EVIDENCE SUMMARY

Should antibody tests be used for COVID-19 seroprevalence studies and monitoring vaccine response among adults?

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RECOMMENDATIONS

We suggest using antibody tests that accurately measure IgG or total antibodies to determine COVID-19 seroprevalence among adults when needed for public health purposes. *(Very low certainty of evidence; Weak recommendation)*

We suggest against using antibody tests detecting IgM to determine COVID-19 seroprevalence among adults when needed for public health purposes. *(Very low certainty of evidence; Weak recommendation)*

We suggest against using lateral flow immunoassay (LFIA) tests to determine COVID-19 seroprevalence among adults when needed for public health purposes. *(Very low certainty of evidence; Weak recommendation)*

We recommend against routine measurement of SARS-CoV-2 antibody titers after vaccination. *(No evidence; Strong recommendation)*

Consensus Issues

Recommendations on antibody testing were made in the context of public health purposes (i.e., to identify the percentage of people in a population who may have been previously infected). The panel was unanimous against the use of antibody tests detecting IgM and LFIA tests while the majority voted for the use of antibody tests detecting IgG or total antibodies with a weak recommendation. Concern was raised on the cross-reactivity of the tests with other coronaviruses. Thus, emphasis was made on ensuring the accuracy of the antibody test kits used.

The panel was initially divided about antibody testing post-vaccination. Six of eleven panelists voted that there is insufficient evidence to recommend for or against it, citing that the public should be given the choice given the lack of evidence. On the other hand, five panelists voted against routine antibody testing post-vaccination despite the lack of evidence due to: (1) the unavailability of a real neutralizing antibody test in the market and (2) the unclear cutoff of antibody level that is predictive of COVID-19 protection. The panelists eventually reached consensus in the second round of voting, with the majority choosing for a strong recommendation because antibody tests post-vaccination lack clinical utility at this point.

PREVIOUS RECOMMENDATION

We suggest using antibody tests with high sensitivity and specificity (e.g., total antibody or IgG assays, ELISA, ECLIA) to determine COVID-19 seroprevalence among adults. *(Very low quality of evidence; Conditional recommendation)*

We recommend against using antibody tests detecting IgM to determine COVID-19 seroprevalence among adults. *(Very low quality of evidence; Strong recommendation)*

We recommend against using rapid antibody tests (e.g., LFIA) to determine COVID-19 seroprevalence among adults. *(Very low quality of evidence; Strong recommendation)*

Previous Consensus Issues

The different recommendations were made considering the different laboratory techniques and antibodies detected when using antibody testing to detect COVID-19.

Majority voted for a strong recommendation against the use of antibody tests detecting IgM to determine COVID-19 seroprevalence among adults despite the very low quality of evidence because IgM may only suggest relatively recent infection. Others voted for a conditional found for antibody tests detecting IgM. One panelist opined that there may still be settings in which IgM antibody tests can be useful because of its good correlation with IgG tests based on local experience in a hospital setting. Meanwhile, the use of rapid antibody tests was not recommended due to the very low quality of evidence resulting from the significant heterogeneity detected across studies.

What's new in this version?

- Six new seroprevalence studies, including one preprint article, were added in this review to determine the diagnostic accuracy of COVID-19 antibody tests.
- An additional search for the accuracy of antibody tests in detecting breakthrough infection among vaccinated individuals was conducted but did not yield any relevant articles.

Key Findings

- This review included 19 observational studies (n=28,566 samples) that evaluated the diagnostic accuracy of antibody tests compared with reverse transcription polymerase chain reaction (RT-PCR) in seroprevalence studies.
- The studies were of moderate to high methodologic quality. The overall certainty of evidence was rated very low due to serious risk of bias (recall bias) and inconsistency.
- Heterogeneity across studies was substantial (I^2>90%). The sensitivity of antibody tests ranged from 14.4 to 100% while specificity ranged from 54.9 to 99.6%.
- No studies evaluating the accuracy of antibody tests in determining vaccine response compared to RT-PCR-diagnosed breakthrough infections were found.

Introduction

In SARS-CoV-2, the structural nucleocapsid and spike proteins were found to be dominant antigens for host immune response, and have become the basis for detecting antibodies to immunoglobulins (Ig) that bind to these proteins.[1] Generally, antibodies are classified as neutralizing antibodies (i.e. cause virus particles to lose infectivity) and binding antibodies.[2] The

latter are detected by lateral flow point-of-care fingerstick tests.[3] The binding antibodies IgM and IgA appear within five days from symptom onset while IgG rises shortly afterwards.[1]

While SARS-CoV-2 serology can be useful in clinical, occupational health, and public health settings, it is not a replacement for virologic testing.[3] Validating antibody tests is important because certain assays may cross-react with other coronaviruses among other concerns. To be clinically useful, antibody tests should have high sensitivity and specificity. Specificity is particularly important in large serosurveillance studies in areas with a low expected prevalence of prior SARS-CoV-2 infection.[2]

As of October 26, 2021, the Philippine Food and Drug Administration (FDA) has approved six rapid antibody tests after performance validation by the Research Institute for Tropical Medicine (RITM).[4]

This review sought to answer the following clinical questions:

(1) Among adults in seroprevalence studies, how accurate are antibody tests in detecting COVID-19 compared to nucleic acid amplification tests (NAAT) such as RT-PCR?
(2) Among adults vaccinated against COVID-19, how accurate are antibody tests in determining vaccine response compared to RT-PCR-diagnosed breakthrough infection?

Review Methods

To identify new studies for this review update, electronic searches were performed on several databases (MEDLINE through PubMed, Cochrane CENTRAL, medRxiv, bioRxiv, ClinicalTrials.gov) from March 25, 2021 to October 15, 2021 using the following terms: seroprevalence, serosurveillance, antibody test, serology, accuracy, sensitivity, specificity, predictive value, COVID-19, and SARS-CoV-2. No language restrictions were applied. To address the question on vaccine response, an additional search was conducted on October 22, 2021 using free text terms (vaccinated, infection, breakthrough), an age filter (19+ years), and the PubMed diagnosis filter for COVID-19.

Studies that used SARS-CoV-2 antibody tests to determine COVID-19 seroprevalence among adults were included. A positive NAAT such as RT-PCR was used as an acceptable reference standard following several previous reviews.[5-7]

Studies on non-human populations and pediatric age groups, assay validation studies, the use of pre-pandemic samples (i.e. for specificity) and specimens other than serum, plasma, or whole blood (e.g. saliva) were excluded. Articles with published data insufficient to construct a 2x2 table for diagnostic accuracy and those that reported less than 100 samples (similar to an earlier rapid review [5]) were also excluded.

Methodological quality of the included studies was assessed using QUADAS-2. The 'meta' package in R (R Foundation for Statistical Computing 2019) was used to determine sensitivity, specificity, and measures of heterogeneity, as well as to generate forest plots. Random effects models were used in conducting univariate meta-analyses. Planned subgroup analyses according to the type of population tested, serology technique used, and antibody detected were performed. To further investigate heterogeneity, exploratory analyses were conducted according to publication status and whether the RT-PCR result was self-reported.

