-19 infection to make an accurate diagnosis via the SARS-CoV-2test."

What are likelihood ratios?

The likelihood ratio is a tool used in evidence-based medicine to assess the value of performing a diagnostic test. It uses PPA and NPA to create a ratio of the probability that a test result is correct to the probability that it is not. A likelihood ratio is the percentage of ill people with a given test result divided by the percentage of well individuals with the same result (true result: false result). Ideally, abnormal test results should be much more typical in ill individuals than in those who are well (high likelihood ratio) and normal test results should be more frequent in well people than in sick people (low likelihood ratio). Likelihood ratios near one have little effect on decision-making; by contrast, high or low ratios can greatly shift the clinician's estimate of the probability of disease. When combined with an accurate clinical diagnosis, likelihood ratios improve diagnostic accuracy in a synergistic manner. (10,11)

Tests can be either positive or negative, so there are two ratios:

- Positive LR (LR+): This tells us how much to increase the probability of having a disease, given a positive test result. The ratio is:
  "Probability a person with the condition tests positive (a true positive) / probability a person without the condition tests positive (a false positive)." (10)
- Negative LR (LR-): This tells us how much to decrease the probability of having a disease, given a negative test result. The ratio is:
  "Probability a person with the condition tests negative (a false negative) / probability a person without the condition tests negative (a true negative)." (10,11)

Likelihood ratios are calculated to determine 2 things: i) how useful a diagnostic test is and ii) how likely it is that a patient has a disease. (10)

Likelihood ratios range from zero to infinity (9999.9). The higher the value, the more likely the test will indicate that the patient has the condition.

Likelihood ratios are calculated from PPA and NPA:
- Positive LR = (PPA / (100 – NPA) (True Positives / False Positives)
- Negative LR = (100 – PPA) / NPA (False Negatives/True Negatives)

What are Confidence Intervals?

"A confidence interval gives an estimated range of values which is likely to include an unknown population parameter." (10,11) Confidence intervals provide a range of possible results: minimum, probable and maximum. They tell the end-user how much faith they can have in the value reported.

Methodology

1. We created a LinkedIn survey asking for "your estimate that a 20-to-30-year-old patient (or a 60-to-70-year-old patient) actually has, or will soon develop COVID-19 infection" - with local prevalence of 3%, with and without known contact, escalating age and COVID-19 symptoms.
2. We used the calculations and definitions in Table 1 to examine results of individual laboratory experiments for molecular tests for SARS-CoV-2as reported to the FIND database (1), and for selected methods in FDA EUA submissions (2,3).

Table 1. Common definitions		
PPA	Positive Percent Agreement (sensitivity) = True positive results / All positive results Drives the True Positive and False Negative rates	
NPA	Negative Percent Agreement (specificity) = True negative results / All negative results Drives the True Negative and False Positive rates	
Pre-test probability	Clinical probability that a person being tested has COVID-19 , before the test is performed. Based on prevalence, contact, symptoms and age.	
Pre-test odds	= Pre-test probability/(1-Pre-test probability) = Probability person is infected/ Probability they are not	
Calculations	**Positive Test**	**Negative Test**
Likelihood Ratio	Positive (LR+) = PPA / (1 - NPA) = True Pos Rate/False Pos Rate	Negative (LR-) = (1-PPA)/NPA = False Neg Rate/True Neg Rate

Post-test odds	= Pre-test odds x LR+
	= Probability person is infected
	   x (True Pos Rate/False Pos Rate)
		= Pre-test odds x LR-
		= Probability person is infected
		   x (False Neg Rate/True Neg Rate)

Post-test probability	= Post test odds Pos Test /
	   (Post-Test Odds Pos +1)
	The probability that a person with a positive test is infected
		= Post test odds Neg Test /
		   (Post-Test Odds Neg Test +1)
		The probability that a person with a negative test is infected

1) We created an Excel spreadsheet and then designed an online application to graph confidence intervals of post-test probability of infection on with a positive or negative test result (on the y-axis) against the clinician's estimate of pre-test probability (on the x-axis.) Confidence intervals for the graph and each of the following indicators are driven by PPA and PNA reported, plus the number of samples tested (14).:

   a) Post-test probability of COVID-19 with positive and negative test result. (This tells the clinician the probability that each patient result from each laboratory indicates SARS-CoV-2 infection – based on their clinical estimate of pre-test probability. **Post-test Probability of COVID-19** (Ideal is 100% with positive test; 0% with negative test)

   b) Number of true positive and negative tests in every ten positive or negative results seen. (Ideally every test result will be true; with poorer tests or lower confidence limits, users may see only five true results in ten, providing little value to the clinician or patient.)

   c) Number of false positive and negative tests in every 10 positive or negative results seen. (Ideally there will be zero false results.)

   d) One in 'x' positive tests is true, and one in 'x' negative tests is True. (Ideally, one in one results will be true; with poorer tests, users may see only one true test in over thirty results, providing little value to the clinician or patient.)

