ANTIBODY TESTS FOR SEROPREVALENCE

RECOMMENDATIONS

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)*.

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 recommendation because of the very low certainty of evidence resulting from the low sensitivity found for IgM 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.

EVIDENCE SUMMARY

Should antibody tests be used for COVID-19 seroprevalence studies among adult populations?

Evidence Reviewers: Cary Amiel G. Villanueva, MD, Myzelle Anne J. Infantado, PTRP, MSc (cand) & Howell Henrian G Bayona, MSc

Key Findings

There is very low-quality evidence from 13 observational studies (n = 24,082) on the use of antibody tests for COVID-19 seroprevalence studies. Four studies were at moderate risk of bias due to issues with defining the reference standard and susceptibility to recall bias; the rest were at low risk of bias. Heterogeneity across all studies were substantial. Sensitivity ranged from 14.4 to 100% while specificity ranged from 59.4 to 99.6%.

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) binding to these proteins [3]. Antibodies are classified as neutralizing antibodies, i.e., cause virus particles to lose infectivity, and binding antibodies [1]. The latter are detected by lateral flow point-of-care fingerstick tests [2]. The binding antibodies IgM and IgA appear within 5 days from the onset of symptoms while IgG rises shortly afterwards [3].

Available laboratory techniques to detect anti-SARS-CoV-2 antibodies include lateral flow immunoassay (LFIA), enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassay (CLIA), Electrochemiluminescence Immunoassay (ECLIA and fluorescent immunoassays (FIA). Neutralization assays are also in use primarily for research purposes.

The United States Food and Drug Administration (FDA) granted emergency use authorization (EUA) for 65 serology tests for COVID-19; the assay sensitivity and specificity ranged from 77.5 to 100% and 94.8 to 100% on laboratory validation [4] Meanwhile, the Philippine FDA approved 115 rapid antibody test (RAT) and 73 immunoassay test kits for commercial use as of 26 March 2021 [5-6].

While not a replacement for virologic testing, SARS-CoV-2 serology can be useful in clinical, occupational health and public health settings [2]. Validating antibody tests is important because certain assays may cross-react with other coronaviruses among other concerns. Antibody tests should have high sensitivity and specificity to be clinically useful; specificity is particularly important in large serosurveillance studies in areas with a low expected prevalence of prior SARS-CoV-2 infection [1].

This review sought to summarize the diagnostic test accuracy of antibody tests for COVID-19 in the use case of seroprevalence surveys in adults.

Review Methods

We conducted a search on several electronic databases (MEDLINE through PubMed, Cochrane CENTRAL) and preprint servers (medRxiv, bioRxiv, ChinaXiv) until March 25, 2021 using the following terms and their variations: seroprevalence, serosurveillance, antibody test, serology, accuracy, sensitivity, specificity, predictive value, COVID-19, SARS-CoV-2. No language restrictions were applied. We also searched trial registries (ClinicalTrials.gov, WHO ICTRP, ChiCTR) on March 26, 2021 for ongoing clinical studies.

Studies that used SARS-CoV-2 antibody tests to determine COVID-19 seroprevalence among adults were included. In the absence of a gold standard for antibodies against SARS-CoV-2, a positive nucleic acid amplification test (NAAT) such as reverse transcription polymerase chain reaction (RT-PCR) was used as an acceptable reference standard following several existing reviews [7-9].

We excluded studies on non-human populations and pediatric age groups, assay validation studies and those that used pre-pandemic samples (i.e. for specificity) and specimens other than serum, plasma, or whole blood (e.g. saliva). 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 [7]) were excluded.

Sensitivity and specificity for individual studies were generated using Review Manager Version 5.4 (RevMan 5.4.1, The Cochrane Collaboration 2020). Measures of diagnostic accuracy pooled across studies were calculated using the 'meta' package in R (R Foundation for Statistical Computing 2019) and random effects models.

