ANTIBODY TESTING for DIAGNOSIS of REINFECTION

RECOMMENDATION

We recommend against the use of SARS-CoV-2 Ab testing to diagnose presumptive COVID-19 reinfection among symptomatic patients previously diagnosed with COVID-19* *(Very low quality of evidence; Strong recommendation)*.

**NAAT (RT-PCR) and Genomic sequencing are the recommended diagnostic tests to confirm COVID-19 reinfection.*

Consensus Issues

The studies reviewed did not perform subgroup analysis according to 4-fold titer rise at a given interval. Due to the very low quality of evidence, the use of SARS-CoV-2 Ab testing to diagnose presumptive COVID-19 reinfection was not recommended.

EVIDENCE SUMMARY

Among symptomatic individuals previously diagnosed with COVID-19, should antibody testing be done to diagnose presumptive COVID-19 reinfection?

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Key Findings

There were no studies that directly assessed the accuracy of SARS-CoV-2 Ab in diagnosing presumptive COVID-19 reinfection, compared to RT-PCR as the reference standard. Only three retrospective observational studies reported on the accuracy of SARS-CoV-2 IgG/IgM Ab in diagnosing COVID-19. There is very low certainty regarding these estimates due to very serious risk of bias concerns, imprecision, indirectness, and inconsistency.

The sensitivity of SARS-CoV-2 IgG/IgM ranged from 0.19 (95% CI 0.4- 0.46) to 0.89 (95% CI 0.71- 0.98) and specificity of 0.50 (95% CI 0.01-0.99) to 1.00 (95% CI 0.89-1.00). Subgroup analysis suggested that the sensitivity of Ab testing was low under the following conditions: (a) when used within 0-15 days from symptom onset, (b) Ab tests that assess IgM, (c) using LFIA technique. Specificity was consistently high (>89%) regardless of the type of antibody detected or if either LFIA or CLIA techniques were done. However, specificity was high only if the test was performed more than 16 days from symptom onset.

Introduction

Several cases of reinfection with SARS-CoV-2 have been reported [1,2] with a median interval from initial COVID-19 infection of 71 days (range: 19-250) [3-5]. Presumptive COVID-19 reinfection is considered in symptomatic individuals previously diagnosed with COVID-19 when other alternative diagnoses are ruled out and a positive NAAT result is found [6]. Confirmed COVID-19 reinfection is then confirmed through genomic sequencing.

Studies suggest that >90% of recovered COVID-19 patients develop anti SARS-CoV-2 antibodies. [7-11). In some reports, duration of IgG/IgM positivity ranges from 1 to 4 months after the acute infection [1, 8,12,]. Correlates of immunity to COVID-19 have not been established, although a positive serologic test may indicate resolving or previous infection [6]. CDC has released the “investigative criteria for suspected cases of SARS-CoV-2 reinfection”. These include presence of symptoms compatible with COVID-19, prior COVID-19 diagnosis, no obvious alternative etiology for COVID-19-like symptoms OR close contact with a laboratory-confirmed COVID-19 and detection of SARS-CoV-2 RNA [6].

Antibody testing may play a role in the diagnosis of COVID-19 in patients who present beyond the first week from symptom onset, particularly where molecular testing has failed to detect the SARS-CoV-2. Confirming COVID-19 diagnosis using serologic test requires detection of SARS-CoV-2 IgM and IgG in serum, or a 4-fold or greater increase in titer observed during convalescence compared with the acute phase. FDA approval is given to Ab tests with at least 90% sensitivity and 95% specificity.

Review Methods

Comprehensive literature search across several electronic databases was done on 17 Feb 2021. Studies considered eligible for this review included cross-sectional studies that evaluated the diagnostic performance of SARS-CoV-2 Ab in symptomatic patients suspected to have COVID-19 reinfection. The reference standard was RT-PCR. We excluded studies on sensitivity or specificity studies alone, used other reference standards with RT-PCR and those that included healthy individuals as control.