Results:

1. LinkedIn Survey:

Seventeen people responded to a LinkedIn survey asking for "your estimate that a 20-to-30-year-old patient (or a 60-to-70-year-old patient) actually has, or will soon develop Covid19 infection" - with escalating Covid19 symptoms, with and without KNOWN contact." The local prevalence was given as 3%. (Questionnaire attached as supplementary material).

Table 2 presents the survey results, showing a clear pattern that pre-test probability increases with patient age, known contact and presence of typical COVID-19 symptoms.

Table 2. Median Votes from LinkedIn Survey	No Symptoms	sore throat, and nasal stuffiness	sore throat, and nasal stuffiness, with reduced taste or smell	sore throat, and nasal stuffiness, with reduced taste or smell, fever, and body ache
Without Contact - Young	3%	35%	79%	85%
Without Contact - Old	5%	50%	80%	90%
WITH Contact - Young	40%	77%	87%	96%
WITH Contact - Old	45%	60%	94%	98%

2. Results reported to FIND and FDA- with calculations:

Ninety-two laboratories reported both PPA and NPA to the FIND database (1) as of October 17, 2020. We removed 19 results from two laboratories in 1 country that reported NPA of 100% based on only one negative. Figure 1 shows the number of known positive and negative samples reported in each laboratory's study. Fifteen of the laboratories (21%) tested fewer than five known negative samples.

Figure 1. Known Samples Tested

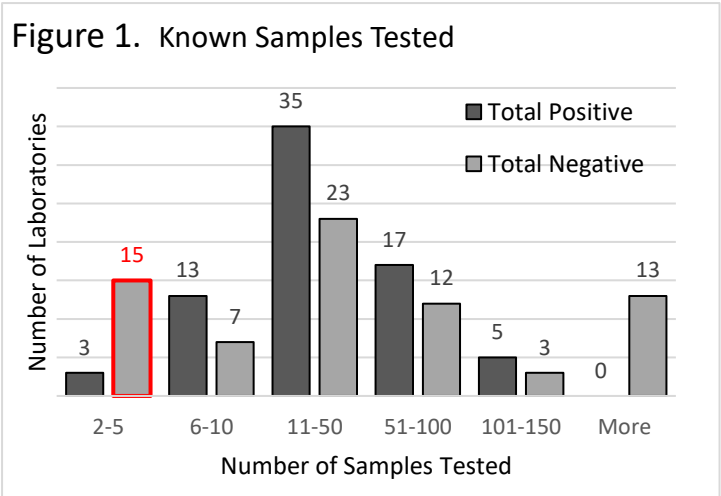
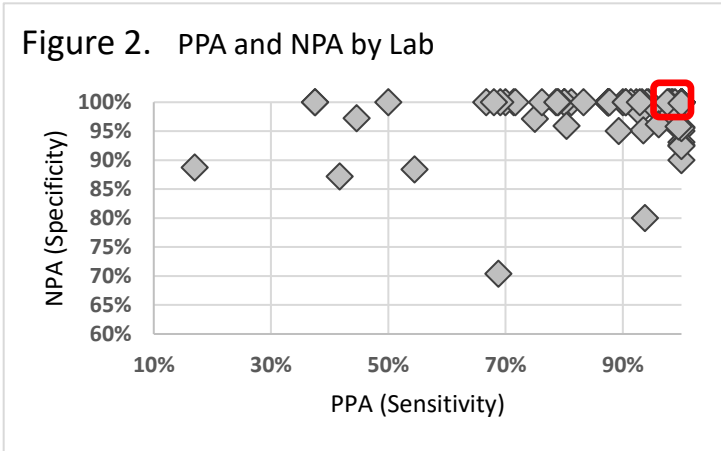



Figure 2 shows the PPA and NPA for 73 studies reported to FIND. Only results within the red box meet the FDA recommendations "FDA defines the acceptance criteria for the performance as 95% agreement at 1x-2x LoD, and 100% agreement at all other concentrations and for negative specimens."(12)

Figure 2.   PPA and NPA by Lab

The authors observed no relationship between the number of samples tested and method quality reflected in the reported PPA and NPA.