Results

Seroprevalence Studies

A. Included Studies

Nineteen studies meeting the inclusion criteria were included in this review. Seven articles were available as preprints.[8-14] There were a total of 23 comparisons of antibody tests with RT-PCR (n=28,566 samples). Seropositivity was as low as 0.80% [15] to as high as 44.58%.[16]

The most common technique used was enzyme-linked immunosorbent assay (ELISA; 6 studies) [8,10,14,17-19] followed by Chemiluminescent immunoassay (CLIA; 3 studies) [12,15,20,21] and electrochemiluminescence immunoassay (ECLIA; 3 studies).[20-22] Other serology tests used were chemiluminescent microparticle immunoassay (CMIA), enzyme-linked fluorescence assay (ELFA), and microneutralization assay.

IgG was the most frequent anti-SARS-Cov-2 antibody detected (10 studies) [8,11,12,15,16,19-21,23,24]. Total antibody detection was used by five studies [9,10,17,20,22]. Only one study each used IgM alone [11] or neutralizing antibody.[25] Two studies used IgM and IgG in combination [13,18], while another study used either IgA or IgG to indicate a positive test.[14] One study used a variety of antibody tests and could not be classified in the prior categories.[26]

B. Methodological Quality

Eight studies were at risk of recall bias because of self-reporting and were therefore deemed as moderate quality.[8-10,14,16,19,24,26] Another study was considered as moderate quality because participants without PCR testing were lumped together with those who had negative RT-PCR results.[20] The rest of the studies had low risk of bias and were deemed to have high methodological quality.

C. Diagnostic Accuracy

The measures of diagnostic test accuracy varied across included studies (n=28,566). The sensitivity of antibody tests ranged from 14.4% (95% CI 9.4-20.6%) to 100% (95% CI 83.2-100%). Meanwhile, specificity of the index test ranged from 54.9% (95% CI 49.5-60.3%) to 99.6% (95% CI 97.8-100%).

Heterogeneity was considerable (I^2=96.3% for sensitivity and I^2=99.1% for specificity) and was not reduced in almost all subgroup analyses. The type of antibody detected may account for some heterogeneity, yet this remained moderate to substantial.

Sensitivity was highest in a small study that used a variety of antibody tests, including for IgM and IgG as well as IgG alone (Sn 91.2%, 95% CI 76.0-97.1%; n=152).[26] This was followed by total antibody (Sn 41.0-98.2%; I^2=97.7%) and IgG tests (Sn 15.6-100%; I^2=96.4%). Meanwhile, specificity was highest in one study that used either IgA or IgG (Sp 97.7%, 95% CI 95.5-98%; n=356).[14]

Among the individual serology techniques used, the point estimates for sensitivity were highest in ELISA (Sn 42.9-98.2%; I^2=91%) and CMIA (Sn 79.5-88.8%; I^2=84%). On the other hand, LFIA had the highest specificity among the subgroups (Sp 92.0-98.6%; I^2=78%).

In seroprevalence studies among general adult populations, the sensitivity of antibody tests ranged from 15.0 to 100%, while specificity ranged from 54.9 to 97.7%. Among healthcare and other frontline workers (i.e. police and fire personnel), sensitivity varied from 39.8 to 98.2% and

specificity from 59.4 to 97.8%. Antibody tests among patient populations had sensitivity ranging from 14.4 to 91.2% and specificity from 92.9 to 99.6%.

D. Ongoing Studies
We found no ongoing studies evaluating the accuracy of antibody tests against NAATs in seroprevalence studies.

Vaccine Response
No available studies evaluated the diagnostic accuracy of antibody tests compared with RT-PCR in determining vaccine response among adults who received COVID-19 vaccination.

Evidence to Decision
Rapid antibody tests for COVID-19 may cost around Php 400 to 700.[27] Neutralization tests however may be as much as Php 2,300.[28] There is no available evidence on the cost-effectiveness and acceptability of antibody tests in the Philippine setting.

Recommendations from Other Groups
Table 1 summarizes the recommendations from various regulatory agencies.

Research Gaps
The seroprevalence studies have considerable variability in the estimates of diagnostic accuracy probably due to methodological differences. Many of these studies rely on self-reporting of RT-PCR results.

Direct evidence on whether the detection of antibodies after COVID-19 vaccination is protective against breakthrough infection is still wanting.

Table 1. Summary of Recommendations from Other Groups

Guideline / Agency	Recommendation	Strength of Recommen-dation	Certainty of Evidence
Philippine Food and Drug Administration	"There are **no currently available** FDA approved COVID-19 test kits in the Philippines that differentiate the antibody protection gained from natural COVID-19 infection and the immunity from vaccination."	-	-
Health Technology Assessment Council (Philippines) 01 August 2020	HTAC does **not recommend** the use of rapid antibody tests "in seroprevalence surveys, return-to-work decisions, or entry-to-country/ province policies due to the lack of evidence regarding the link of presence of antibodies and the immunity to subsequent infection AND on the persistence of protection from COVID-19."	-	-
U.S. Centers for Disease Control and Prevention 21 September 2021	"Antibody tests **can be used** in seroprevalence studies to estimate the cumulative incidence of infection (or vaccination) in a community."	-	-
Infectious Disease Society of America 18 August 2020	"The IDSA panel makes **no recommendation** either for or against using IgM antibodies to detect evidence of past SARS-CoV-2 infection"	Conditional	Very Low
	"The IDSA panel suggests **against using IgA** antibodies to detect evidence of past SARS-CoV-2 infection"	Conditional	Very Low
	"The IDSA panel suggests **against using IgM or IgG** antibody combination tests to detect evidence of past SARS-CoV-2 infection"	Conditional	Very Low
	Benefits and Harms: "IgA tests or LF devices with both IgM and IgG targets… all suffer from lower specificity compared to tests involving IgG or total antibody targets, leading to increased false positive rates. These tests would falsely increase seroprevalence and potentially mislead public health officials, policymakers, and the general public."	-	-

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Appendix 1. Evidence to Decision

FACTORS	JUDGEMENT						RESEARCH EVIDENCE/ADDITIONAL CONSIDERATIONS
Problem	No (3)	Yes (7)					
Certainty of Evidence	High	Moderate	Low (2)	Very low (8)			The overall certainty of evidence is very low after downgrading for serious risk of bias (recall bias) and very serious inconsistency (I^2 > 90%).
Accuracy	Very Accurate	Accurate	Inaccurate (6)	Very Inaccurate (1)	Uncertain (3)		In seroprevalence studies among adults, point estimates of the sensitivity of antibody tests ranged from 14.4 to 100%. Their specificity ranged from 54.9 to 99.6%. There was substantial heterogeneity across studies.
Values	Important uncertainty or variability (4)	Possibly important uncertainty or variability (5)	Possibly NO important uncertainty or variability (1)	No important uncertainty or variability			
Resources Required	Uncertain (1)	Large cost (3)	Moderate Cost (6)	Negligible cost	Moderate savings	Large savings	Rapid antibody tests for COVID-19 may cost around Php 400 to 700. Neutralization tests however may be as much as Php 2,300.
Certainty of evidence of required resources	No included studies (7)	Very low (1)	Low (2)	Moderate	High		
Cost effectiveness	No included studies (6)	Favors the comparison (1)	Does not favor either antibody testing or the comparator (3)	Favors antibody testing			No local cost-effectiveness studies found.
Equity	Uncertain (5)	Reduced	Probably no impact (5)	Increased			
Acceptability	Uncertain (6)	No (3)	Yes (1)				
Feasibility	Uncertain (4)	No (3)	Yes (3)				