To address heterogeneity, we conducted a priori subgroup analyses according to population tested, serology technique used, antibody detected, symptoms and COVID-19 disease prevalence. Additional exploratory analyses were done where possible based on risk of bias, publication status and use of in-house assays. We did not analyze according to timing of testing in relation to RT PCR as most seroprevalence studies in practice use only antibody tests which are less expensive than NAATs.

Results

Characteristics of included studies

We included 7 publications and 6 preprint articles meeting our eligibility criteria (**Appendix 1**) with a total of 24,082 samples from 9 countries. All were cross-sectional studies apart from one prospective cohort study [10]. One study was excluded due to high risk of bias because the majority of the participants had no RT PCR testing done but were assumed to be negative on the reference standard [11].

Healthcare and other frontline workers (i.e., police and fire personnel) were included in five studies (n = 20,199) [10-11,13-15]. There were 4 studies each that tested patients in hospital and isolation centers [16-19], and the general population [20-23]. Measured seroprevalence ranged from 1.60% among cancer patients [18] to 40.7% among healthcare workers who were symptomatic or had exposure to household contacts with symptoms [15].

ELISA was used in 4 seroprevalence studies [10-11,15,17] and was the most common serology technique used. Meanwhile, CLIA [14,18,20] and LFIA [19,22-23] were used in 3 studies each. Two studies tested antibodies using ECLIA [14,16], and only one study used a microneutralization assay to detect SARS-CoV-2 neutralizing antibodies [21]. One study defined seropositivity as antibody detection on either ECLIA or ELISA [13]. Rapid test kits were used by four studies: three with LFIAs [19,22-23] while one with a rapid microneutralization assay [21].

Seven studies used IgG assays [10,13-14,18-20,22] while only one study tested for IgM alone [19]. Three studies used assays that measured total antibodies [11,15-16]. IgM or IgG combination tests, i.e., seropositivity is defined by detecting either antibody class, were used in 2 studies [17,23]. Only one seroprevalence study included measured neutralizing antibodies [21].

In three studies, antibody testing was performed together with PCR upon enrollment [13-14,23]. Timing varied from unspecified durations before RT PCR testing to 53 days (median) after RT PCR testing. Three studies included asymptomatic participants at the time of enrollment. [13,21-22] Among studies that reported symptoms, 21.2% [10] to 87.7% [15] of participants had at least one symptom attributed to SARS-CoV-2.

Methodological quality

Four studies were judged to have moderate risk of bias; three were subject to recall bias due to self-reported RT PCR results [10,13,15]. In another study [14], one of two healthcare worker

cohorts lumped together participants without PCR testing together with those who tested negative on PCR. The 9 remaining studies were at low risk of bias [11,16-23].

Diagnostic accuracy

Sensitivity across 13 studies (n = 24,082) ranged from ranged from 14.4 to 100% while specificity ranged from 59.4 to 99.6% with substantial heterogeneity (I^2 = 96.2% and 98.2% respectively). Rapid antibody tests (RATs) also had significant heterogeneity (I^2 = 87.7% for sensitivity and 95.7% for specificity) across 4 studies (n = 1,861). The sensitivity of RATs varied widely from 1.44 to 100% while specificity values were from 76.2 to 98.6%.

Subgroup analysis

Heterogeneity was large in nearly all subgroups (Table 1). In studies involving healthcare workers, sensitivity ranged from 40 to 98% while specificity ranged from 59 to 98% (Figure 1, Appendix 3). Among patients in hospitals and isolation centers, antibody tests had sensitivities ranging from 14 to 90% and high specificities from 93 to 100%. Sensitivity varied the most among the general population (15 to 100%) while specificity ranged from 76 to 96% in this subgroup.