The authors compared the Information For Use (IFU) documents provided to FDA for two manufacturers (2,3), to five FIND (1) laboratory studies for manufacturer 1 (Mfg-1) and six FIND laboratory studies for manufacturer 2 (Mfg-2.) To calculate PPA, Positive Percent Agreement, Mfg-1 tested "30 contrived clinical nasopharyngeal (NP) swabs prepared by spiking clinical NP swab matrix with purified viral RNA containing target sequences from the SARS-CoV-2genome at concentrations approximately 2x LOD (20 samples) and 5x LOD (10 samples)." Mfg-2 tested "45 patient samples collected during COVID-19 pandemic in the US that had previously been characterized as positive for SARS-CoV-2by an EUA RT-PCR test." Mfg-1 reported PPA of 100% (30/30). Mfg-2 reported PPA of 97.8% (44/45).

To prove NPA, Negative Percent Agreement, Mfg-1 reported that "Thirty Negative NP swab samples were also tested in this study." Mfg-2' IFU reported testing 45 samples, saying "Fifteen of the 45 SARS-CoV-2negative NP swab specimens were collected before December 2019 and are expected to be negative for SARS-CoV-2. The others had previously been characterized as negative for SARS-CoV-2by an EUA RT-PCR test." Mfg-1 reported NPA of 100% (30/30). Mfg-2 reported NPA of 95.6% (43/45). These results were driven by each manufacturer's test methods, the type and number of samples tested plus the competency of staff and instrument performance at the manufacturers' sites.

Figure 3 shows the reported PPA on the x-axis, and NPA on the y-axis, by Manufacturers 1 and 2, and in the FIND studies for all labs reporting studies from the same manufacturers. The circles representing 'Manufacturer 1' labs are coloured blue; the manufacturer is shown as the clear circle. The diamonds represent 'Manufacturer 2'; labs are coloured yellow; the manufacturer is shown as the clear diamond. Labs A, B, C and D are examined in greater detail in Tables 3, 4, 5 and 6.

Figure 4 shows the number of known samples tested by Manufacturers 1 and 2, and in the FIND studies for all labs reporting studies from the same manufacturers.

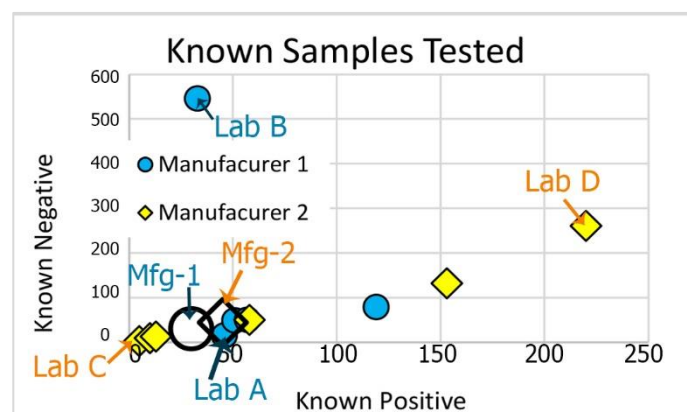
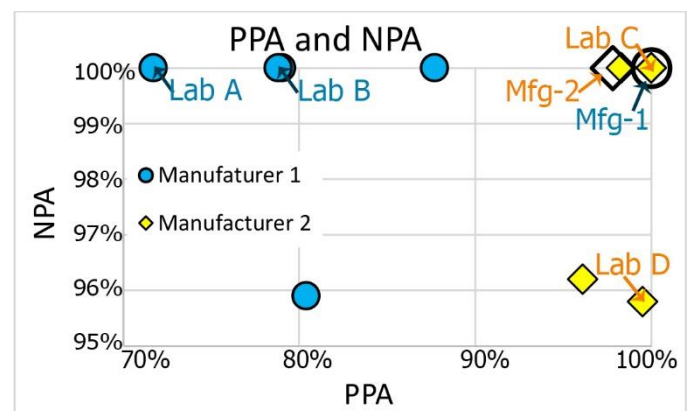



Table 3 presents the data reported by the selected manufacturers and two laboratories using each test method. The number of known samples that each laboratory tested drives the confidence intervals around PPA and NPA which, in turn, drive the confidence intervals around likelihood ratios, which drive post-test probability and other indicators. Notice the wide variation in the number of known samples tested in rows 1 and 2. In row 3, notice that the labs A and B reported PPA values below Mfg-1 while Labs C and D reported higher PPA than Mfg-2.