Appendix 2. Search Yield and Results

Database	Search Terms	Date and Time	Yield
PubMed	((sensitivity) OR (specificity) OR (accuracy) OR (predictive value)) AND ((((antibody test) OR (serology)) AND ((seroprevalence) OR (serosurveillance))) AND ((((("COVID-19" [Supplementary Concept] OR "COVID-19 drug treatment" [Supplementary Concept] OR "COVID-19 serotherapy" [Supplementary Concept] OR "severe acute respiratory syndrome coronavirus 2" [Supplementary Concept] OR "2019-nCoV" OR "2019nCoV" OR "cov 2" OR "Covid-19" OR "sars coronavirus 2" OR "sars cov 2" OR "SARS-CoV-2" OR "severe acute respiratory syndrome coronavirus 2" OR "coronavirus 2" OR "COVID 19" OR "COVID-19" OR "2019 ncov" OR "2019nCoV" OR "corona virus disease 2019" OR "cov2" OR "COVID-19" OR "COVID19" OR "nCov 2019" OR "nCoV" OR "new corona virus" OR "new coronaviruses" OR "novel corona virus" OR "novel coronaviruses" OR "SARS Coronavirus 2" OR "SARS2" OR "SARS-COV-2" OR "Severe Acute Respiratory Syndrome Coronavirus 2") OR ((19[tiab] OR 2019[tiab] OR "2019-nCoV" OR "Beijing" OR "China" OR "Covid-19" OR epidem*[tiab] OR epidemic* OR epidemy OR new[tiab] OR "novel"[tiab] OR "outbreak" OR pandem* OR "SARS-CoV-2" OR "Shanghai" OR "Wuhan") AND ("Coronavirus Infections"[Mesh] OR "coronavirus"[MeSH Terms] OR coronavirus*[all] OR corona-virus*[all] OR cov[tiab] OR pneumonia-virus*[tiab]))) AND 2019/12/1:3000/12/31[PDAT]))) Filter: From 03/25/2021 Species: Humans	15 October 2021 21:34	103
PubMed	(((antibody test) OR (serology)) AND (vaccinated)) AND (infection OR breakthrough) AND (LitCDIAGNOSIS[filter]) Filter: Adult: 19+ years	22 October 2021 10:17	219
The Cochrane Library	((COVID-19) OR (SARS-CoV-2)) AND ((serology) OR (antibody test)) Filter: From 03/25/2021 Filter: From 2021 (trials)	15 October 2021 20:46	5 reviews 6 protocols 94 trials 2 special collections
MedRxiv & BioRxiv	(COVID-19 OR SARS-CoV-2) AND (serology) AND (seroprevalence OR serosurveillance) Filter: From 03/25/2021	15 October 2021 20:50	390
ClinicalTrials.gov	seroprevalence AND (antibody test) | COVID-19	26 October 23:13	72

Appendix 3. Characteristics of Included Studies

Study ID, Study Design, and Country	Sample Size	Population	Index Test	Reference Standard	Outcome
Caillard 2021* Cross-sectional France	152	Kidney transplant recipients with functioning grafts from a single center **Age**: Not specified **Symptoms**: 81% of seropositive patients	**Various antibody tests**: Various tests including (1) Architect Abbott Elecsys: SARS-CoV-2 IgG (Abbott Laboratories, Abbott Park, USA); (2) Euroimmun (HUS); (3) Euroimmun (Lübeck, Germany); (4) Elecsys anti SARS Cov2 Roche Cobas (Roche Diagnostic GmbH, Mannheim, Germany); (5) Orient Gene Biotech COVID-19 IgG/IgM Rapid Test Cassette (Orient Gene Biotech, Zhejiang, China); (6) Biosynex COVID-19 BSS (Biosynex, Fribourg, Switzerland); (7) VIDAS Anti-SARS CoV-2 IgG and IgM (BioMérieux, Marcy-l'Etoile, France); (8) CLIA Maglumi ; MAGLUMI 2019 nCov IgM and IgG (SNIBE - Shenzhen New Industries Biomedical Engineering, Shenzhen, China); (9) Platelia SARS-CoV-2 Total Ab method (Bio-Rad, HERCULES,USA) **Antibody detected**: Varied **Target antigen**: Varied **Timing**: Not specified	Self-reported RT-PCR using a nasopharyngeal swab	Diagnostic accuracy
Carrat 2021* Prospective cohort France	242	General adult population from three regions **Age**: 56% were < 60 years **Symptoms**: 47% of seropositive patients with median 56 days (IQR 40-61) from specimen collection	**ELISA**: EuroimmunVR (Lübeck, Germany) **Antibody detected**: IgG (optical density ratio ≥ 1.1) **Target antigen**: Spike protein **Timing**: Not specified	Self-reported RT-PCR result	Diagnostic accuracy
Khan 2021* Cross-sectional India	1,088	General adult population **Age**: 42.9% were 30-49 years **Symptoms**: 7.6% with COVID-19-like symptoms within 3 months prior	**CMIA**: Abbott SARS-COV-2 IgG assay **Antibody detected**: IgG (cut-off index ≥ 1.4) **Target antigen**: Nucleocapsid **Timing**: Not specified	Self-reported RT-PCR result	Diagnostic accuracy
Lorent 2021* Cross-sectional Poland *Preprint*	356	Adult volunteers in the Poznan metropolitan area **Age**: Mean 38.7 years +/- 12.7 **Symptoms**: Asymptomatic	**ELISA**: EuroImmun anti-SARS-CoV-2 assay **Antibody detected**: IgA or IgG **Target antigen**: Spike **Timing**: Not specified	Self-reported previous RT-PCR result	Diagnostic accuracy