Results

Characteristics of included studies

There were no studies that directly assessed the accuracy of SARs-CoV-2 Ab in diagnosing presumptive COVID-19 reinfection, compared to RT-PCR as the reference standard.

Indirect evidence comes from 3 observational cross-sectional studies on the diagnostic performance of SARS-CoV-2 Ab testing on COVID-19 infection involving 429 patients [13-15]. It was not specified if these patients already had a previous COVID-19 infection. Two were pre-prints [14,15]. A total of 429 symptomatic, hospitalized and non-hospitalized patients were included. One study assessed combined IgG/IgM using lateral flow qualitative immunoassay technique (LFIA) [15], another assessed IgM or IgG separately using chemiluminescence immunoassay [13], while the remaining study assessed combined IgG/IgM but with an unspecified, rapid analytic technique [14]. The reference standard used for all 3 studies was RT-PCR. The time interval between the conduct of the Ab testing and the RT-PCR varied from 15 days [13], simultaneous [15], or an unspecified interval [14]. Ab testing was done up to 30 days from symptom onset [13,14].

Methodological quality

Overall quality of evidence was rated very low; downgrading occurred due to indirectness, very serious risk of bias, inconsistency and imprecision due to small sample sizes [14]. It was unclear whether Ab testing was interpreted without knowledge of the RT-PCR results in two studies [13,14], and the time interval between the conduct of the index test and reference standard was unreported. Incomplete data was noted in 17/267 (6%) of patients in 2 studies [13,15]. Inconsistency was attributed to specimen site (URT in 2 studies and URT or LRT in 1 study) and

difference in timing of testing of index and reference tests (simultaneous vs 15-day interval vs unclear) and type of Ab test.

Outcomes

Diagnostic accuracy
SARS-CoV2 IgG/IgM, IgG or IgM Ab had a sensitivity ranging from 0.29 (95% CI 0.18- 0.41) to 0.89 (95% CI 0.71- 0.98) and specificity of 0.50 (95% CI 0.01-0.99) to 1.00 (95% CI 0.89-1.00). We did not calculate for pooled sensitivity and specificity estimates due to the limited number of studies as well as significant heterogeneity noted from visual inspection of the forest plots.

Subgroup analysis according to time of testing from symptom onset showed that the sensitivity of Ab testing was lowest when performed on day 0 to day 7 (Sn=19% (95% CI 0.04-0.46)), highest on day 8 to 15 (Sn=100%, 95% CI 0.54-1.00). Performing the test beyond day 16 showed moderate-to-high sensitivity values ranging from 0.48 to 1.00. In terms of the type of technique used, LFIA showed poor sensitivity (29% [95%CI 18,41]). Ab tests that used IgM also showed poorer sensitivities (<70%) compared to IgG (>70%).

Specificity was consistently high (>89%) regardless of the type of antibody detected or if either LFIA or CLIA techniques were done. However, lower and less precise specificity estimates were seen in studies that used Ab testing within the first 15 days from symptom onset. Specificity was high when Ab testing was used more than 16 days from symptom onset (90% [95%CI 82,95]).

Table 1. Accuracy of COVID-19 antibody tests for seroprevalence stratified by potential sources of heterogeneity

		IgG		IgM		Combined IgG/IgM	
Subgroup	No. of studies (Sample size)	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Symptom onset							
0-7 days	1 (25)	-	**-**	**-**	-	**19%** (4-46)	78% (40-97)
8-15 days	1 (8)	**-**	**-**	**-**	-	**100%** (54-100)	50% (0.01-0.99)
> 16 days	2 (142)	**89%** (71-98)	**91% (76-98)**	**48%** (29-68**)**	**100%** (89-100)	**86%** (79-91)	90% (82-95)
Laboratory technique							
LFIA	1 (190)					**29%** (18-41)	**89%** (82-94)
CLIA	1(60)	89% (71-98)	**91% (76-98)**	**48% (29-68)**	**100% (89-100)**		
Unspecified	1 (179)					86% (77-92)	91% (83-96)

Recommendations from Other Groups

CDC (Oct 27, 2020) **does not recommend** antibody testing to diagnose current COVID-19.