Table 3. Reported values		Mfg-1	Lab A	Lab B	Mfg-2	Lab C	Lab D
1	Known Positive Samples	30	46	33	45	5	220
2	Known Negative Samples	30	15	546	45	3	261
3	PPA Reported	100.0%	71.7%	78.8%	97.8%	100.0%	99.5%
4	NPA Reported	100.0%	100.0%	100.0%	95.6%	100.0%	95.8%
5	Pos Likelihood Ratio (LR+)	9999.9	9999.9	9999.9	22.2	9999.9	23.7
6	Neg Likelihood Ratio (LR-)	0.00	0.28	0.21	0.023	0.000	0.005

Table 4 presents the probable post-test interpretation of results for patients with pre-test probability of 3% and for pre-test probability of 50%. Red numbers in the table indicate variation from others and/or less-than-ideal test performance. Notice in Row 4 that a positive test result from Mfg-2 and Lab D indicates less than a 50% post-test probability of disease, when pre-test probability is only 3%. On row 7, notice that only 4 of 10 positive tests are true. This relates in Row 10 to the fact that only one of every 2.5 positive tests seen is true. When pre-test probability raises to 50%, (rows 12-21) post-test probability rises to over 90% for these labs. Even with a negative test, post-test probability is approximately 20% in Labs A and B (row 15). In row 18, with pre-test probability of 50%, only 8 of 10 negative tests are true for Labs A and B.

Table 4. Probable Post-Test Interpretation of Results							
1	**Post-Test Projections: With 3% Pre-Test Probability**						
2	**Post-test Probability of COVID-19** (Ideal is 100% with positive test; 0% with negative test)						
3	**Reported values**	**Mfg-1**	**Lab A**	**Lab B**	**Mfg-2**	**Lab C**	**Lab D**
4	With Positive Test	100%	100%	100%	41%	100%	42%
5	With Negative Test	0.0%	0.9%	0.7%	0.1%	0.0%	0.0%
6	**Number of True Results in every Ten Tests Reviewed** (Ideal is ten of ten)						
7	Positive Test	10.0	10.0	10.0	4.1	10.0	4.2
8	Negative Test	10.0	9.9	9.9	10.0	10.0	10.0
9	**One in ___ Test results is/are True** (Ideal is one in one)						
10	Positive Test	1.0	1.0	1.0	2.5	1.0	2.4
11	Negative Test	1.0	1.0	1.0	1.0	1.0	1.0
12	**Post-Test Projections: With 50% Pre-Test Probability**						

13	**Post-test Probability of COVID-19** (Ideal is 100% with positive test; 0% with negative test)						
14	With Positive Test	100%	100%	100%	96%	100%	96%
15	With Negative Test	0.0%	22.1%	17.5%	2.2%	0.0%	0.5%
16	**Number of True Results in every Ten Tests Reviewed** (Ideal is ten of ten)						
17	Positive Test	10.0	10.0	10.0	9.6	10.0	9.6
18	Negative Test	10.0	7.8	8.3	8.7	10.0	9.9
19	**One in 'x' Test results is/are True** (Ideal is one in one)						
20	Positive Test	1.0	1.0	1.0	1.0	1.0	1.0
21	Negative Test	1.0	1.3	1.2	1.0	1.0	1.0

Table 5 presents confidence intervals for PPA, NPA and likelihood ratios. Numbers in red in the table differ from others, are far from ideal, and/or do little to assist the clinician with diagnosis.

Table 5. Confidence Limits for Drivers of Post-Test Interpretation							
		Mfg-1	**Lab A**	**Lab B**	**Mfg-2**	**Lab C**	**Lab D**
1	PPA Low Confidence Interval	88.0%	56.6%	61.2%	87.8%	54.6%	97.3%
2	PPA High Confidence Interval	100.0%	83.8%	90.9%	100.0%	100.0%	100.0%
3	NPA Low Confidence Interval	88.0%	78.5%	99.3%	84.5%	41.9%	92.5%
4	NPA High Confidence Interval	100.0%	100.0%	100.0%	100.0%	100.0%	97.9%
5	LR+ From Low PPA NPA	7.33	2.63	82.60	7.33	0.94	12.91
6	LR+ From High PPA NPA	999.9	999.9	999.9	999.9	999.9	999.9
7	LR- From Low PPA NPA	0.14	0.55	0.39	0.14	1.08	0.39
8	LR- From High PPA NPA	0.00	0.16	0.09	0.00	0.00	0.09

Table 5, in rows 5 and 7, shows that Lab C has a low possible positive likelihood ratio of 0.94 and a high possible negative likelihood ratio of 1.08. When pre-test probability is 50%, the odds are 1:1 that the patient is infected. Multiply the pre-test odds times the likelihood ratio to calculate post-test odds – which will be essentially unchanged in this case. The low range of post-test possibility with a positive test overlaps the high possibility with a negative test (Table 6 Rows 4 & 7, 20 & 24,) A positive and negative test result, as verified in Lab C, may not be able to differentiate infected from non-infested patients. This is not due to an inherent weakness in the test method, but to the low number of samples used by Lab C to verify method performance in their hands.