Nasrallah 2021* Cross-sectional Qatar	393	Volunteers **Age**: Not specified **Symptoms**: Not specified	(1) **ELFA**: BioMérieux VidasIII assay **Antibody detected**: IgG (>= 1.0) **Target antigen**: Spike (2) **CLIA**: Mindray CL-900i anti-ARS-CoV-2 IgG **Antibody detected**: IgG (>= 10.0) **Target antigen**: Nucleocapsid and sike (3) **ECLIA:** Roche Elecsys Anti-SARS-CoV-2 (index >= 1.0) **Antibody detected**: IgG **Target antigen**: Nucleocapsid **Timing**: At time of enrollment	RT-PCR using nasopharyngeal and oropharyngeal swabs	Diagnostic accuracy
Papasavas 2021* Prospective cohort USA	1,317	Healthcare workers and allied professionals **Age**: Median 43 years (range 18-81) **Symptoms**: 75.2% among seropositive	**CMIA**: Abbott Archiect i2000 (Abbott Park, IL) **Antibody detected: IgG (index ≥ 1.4)** **Target antigen**: Nucleocapsid **Timing**: Not specified	Self-reported previous RT-PCR result	Diagnostic accuracy
Afzal 2020 Cross-sectional Pakistan	426	Patients in outpatient and emergency departments **Age**: Mean 42.43 years +/- 16.67 **Symptoms**: 43.6% among included participants	**ECLIA***:* Roche Cobas e601 immunoassay analyzer **Antibody detected**: Total antibody (reactive if cut-off > 1.000) **Target antigen**: Nucleocapsid **Timing**: 15-21 days after RT PCR result	RT-PCR result within 15-21 days presented by patient	Diagnostic accuracy
Flannery 2020 Cross-sectional USA	1,109	Pregnant women presenting for delivery **Age**: Median 31 (IQR 27-35) **Symptoms**: Not specified	In-house **ELISA** modified from protocol by Amanat et al. 2020 **Antibody detected**: IgM or IgG (seropositive if either IgG or IgM > 0.48 arbitrary units) **Target antigen**: Spike **Timing**: 67% taken within 6 days after RT PCR result	RT-PCR using nasopharyngeal specimen (device not specified)	Diagnostic accuracy
Fong 2020 Cross-sectional Italy	250	Cancer patients consecutively enrolled **Age**: Median 69 years (oncology) and 71 years (hematology) **Symptoms**: Not specified	**CLIA**: Abbott Architect SARS-CoV-2 IgG assay **Antibody detected**: IgG **Target antigen**: Nucleocapsid **Timing**: Not specified	RT-PCR using nasopharyngeal swabs (device not specified)	Diagnostic accuracy

Gonzalez 2020 Cross-sectional Colombia *Preprint*	237	University staff **Age**: Mean 36.14 years +/- 9.66 **Symptoms**: 10/32 seropositive individuals were symptomatic	**CLIA**: Abbott IgG Architect SARS-CoV-2 Assay (Abbott, Abbott Park IL, USA) **Antibody detected**: IgG (seropositive if > 1.40) **Target antigen**: Nucleocapsid **Timing**: 91 days after RT PCR (average)	RT-PCR using nasopharyngeal swabs: U-TOP COVID-19 detection Kit (SeaSun Biomaterial Inc., Daejeon, South Korea); Ct threshold not specified	Diagnostic accuracy
Ige 2020 Cross-sectional Nigeria *Preprint*	521	Patients in community isolation centers **Age**: Mean age 35.2 years +/- 15 **Symptoms**: Not specified	**LFIA**: Innovita® (Biological 116 Technology CO., LTD, China) **Antibody detected**: IgM & IgG **Target antigen**: Spike and nucleocapsid **Timing**: At time of enrollment	PCR using oral and nasopharyngeal swabs: Liferiver extraction kits (Shanghai, China) and primers from Genefinders Company LTD (South Korea); Ct threshold not specified	Diagnostic accuracy
Jespersen 2020 Cross-sectional Denmark	4,797	Healthcare and administrative personnel at hospitals, prehospital services, and specialist practitioners **Age**: Not specified **Symptoms**: Not specified	**ELISA**: Wantai Biological Pharmacy Enterprise Co, Ltd (Beijing, China) **Antibody detected**: Total antibody (seropositive if A/CO ≥ 1.1) **Target antigen**: Spike **Timing**: Not specified	RT-PCR using oropharyngeal swab, nasopharyngeal swab or tracheal aspirate: Cobas® SARS-CoV-2 test (Cobas® 6800 System) or in-house PCR analysis; Ct threshold not specified	Diagnostic accuracy

Morgat 2020 Prospective cohort study Belgium *Preprint*	699	Healthcare workers (48% worked in a COVID-19 ward) **Age**: median 39.5 (IQR 32-49) **Symptoms**: 51/241 (21.2%) had at least one symptom	**ELISA**: Euroimmun (anti-SARS-CoV-2 IgG ELISA, reference EI 2606-9601 G, Medizinische Labordiagnostika AG) **Antibody detected**: IgG (seropositive if S/N ratio ≥ 1.1) **Target antigen**: Spike **Timing**: Not specified	Self-reported previous PCR result	Diagnostic accuracy
Mulchandani 2020 Cross-sectional UK *Preprint*	2,847	Frontline workers, i.e. police and fire, healthcare **Age**: Median 43 years (range 19-73) **Symptoms**: None in the last 7 days; 33% previously with symptoms compatible with COVID-19	[1] **ECLIA**: Roche Elecsys ® Anti-SARS-CoV-2 **Antibody detected**: Total antibody, predominantly IgG (positive if COI ≥ 1.0) **Target antigen**: Nucleocapsid [2] **ELISA**: EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) assays **Antibody detected**: IgG (positive if ratio > 0.8) **Target antigen**: Spike *Considered seropositive if positive on either assay (N.B. listed under IgG subgroup)* **Timing**: Median 75 days (IQR 63-92 days) from symptom onset among symptomatic	Self-reported previous PCR result via nasal and/or throat swab	Diagnostic accuracy
Percivalle 2020 Cross-sectional Italy	390	Asymptomatic blood donors **Age**: Median 46 years, range 19-70 **Symptoms**: None during enrollment	In-house SARS-CoV-2 **microneutralization assay** **Antibody detected**: Neutralizing antibodies (positive titer >= 1:10) **Target antigen**: - **Timing**: At time of enrollment (paired with nasal swab)	RT-PCR using nasal swabs: QIAGEN (Qiagen, Hilden, Germany); Ct threshold not specified	Diagnostic accuracy
Robinson 2021 UK	10,640	Hospital staff: (1) Western Sussex Hospitals NHS Foundation Trust (WSHT) (2) Brighton and Sussex University Hospitals (BSUH) **Age**: Not specified **Symptoms**: 28.7% among recruited	[1] **CLIA**: Abbott ARCHITECT i2000 (Abbott, California) **Antibody detected**: IgG (seropositive if COI > 1.4) **Target antigen**: Nucleocapsid **Timing**: 97 days after symptom onset in patients with positive RT PCR (median, WSHT cohort) [2] **ECLIA**: Cobas e411 analyser (Roche Anti-SARS-Diagnostics, Mannheim Germany) and Roche Elecsys® CoV-2 sandwich immunoassay **Antibody detected**: IgM & IgG (seropositive if COI > 1.0; N.B. listed under Total Antibody subgroup) **Target antigen**: Nucleocapsid **Timing**: 53 days after PCR, 61 days after symptom onset (median, WSHT cohort)	RT-PCR using nasopharyngeal swabs (device not specified)	Diagnostic accuracy