PSMID (July 20, 2020) **does not recommend** antibody tests as stand-alone tests for the diagnosis of COVID-19 [16].

COVID-Low and Middle Income Countries (LMIC) Task Force (22 Jan 2021) suggests **against using SARSCoV-2 Ab testing** for the detection of active or past SARS-CoV-2 infection, until there is better evidence for its usefulness [17].

CDC proposes **investigative criteria for identifying cases** suspected of COVID-19 reinfection. These include presence of symptoms compatible with COVID-19, prior COVID-19 diagnosis, no obvious alternative etiology for COVID-19-like symptoms OR close contact with a laboratory-confirmed COVID-19 and detection of SARS-CoV-2 RNA [6].

Research Gaps

There are no ongoing studies on the accuracy of Ab testing in diagnosing COVID-19 presumptive reinfection.

References

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Appendix 1. Characteristics of Included Studies

Study ID and Country	Study Design	Population	Index Test/s	Reference Standard	Testing Interval (days)
Jin 2020 China	Retrospective Cross-sectional	n = 76 Median age 47 yo (IQR range 35-59) Hospitalized patients	SARS-CoV-2 IgG SARS-CoV-2 IgM CLIA on day 18 from symptom onset (IQR 11-23 days)	RT-PCR from oral swab or sputum on day 3 of symptom onset (IQR 2-7 days)	15 days
Liu Ying 2020	retrospective observational	n=179 inpatient and outpatient cases mild to critical 23-80 years old	SARS-CoV-2 IgG/IgM (unspecified analytic technique) 30 days from symptom onset (+/- 17 days) in PCR -positive patients 8 days (+/- 14 days) in PCR-negative patients	RT- PCR from nasal and pharyngeal swabs	unreported time interval
Paradiso 2020	observational	n=191 14 (8.7%) asymptomatic median age 58.5 years symptomatic patients seen in the emergency room	SARS-CoV-2 IgG/IgM (lateral flow qualitative immunoassay)	RT-PCR from nasopharyngeal/ oropharyngeal swab	simultaneous

Appendix 2. GRADE Evidence Profile

Sensitivity	-- (95% CI: -- to --)
Specificity	-- (95% CI: -- to --)

Prevalences	0 %		

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 0%	
True positives (patients with [Presumptive COVID-19 Reinfection])	3 studies 187 patients	cross-sectional (cohort type accuracy study)	very serious [a,b,c,d]	serious [e]	serious [f]	serious [g]	none	0 (0 to 0)	⊕◯◯◯ VERY LOW
False negatives (patients incorrectly classified as not having [Presumptive COVID-19 Reinfection])								0 (0 to 0)	
True negatives (patients without [Presumptive COVID-19 Reinfection])	3 studies 187 patients	cross-sectional (cohort type accuracy study)	very serious [a,b,c,d]	serious [e]	serious [f]	not serious	none	0 (0 to 0)	⊕◯◯◯ VERY LOW
False positives (patients incorrectly classified as having [Presumptive COVID-19 Reinfection])								1000 (1000 to 1000)	

Explanations

a. It was unclear if consecutive patients or random sample of patients were enrolled.
b. It was unclear if the reference and index tests were interpreted independently from each other.
c. Different specimens were used, either nasopharyngeal or oropharyngeal, or sputum.
d. Interval between timing of tests was not reported in 2 studies.
e. Did not state if patients were previously diagnosed with COVID-19.
f. Heterogeneity can be attributed to different Ab testing technique, timing of testing and type of Ab (IgG/IgM or IgG only or IgM only.
g. Small sample size in 2 studies

References

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3. Paradiso A, De Summa S,Loconsole D,Procacci V,Sallustio A,Centrone F,et al. Clinical meanings of rapid serological assay in patients tested for SARS-Co2 RT-PCR. medRxiv 2020.04.03.20052183; 2020.