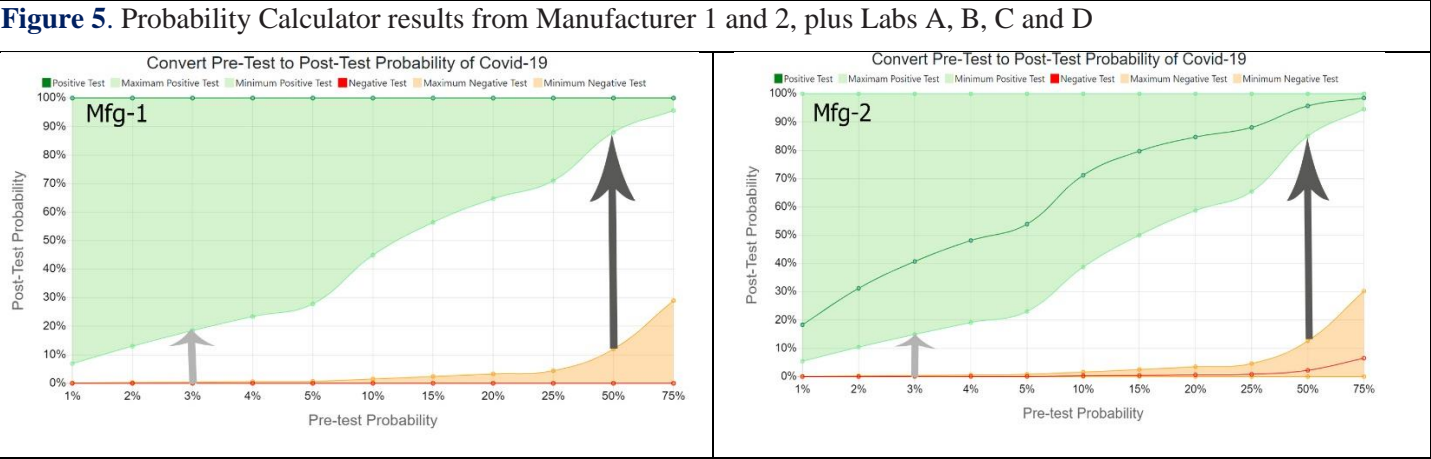
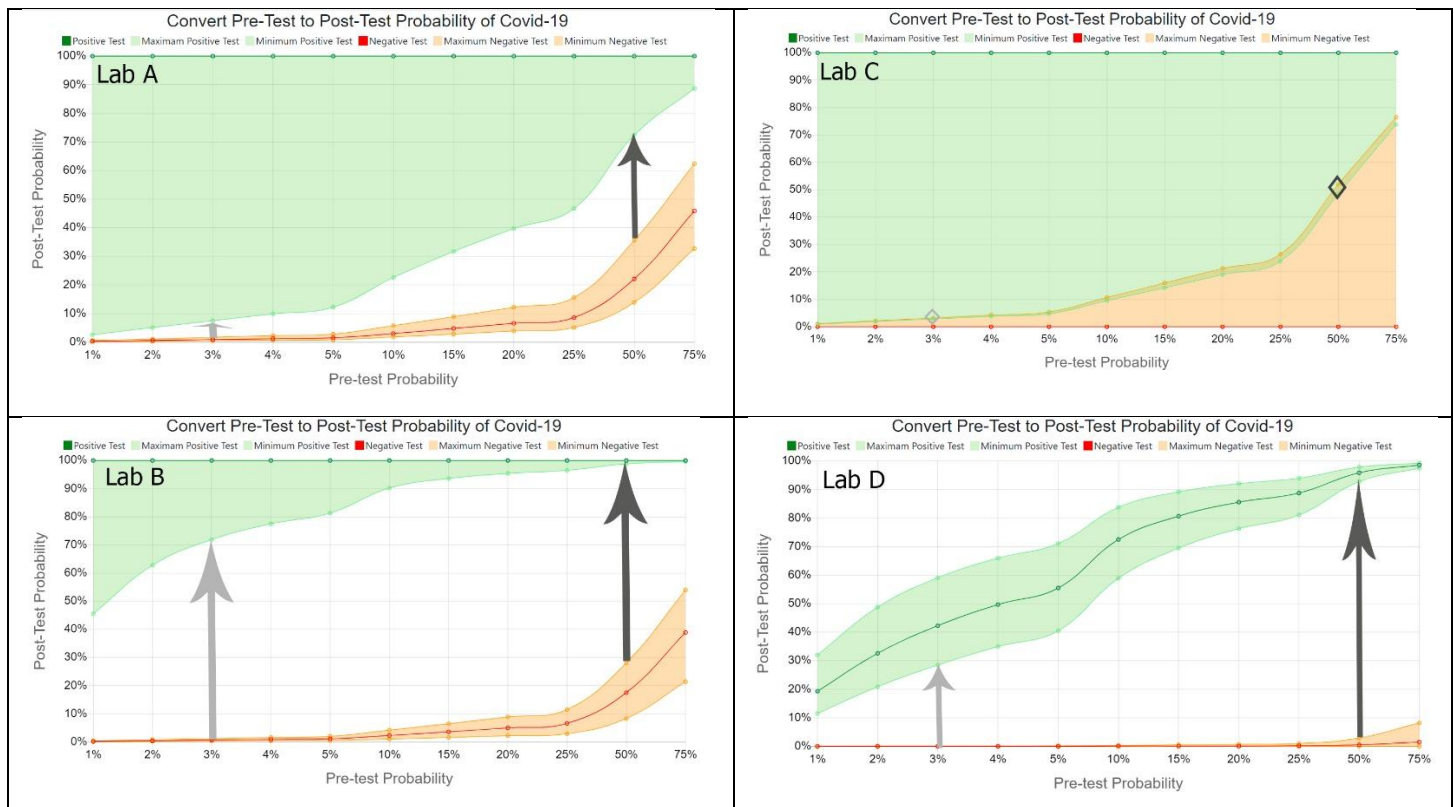
Where Table 4 presented the 'Probable' Post-Test Interpretation of Results, Table 6 shows the range of possibilities with low and high confidence intervals. Notice in row 4 that, where Mfg-1 and Lab A both reported 100% PPA, confidence intervals show that could actually be as low as 18% or 7.5%; in Lab C, the reported 100% may actually be as low as 2.8% due to the low number of samples tested. Row 9 shows that there may be less than two or three true results in every ten positives reviewed by clinicians.