Santarelli 2021 Cross-sectional USA	108	General adult population ≥ 18 years, convenience sample **Age**: Mean 49.4 years **Symptoms**: None during enrollment; 33% of seropositive participants had symptoms within the past two months	**LFIA**: VITROS Anti-SARS-CoV-2 IgG test (Ortho-Clinical Diagnostics Inc.) **Antibody detected**: IgG Target antigen: Spike **Timing**: Not specified	RT-PCR result from review of medical records	Diagnostic accuracy
Shields 2020 Cross-sectional UK *Preprint*	216	Healthcare workers who were not hospitalized for COVID-19 but previously self-isolated due to symptoms experienced by themselves or household contacts **Age**: Median 41.0 (IQR 31-50) **Symptoms**: 87.7% among recruited with at least one SARS-CoV-2 symptom	**ELISA**: IgGAM ELISA that measures the total antibody response (Product code: MK654, The Binding Site (TBS), Birmingham) **Antibody detected**: IgG, IgA & IgM (positive if ratio > 1; N.B. listed under Total Antibody subgroup) **Target antigen**: Spike **Timing**: Not specified	Self-reported previous PCR result	Diagnostic accuracy
Silva 2020 Cross-sectional Brazil *Preprint*	321	Staff at the Adolfo Lutz Institute (analytical laboratory) and Ministry of Health **Age**: Median 50 years (IQR 40-57) **Symptoms**: 48% among recruited with at least one symptom	**LFIA**: SARS-CoV-2 Wondfo (Guangzhou Wondfo Biotech Co., Ltd., China) **Antibody detected**: IgG or IgM **Target antigen**: Spike **Timing**: At time of enrollment	RT-PCR using NP swab, OP swab or throat wash (Allplex 2019-nCoV Assay (Seegene, Korea); Ct up to 37	Diagnostic accuracy

* New study added in this update

Appendix 4. Study Appraisal

Table 1. Risk of bias assessment of newly included studies using QUADAS-2

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Caillard 2021	☺	?	?	?	☺	☺	☺
Carrat 2021	☺	☺	?	☺	☺	☺	☺
Khan 2021	☺	☺	?	☺	☺	☺	☺
Lorent 2021	☺	?	?	☺	☺	☺	☺
Nasrallah 2021	☺	☺	☺	☺	☺	☺	☺
Papasavas 2021	☺	☺	?	☺	☺	☺	☺

☺ Low Risk ☹ High Risk ? Unclear Risk

Table 2. Risk of bias assessment of previously included studies using validity criteria from DOH Manual / Painless Evidence-Based Medicine (2nd edition)

Study	Acceptable reference standard	Definitions of index test and reference standard independent	Performance of index test and reference standard independent	Interpretation of index test and reference standard independent
Afzal 2020 [22]	Low	Low	Low	Low
Flannery 2020 [18]	Low	Low	Low	Low
Fong 2020 [15]	Low	Low	Low	Low
Gonzalez 2020 [12]	Low	Low	Low	Low
Ige 2020 [11]	Low	Low	Low	Low
Jespersen 2020 [17]	Low	Low	Low	Low
Mortgat 2020 [8]	Unclear	Low	Low	Low
Mulchandani 2020 [9]	Unclear	Low	Low	Low
Percivalle 2020 [25]	Low	Low	Low	Low
Robinson 2021 [20]	Unclear	Low	Low	Low
Santarelli 2021 [23]	Low	Low	Low	Low
Shields 2020 [10]	Unclear	Low	Low	Low
Silva 2020 [13]	Low	Low	Low	Low

Appendix 5. GRADE Evidence Profile

Should antibody tests be used to screen for COVID-19 in seroprevalence studies?

Sensitivity: 0.14 to 1.00
Specificity: 0.55 to 1.00
Prevalences: 1%, 10%, 40%

Outcomes	No of studies (No 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 1%	Pre-test probability of 10%	Pre-test probability of 40%	
True positives (patients with COVID-19)	19 studies (2581 patients)	cohort & case-control type studies	serious[a]	not serious	very serious[b]	serious	none	**1 to 10**	**14 to 100**	**58 to 400**	⊕◯◯◯ Very low
False negatives (patients incorrectly classified as not having COVID-19)								**0 to 9**	**0 to 86**	**0 to 342**	
True negatives (patients without COVID-19)	19 studies (25985 patients)	cohort & case-control type studies	serious[a]	not serious	very serious[b]	not serious	none	**544 to 986**	**494 to 896**	**329 to 598**	⊕◯◯◯ Very low
False positives (patients incorrectly classified as having COVID-19)								**4 to 446**	**4 to 406**	**2 to 271**	

Explanations

a. Several studies vulnerable to recall bias (self-reported RT PCR), and one study assumed those not tested with RT PCR as being negative for the reference standard
b. Substantial heterogeneity across studies (I^2>90%)

Appendix 6. Forest Plots

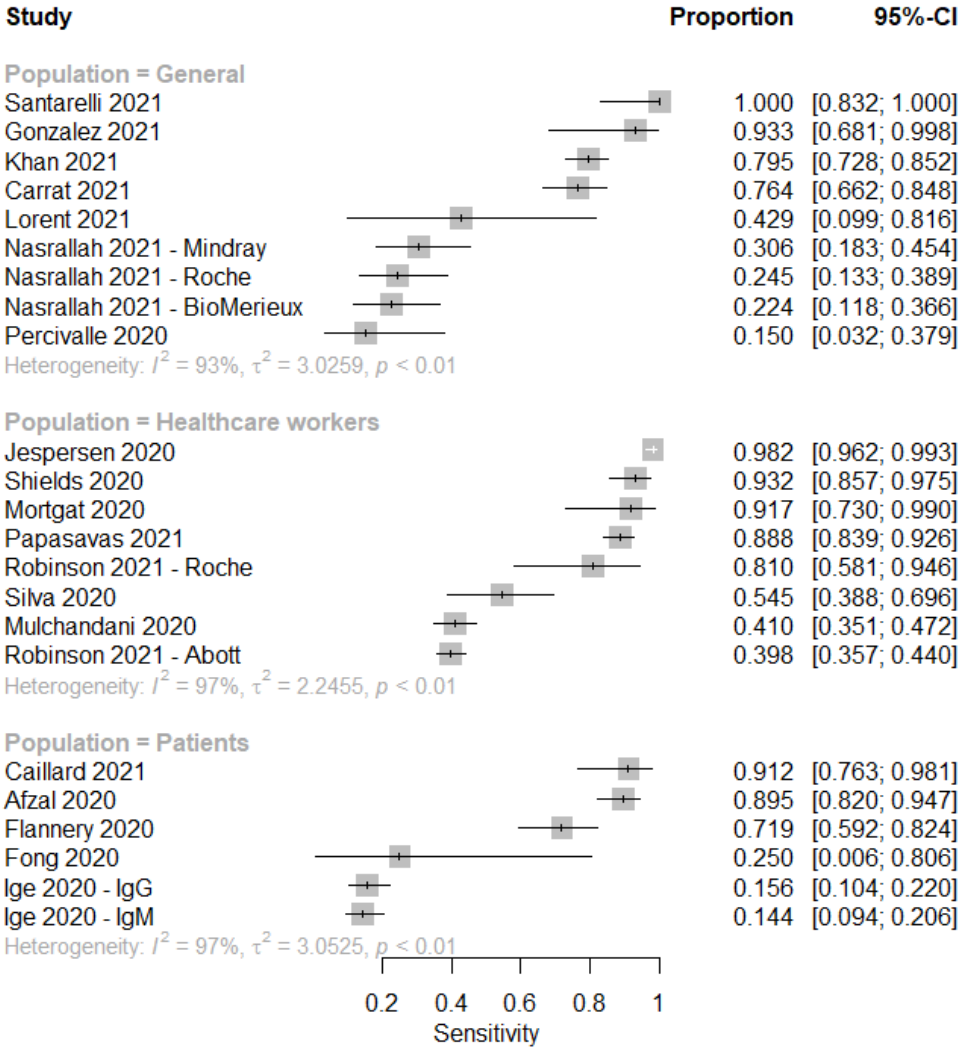

Figure 1. Sensitivity of antibody tests in COVID-19 seroprevalence studies according to population