Appendix 3. Forest Plots

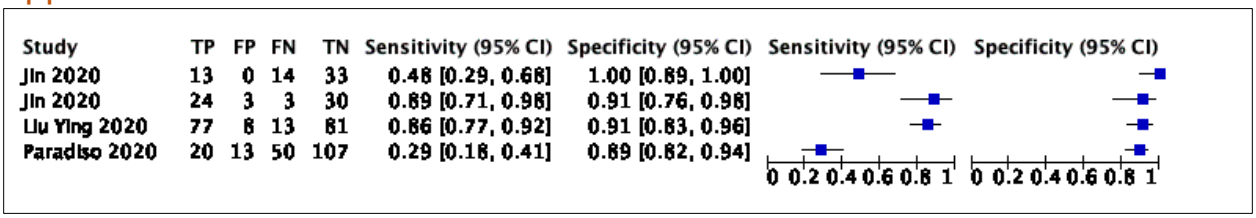


Figure 1. Forest Plot of the Overall Sensitivity and Specificity of SARS-CoV-2 Ab

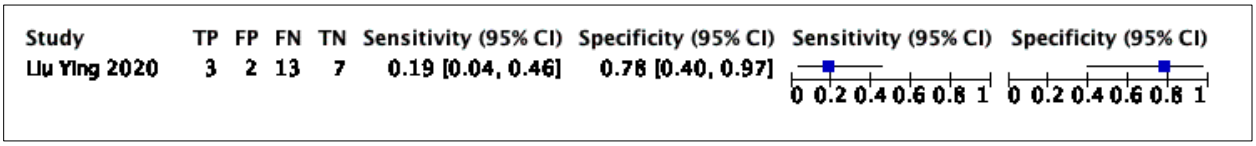


Figure 2. Forest Plot of 4 SARS-CoV-2 IgG IgM Day 0-7

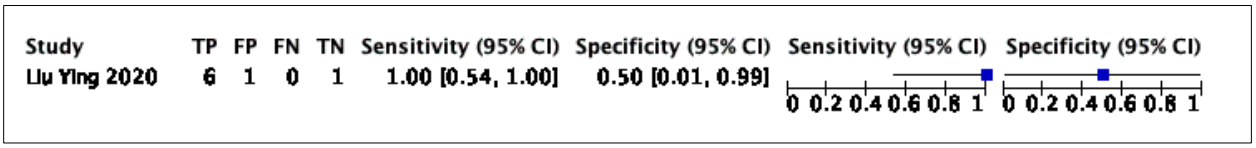


Figure 3. Forest Plot of 5 SARS-CoV-2 IgG IgM Day 8-15

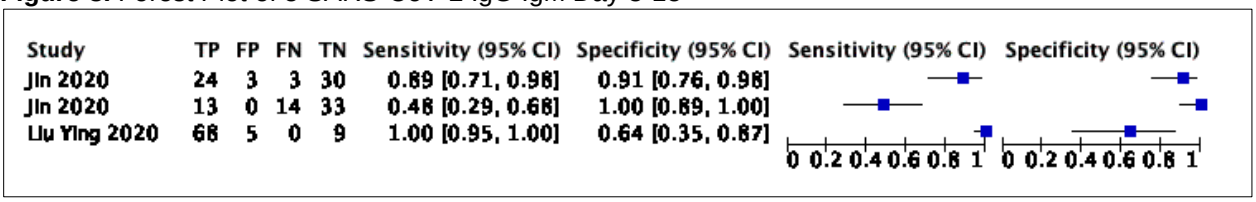


Figure 4. Forest Plot of 6 SARS-CoV-2 IgG IgM (>/=) Day 16