	Table 6. Range of Possible Interpretation of Test Results with Confidence Intervals						
1	**Post-Test Projections: With 3% Pre-Test Probability**						
2	**Post-test Probability of Covid-19** (Ideal is 100% with positive test; 0% with negative test)						
3		**Mfg-1**	**Lab A**	**Lab B**	**Mfg-2**	**Lab C**	**Lab D**
4	With Positive Test- Low	18.5%	7.5%	71.9%	14.9%	2.8%	28.5%
z	- High	100%	100%	100%	100%	100%	59%
6	With Negative Test - Low	0.00%	0.50%	0.30%	0.00%	0.00%	0.00%
7	- High	0.40%	1.70%	1.20%	0.40%	3.20%	0.10%
8	**Number of True in Results in every Ten Tests Reviewed** (Ideal is ten)						
9	Positive Test- Low	1.8	0.8	7.2	1.5	0.3	2.9
10	- High	10	10	10	10	10	5.9
11	Negative Test - Low	10	9.8	9.9	10	9.7	10
12	- High	10	10	10	10	10	10
13	**One in every 'x' Test results is/are True** (Ideal is one)						
14	Positive Test- Low	1.0	1.0	1.0	1.0	1.0	1.7
15	- High	5.4	13.3	1.4	6.7	35.4	3.5
16	Negative Test - Low	1.0	1.0	1.0	1.0	1.0	1.0
17	- High	1.0	1.0	1.0	1.0	1.0	1.0
18	**Post-Test Projections: With 50% Pre-Test Probability**						
19	**Post-test Probability of Covid-19** (Ideal is 100% with positive test; 0% with negative test)						
20	With Positive Test- Low	88.0%	72.4%	98.8%	85.0%	48.5%	92.8%
21	- High	100%	100%	100%	100%	100%	98%
22	With Negative Test - Low	0.00%	13.90%	8.30%	0.00%	0.00%	0.00%
23	- High	12.0%	35.6%	28.1%	12.6%	52.0%	2.9%
24	**Number of True Results in every Ten Tests Reviewed** (Ideal is ten)						
25	Positive Test- Low	8.8	7.2	9.9	8.5	4.8	9.3
26	- High	10.0	10.0	10.0	10.0	10.0	9.8
27	Negative Test - Low	8.8	6.4	7.2	8.7	4.8	9.7
28	- High	10	8.6	9.2	9.8	10	10
29	**One in every 'x' Test results is/are True** (Ideal is one)						
30	Positive Test- Low	1.0	1.0	1.0	1.0	1.0	1.0
31	- High	1.1	1.4	1.0	1.2	2.1	1.1
32	Negative Test - Low	1.0	1.2	1.1	1.0	1.0	1.0
33	- High	1.1	1.6	1.4	1.1	2.1	1.0