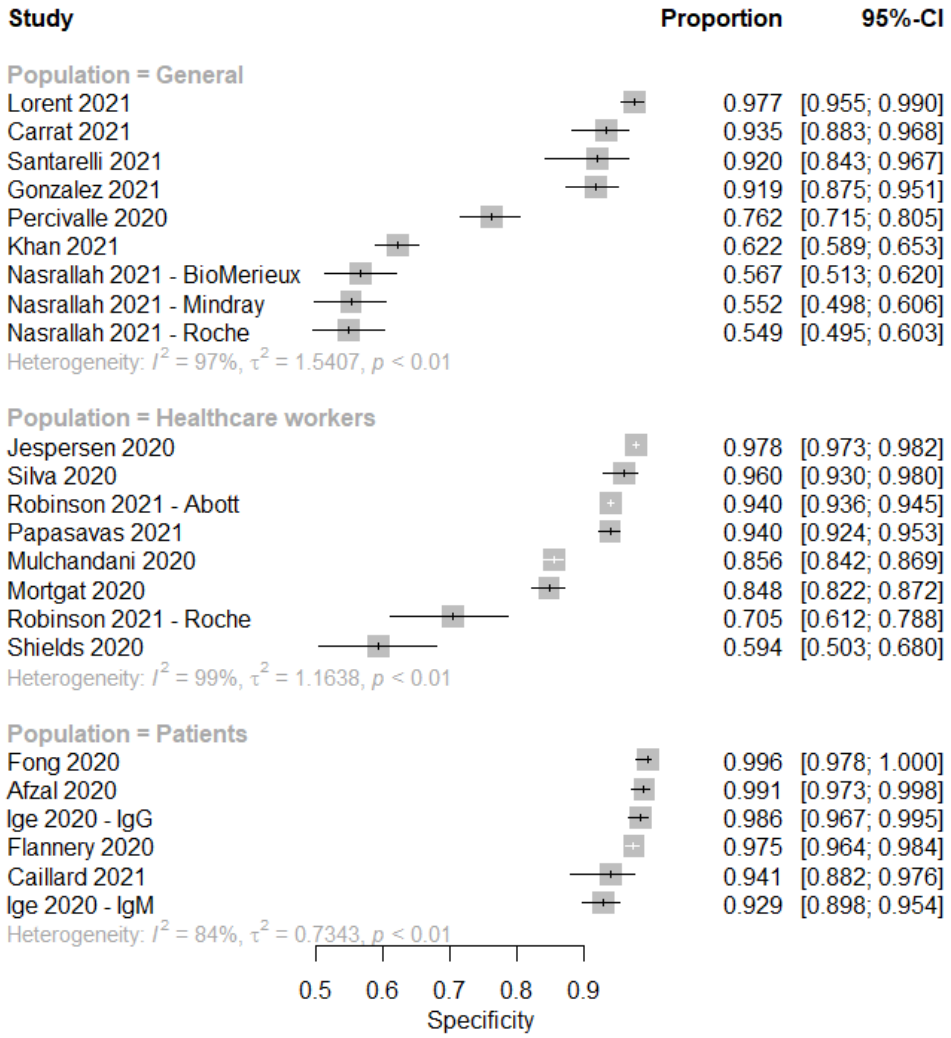

Figure 2. Specificity of antibody tests in COVID-19 seroprevalence studies according to population

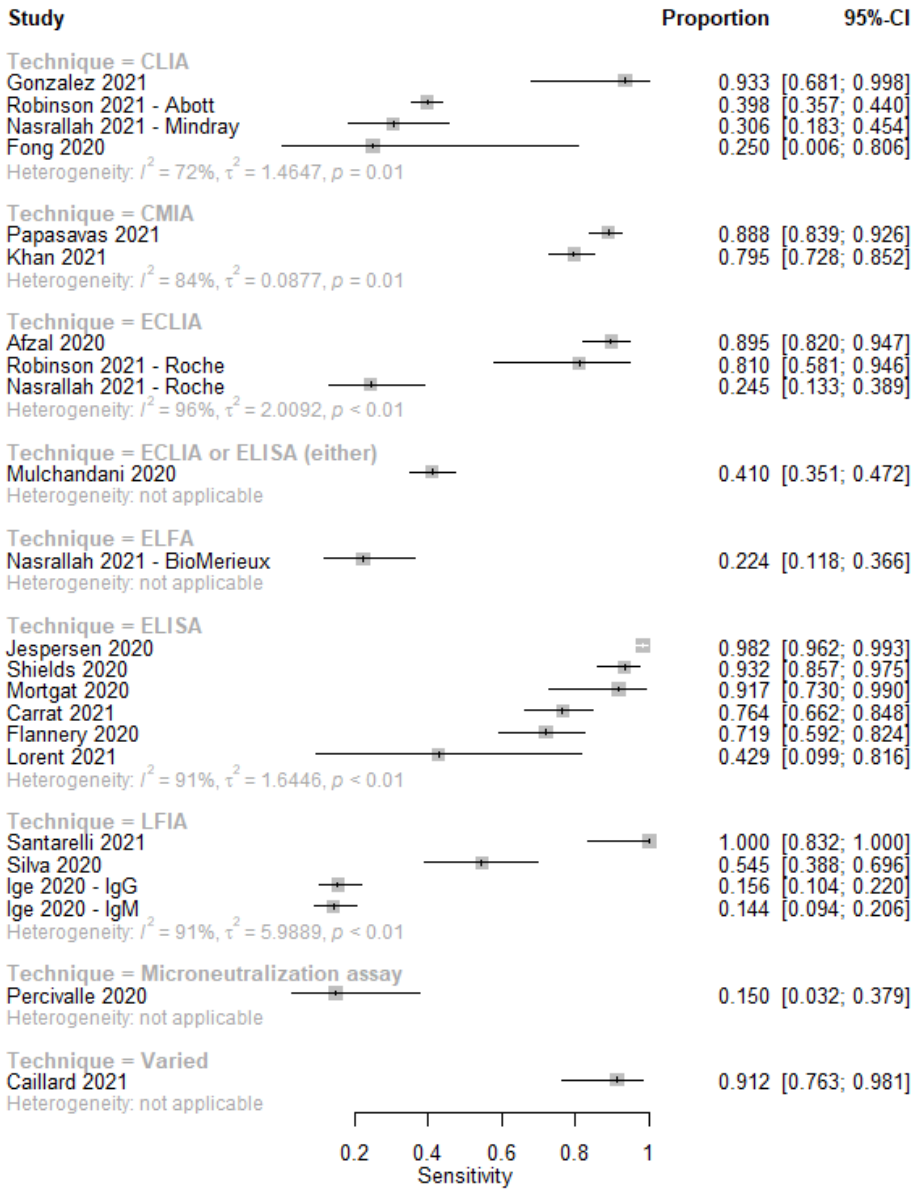

Figure 3. Sensitivity of antibody tests in COVID-19 seroprevalence studies according to serology technique used