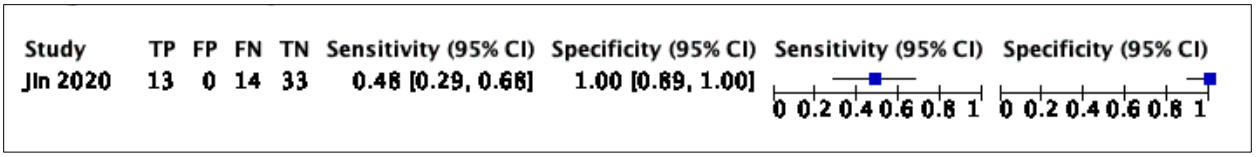


Figure 5. Forest Plot of 2 SARS-CoV-2 IgM Ab

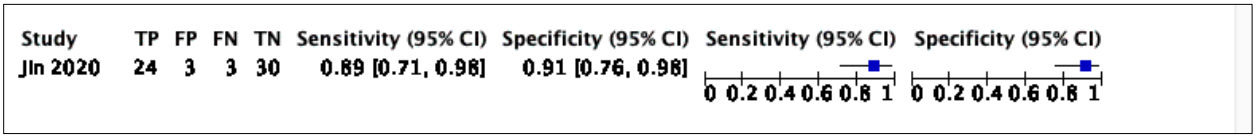


Figure 6. Forest Plot of 1 SARS-CoV-2 IgG Ab

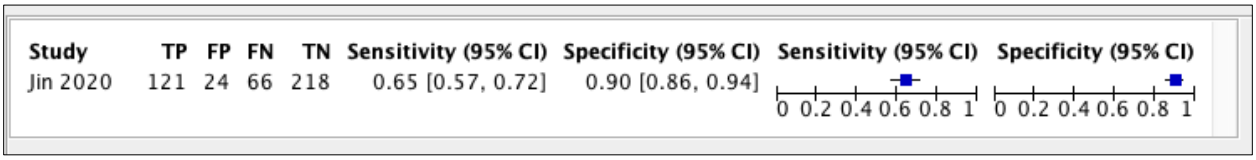
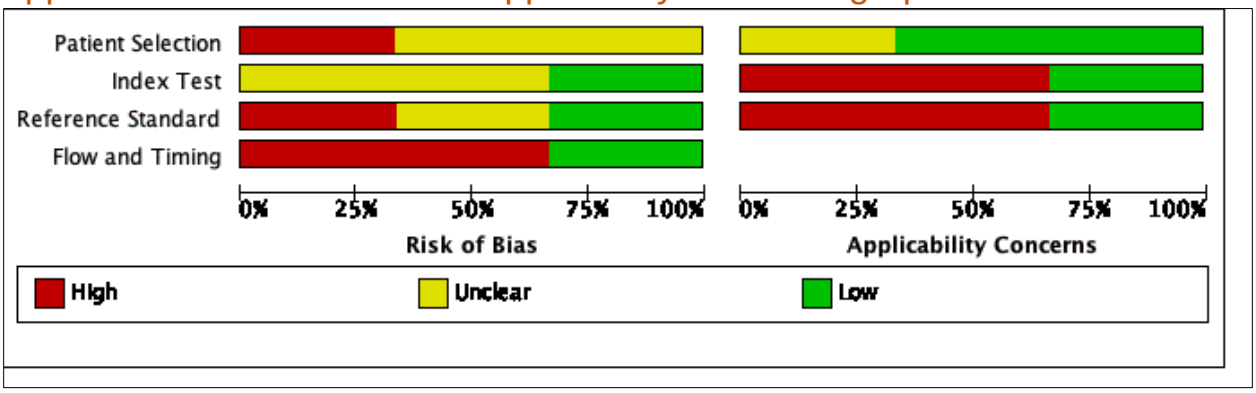


Figure 7. Forest Plot of the Sensitivity and Specificity of SARS-CoV-2 IgG Ab

Appendix 4. Risk of bias and applicability concerns graph



Appendix 5. Risk of bias and applicability concerns summary