3. To overcome the complexity of calculations that prohibit most laboratories from reporting post-test probability with confidence intervals, the authors designed an online probability calculator to create graphics and text that could accompany each laboratory's test result to let clinicians and public health staff visualize the probability that each positive, or negative, COVID-19 test result indicates an infected person, based on their estimate of pre-test probability. It is available at https://awesome-numbers.com/post-test-probability-calculator/. Users provide the number of known samples tested plus PPA and PNA determined; reports contain data as shown in Tables 3, 4, 5 and 6.

The calculator report clearly conveys information to interpret results, based on the test used and verified in each laboratory. In the graphic, the x-axis is the pre-test probability, as estimated by the clinician or public health professional. The Y-axis is the post-test probability. The shaded green area shows confidence intervals that a positive COVID-19 test result indicates an infected person. The pale orange shaded area shows confidence intervals that negative COVID-19 test results are false and do indicate an infected person.

Figure 5 shows Probability Calculator graphs from Manufacturer 1 and 2, plus Labs A and B who reported data from Manufacturer 1, and Labs C and D who reported data from Manufacturer 2. The arrows show the gap between the highest probability that a negative test represents an infected person and lowest probability with a positive test.

Figure 5. Probability Calculator results from Manufacturer 1 and 2, plus Labs A, B, C and D

<mark>Discussion:</mark>

Importance of PPA (sensitivity) and NPA (specificity):

PPA, Positive Percent Agreement (sensitivity), drives the rate of true positive and false negative test results. NPA, Negative Percent Agreement (specificity), drives the rate of true negative and false positive test results. PPA and NPA combine to drive the probability, number and cost of false-positive and -negative test results. (13) PPA and PNA are typically used by laboratory directors to compare inherent method quality and select test methods. They can also be used to calculate likelihood ratios that in turn drive post-test probability of COVID-19 plus the graphs and other metrics displayed in Tables 3, 4, 5 and 6 and Figure 5.

Test methods are often verified by manufacturers under ideal conditions with hospital or contrived samples containing higher viral loads than those from asymptomatic individuals living in the community. As such, PPA and NPA in test laboratories might differ significantly from values reported by manufacturers. Notice in Figure-3 that none of the five laboratories reporting to FIND attained the 100% PPA claimed in the FDA IFU by Manufacturer 1 (2). In contrast, laboratories C and D reported higher PPA values than Manufacturer 2 (3). Thus, the PPA and NPA values reported by manufacturers cannot be assumed to accurately reflect performance in each laboratory.

PPA and NPA do not help clinicians decide if a specific positive or negative test result is true.   PPA and NPA can be converted to likelihood ratios which can be used to convert clinical pre-test probability of disease for a specific patient to post-test probability.

Relevance of likelihood ratios:

Likelihood ratios allow one to convert pre-test to post-test odds of infection. The mathematics of this process are complicated, but the logic is clear. (15) When pre-test probability is 50%, the odds are 1:1 that the patient is infected. One of every two people with 'these' clinical symptoms is expected to be positive before testing (50%). If the positive likelihood ratio is approximately 24, as in Lab D in Table 3, multiplying the pre-test odds x the positive likelihood ratio produces post-test odds of 24:1. Twenty-four of every 25 people with a positive test are actually infected; 24/25 = 96% post-test probability. Lab D's negative likelihood ratio is 0.005; multiplying the pre-test odds by the negative likelihood ratio produces post-test odds of 0.005:1. The post-test probability of infection with a negative test result is only 0.5%.

Importance of number of known samples tested:

The number of known positive and negative samples tested determines the confidence intervals around PPA and NPA (14).   Figure 1 and Table 3 illustrate the dramatic difference in number of samples tested by individual sites reporting to FIND (1). Labs A, B and C each reported 100% NPA. Lab A made that assessment by testing 15 known negative samples, while Lab B tested 546 known negatives and Lab C tested only three. The lower limit of confidence for NPA in Lab A is 78.5% compared to 99.3% in Lab B and only 41.9% in Lab C (Table 5.) The low number of known samples tested in Lab C do not allow this lab to verify acceptable method performance.