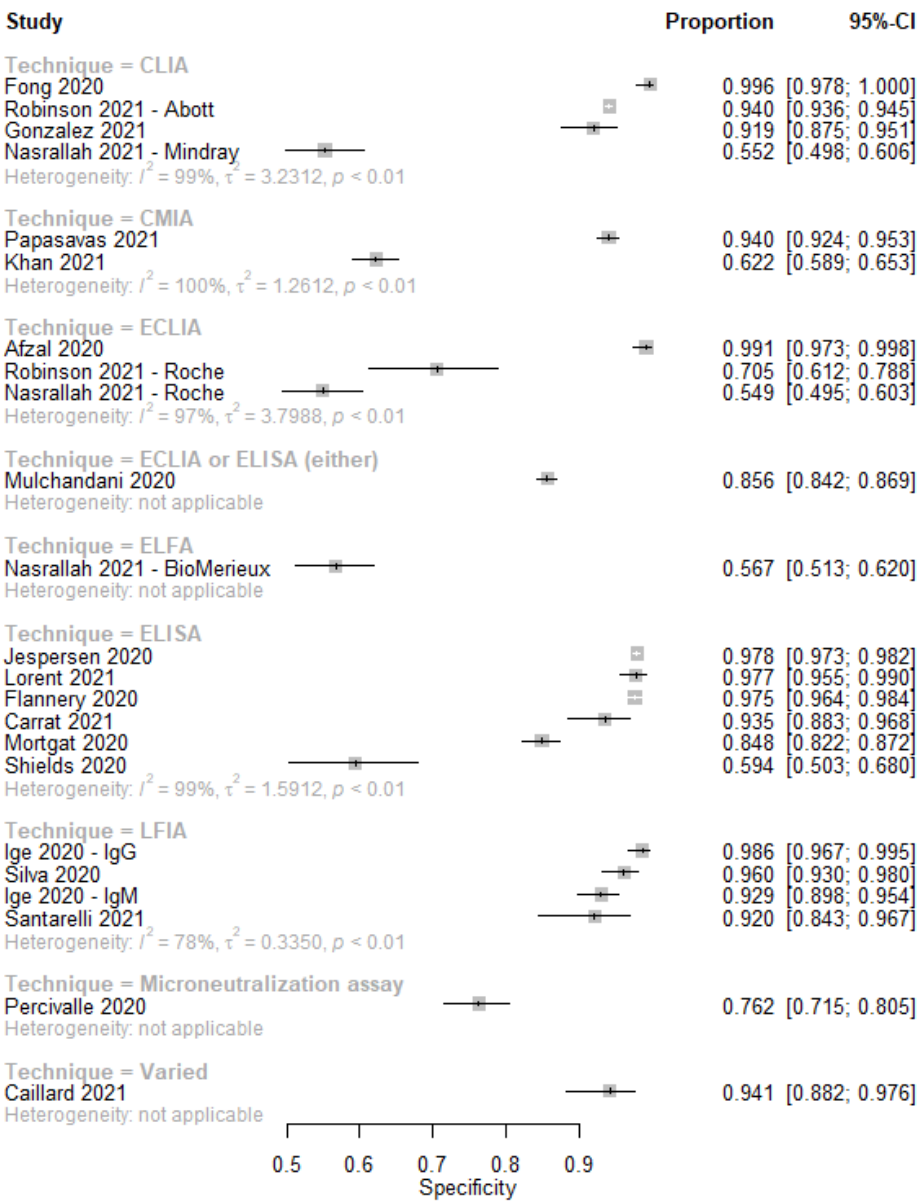

Figure 4. Specificity of antibody tests in COVID-19 seroprevalence studies according to serology technique used

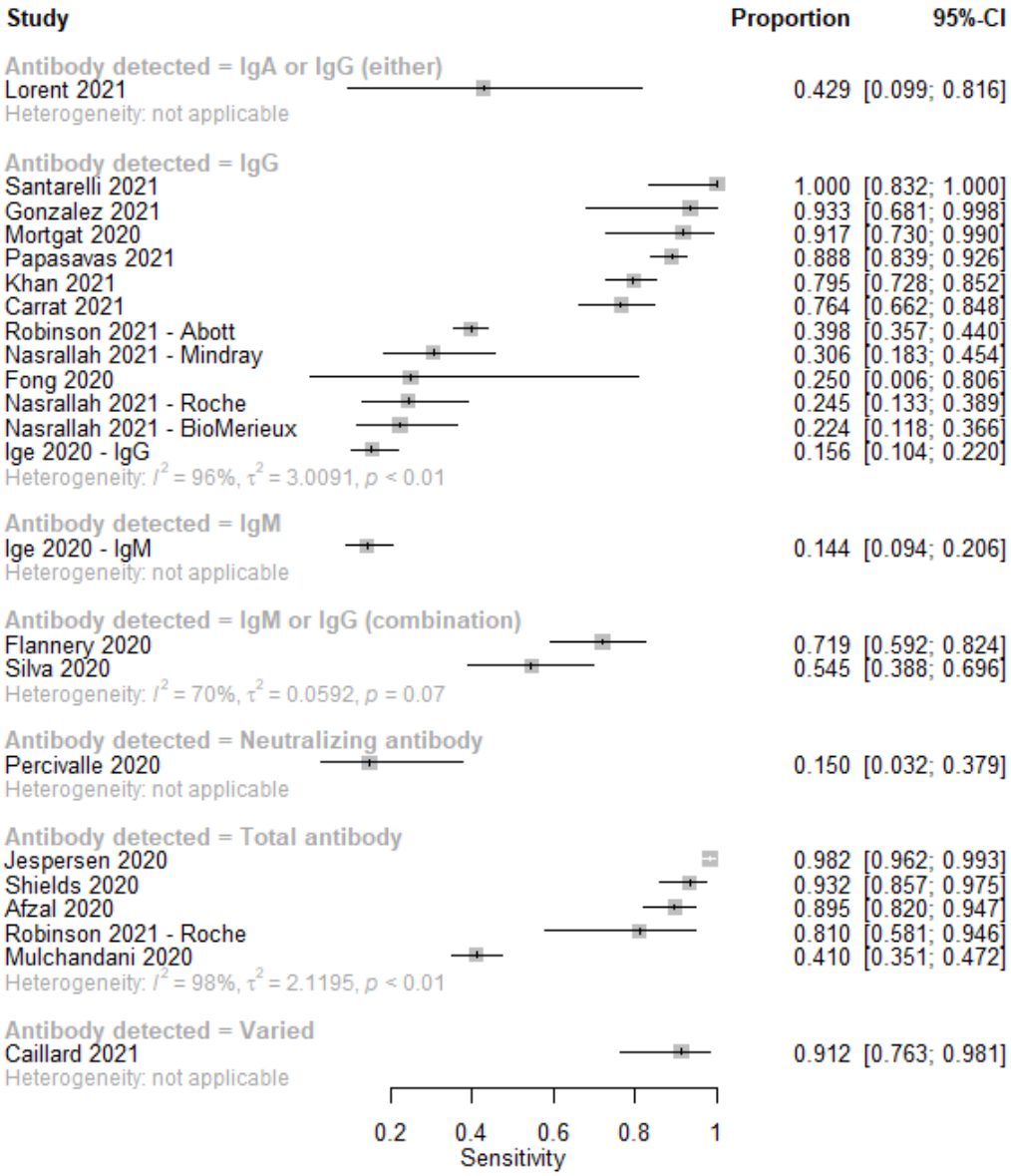

Figure 5. Sensitivity of antibody tests in COVID-19 seroprevalence studies according to type of antibody detected

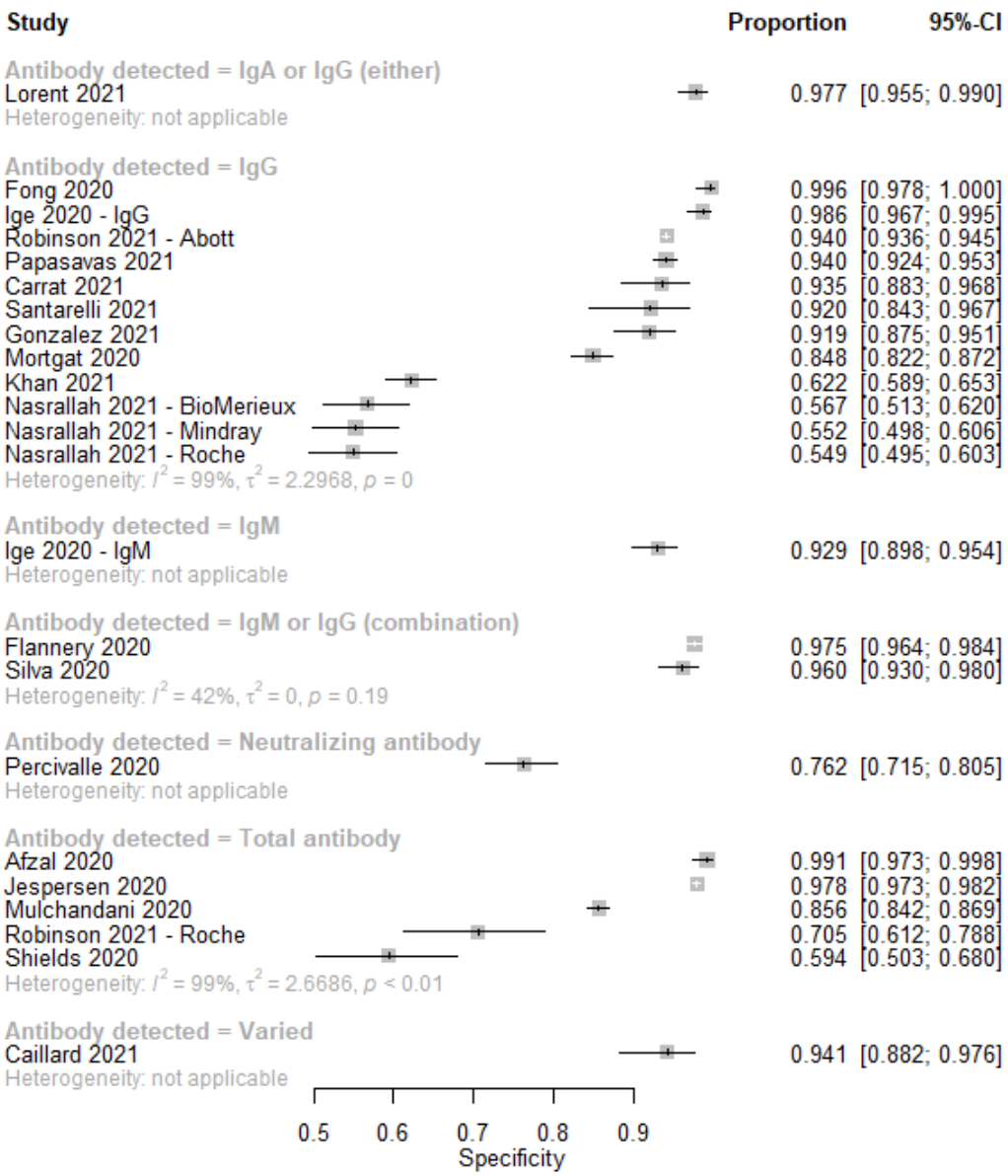

Figure 6. Specificity of antibody tests in COVID-19 seroprevalence studies according to type of antibody detected

Appendix 7. Subgroup Analyses

Subgroup	No. of comparisons	Sensitivity (95% CI)	I^2	Specificity (95% CI)	I^2
Population					
Healthcare workers	8	0.817 (0.603 – 0.929)	97.3%	0.897 (0.803 – 0.949)	98.9%
General	9	0.590 (0.300 – 0.828)	92.9%	0.817 (0.662 – 0.911)	96.9%
Patients	6	0.541 (0.215 – 0.835)	97.3%	0.978 (0.954 – 0.990)	84.5%
Serology Technique					
CLIA	4	0.501 (0.209 – 0.792)	72.0%	0.934 (0.697 – 0.989)	99.4%
CMIA	2	0.847 (0.772 – 0.901)	84.2%	0.835 (0.514 – 0.960)	99.6%
ECLIA	3	0.697 (0.302 – 0.925)	96.2%	0.869 (0.413 – 0.984)	96.8%
ECLIA or ELISA (either)	1	0.410 (0.353 – 0.470)	-	0.856 (0.842 – 0.869)	-
ELFA	1	0.224 (0.129 – 0.362)	-	0.567 (0.514 – 0.618)	-
ELISA	6	0.870 (0.690 – 0.953)	91.1%	0.935 (0.838 – 0.976)	98.8%
LFIA	4	0.545 (0.084 – 0.940)	90.6%	0.957 (0.921 – 0.978)	77.8%
Microneutralization assay	1	0.150 (0.049 – 0.376)	-	0.762 (0.716 – 0.803)	-
Varied	1	0.912 (0.760 – 0.971)	-	0.941 (0.881 – 0.971)	-
Antibody Detected					
IgA or IgG (either)	1	0.429 (0.144 – 0.770)	-	0.977 (0.955 – 0.98)	-
IgG	12	0.637 (0.383 – 0.832)	96.4%	0.890 (0.771 – 0.951)	99.3%
IgM	1	0.144 (0.098 – 0.205)	-	0.929 (0.898 – 0.952)	-
IgM or IgG (combination)	2	0.643 (0.516 – 0.753)	70.4%	0.972 (0.962 – 0.980)	42.5%
Neutralizing antibody	1	0.150 (0.049 – 0.376)	-	0.762 (0.716 – 0.803)	-
Total antibody	5	0.881 (0.663 – 0.965)	97.7%	0.907 (0.696 – 0.977)	99.1%
Varied	1	0.912 (0.760 – 0.971)	-	0.941 (0.881 – 0.971)	-
Self-Reported RT PCR (post hoc)					
No	15	0.584 (0.334 – 0.797)	95.6%	0.924 (0.843 – 0.965)	99.2%
Yes	8	0.808 (0.662 – 0.901)	95.8%	0.883 (0.778 – 0.942)	98.4%
Preprint (post hoc)					
No	15	0.708 (0.490 – 0.860)	96.3%	0.904 (0.807 – 0.955)	99.4%
Yes	8	0.603 (0.303 – 0.841)	95.3%	0.924 (0.844 – 0.964)	95.7%