Impact of confidence intervals:

Confidence intervals determine the range of possibilities for PPA and NPA, which drive likelihood ratios that drive post-test probability of COVID-19 with positive and negative test results. Post-test probability drives the number of true and false positive and negative tests in every 10 positive or negative results seen, and how many positive or negative test results would be seen to find one true test result. Confidence intervals allow users to visualize the gap between the post-test probability that a positive, or negative, test indicates an infected person.

Value of graphs and metrics reported by the Probability Calculator:

Instead of either taking all positive or negative test results at face value or developing personal experience to 'guess' if results are true or false, clinicians can visualize a reliable scientific range of possibilities.   Glancing at

the six graphs in Figure 5 clarifies that when pre-test probability is only 3%, the probability of a person having COVID-19, even with a positive test, is less than 50% - except in Lab B where they tested enough samples to prove test reliability. These graphics and data eliminate the use of Fagan's Nomogram (7), which is typically used with likelihood ratios but are cumbersome for front-line use and does not include confidence intervals.

Laboratory directors and public health officials who are challenged to select and verify test methods can clearly see the ability of each test to project, or rule out, COVID-19 infection.   Lab C shows no gap at all between the range of possibilities that a positive or negative test indicates an infected person. The test has not been verified to provide useful information by testing only five known-positive and three known-negative samples. Laboratory directors and clinicians can have little confidence in the values reported.

Clinicians can benefit from understanding the number of true and false positive results they can expect to see in every ten positive or negative results.   Clinicians may see as few as one or two true positives in every ten positive test results according to the Manufacturer-1 and Lab A, while Lab B can be relied on to produce over seven of ten true positives (Table 6 Row 9.) Knowing the frequency of true test results reported could certainly change test selection and interpretation.   According to data from Mfg-1, clinicians may see only one true positive result in every 5.4 tests reviewed; for Lab A, that could be one in 13.4 results (Table 6, Row 15.) Lab B, who tested more known negative samples and has less variation due to confidence intervals, clinicians will see one true positive in every 1.4 tests. In contrast, Lab C may produce only one true positive in every 35 positive results. This information, however, is not available by only examining the reported PPA and NPA values.

In the USA, Clinical Laboratory Improvement Amendments (CLIA) mandates that laboratory director responsibilities include "ensuring that your laboratory develops and uses a quality system approach to laboratory testing that provides accurate and reliable patient test results." (16) Accuracy is the number of true results as a portion of all test results created.(17) The authors were shocked to discover that most laboratories are not required to verify that they can, at least, reproduce PPA and NPA claims from manufacturers. A Linked-In survey confirmed that 83% of 23 respondents agreed that "Testing labs should confirm that they can attain or exceed manufacturer's claims for PPA and NPA, sensitivity & specificity, for COVID-19." This does not preclude the laboratory director from performing this study as part of good lab practice. In fact, Stephanie L. Mitchell et al published an article in the Journal of Clinical Microbiology (18) outlining a process to verify method PPA and NPA with ten positive and negative samples. In order to ensure method accuracy, we recommend that each testing laboratory confirm PPA and NPA with sufficient known samples to provide

reliable post-test probability of disease. We concur with this article (18) that each report should be accompanied by a statement from the laboratory indicating that test performance has been verified. The Clinician's Probability Calculator fulfils this need.

Conclusion

Due to the emergency pandemic, people are being tested in settings with little professional oversight. Despite best efforts, false positive and false negative test results are unavoidable. (4,5) The manufacturers' reported results for PPA and NPA are not always achieved, or even verified, in the clinical settings. Confidence intervals around values reported vary greatly. A positive or negative test result from one laboratory conveys a higher probability for the presence or absence of disease than the same result from another laboratory, depending on clinical pre-test probability of disease plus proven method PPA and NPA in each laboratory.   Post-test probability, likelihood ratios and confidence intervals are not intuitive; they are mathematically complex and seldom used in clinical settings. The authors recommend that testing laboratories verify PPA and NPA with sufficient numbers to verify acceptable performance. We hope that laboratory professionals, clinicians and public health professionals will find the Clinician's Probability Calculator (https://awesome-numbers.com/post-test-probability-calculator) useful to verify test performance and create reports to help guide them with interpretation of the relative probability that positive, or negative, COVID-19 test results indicate infected persons.

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