Among the techniques used to detect antibodies against SARS-CoV-2, ECLIA had the highest point estimates for sensitivity (81 to 90%); specificity was from 71 to 99% using this technique (Figure 2, Appendix 3). Meanwhile, sensitivity and specificity for studies utilizing CLIA ranged from 25 to 93% and 92 to 100% respectively. Seroprevalence studies using ELISA had moderate sensitivities (72 to 98%) and specificities (59 to 98%). Sensitivity for LFIA studies varied widely from 14 to 100% while their specificity values were narrow from 92 to 99%. The sole study testing for neutralizing antibodies using a microneutralization assay had a low sensitivity of 15% (95% CI: 3-38) and moderate specificity of 76% (95% CI: 72-80).

Assays detecting total antibodies to SARS-CoV-2 had sensitivity values from 90 to 98% and specificity of 59 to 99% (Figure 3, Appendix 3). The sensitivity of IgG assays, the most used among the included seroprevalence studies, ranged from 16 to 100% while specificity ranged from 86 to 100%. The only study reporting assay for IgM alone had a low sensitivity of 14% (95% CI: 9-21) but a high specificity of 93% (95% CI: 90-95). Seropositivity on combination assays (either IgM or IgG) had sensitivities ranging from 55 to 72% and specificities from 96 to 98%.

There was insufficient data available to determine test performance in patients with or without symptoms, and according to timing of antibody testing from symptom onset. We also could not analyze test performance according to COVID-19 prevalence as the studies did not report the prevailing disease proportion by RT PCR in the countries where they were performed.

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

Subgroup	No. of studies (Sample size)	Sensitivity (95% CI)	I^2	Specificity (95% CI)	I^2
Population					
Healthcare workers	5 (20,199)	**82.1%** (55.1-94.5)	97.2%	**89.0%** (75.8-95.4)	99.1%
Patients (hospitals and isolation centers)	4 (2,827)	**43.2%** (14.8-76.8)	97.5%	**98.2%** (95.8-99.2)	86.7%
General population	4 (1,125)	**81.2%** (20.9-98.6)	79.4%	**90.8%** (82.1-95.5)	94.5%

Subgroup	No. of studies (Sample size)	Sensitivity (95% CI)	I^2	Specificity (95% CI)	I^2
Laboratory technique					
Lateral flow immunoassay (LFIA)	3 (1,471)	**54.5%** (8.4-94.0)	90.6%	**95.7%** (92.1-97.8)	77.8%
Enzyme-linked immunosorbent assays (ELISA)	4 (6,821)	**91.5%** (77.0-97.2)	92.7%	**93.6%** (79.3-98.3)	98.9%
Chemiluminescent immunoassay (CLIA)	3 (11,994)	**59.0%** (19.3-89.7)	77.8%	**96.7%** (87.7-99.2)	78.4%
Electrochemiluminescence immunoassay (ECLIA)	2 (559)	**88.1%** (81.2-92.7)	15.9%	**94.1%** (51.3-99.6)	97.4%
ELISA or ECLIA (either positive)	1 (2,847)	**41.0%** (35.1-47.2)	-	**85.6%** (84.2-86.9)	-
Microneutralisation assay	1 (390)	**15.0%** (3.2-37.9)	-	**76.2%** (71.5-80.5)	-
Antibody detected					
Total antibody	3 (5439)	**95.1%** (88.5-98.0)	84.8%	**95.0%** (69.1-99.4)	99.3%
IgG	7 (16169)	**68.0%** (35.6-89.1)	90.3%	**94.2%** (87.3-97.4)	97.6%
IgM	1 (521)		-		-
IgM or IgG (combination)	2 (1430)	**64.3%** (51.6-75.3)	70.4%	**97.2%** (96.2-98.0)	42.5%
Neutralizing antibody	1 (390)	**15.0%** (3.2-37.9)	-	**76.2%** (71.5-80.5)	-

Heterogeneity remained substantial in other exploratory subgroups (**Appendix 4**): self-reported PCR subject to recall bias (I^2 = 96.4% for sensitivity and 97.3% for specificity) or those without recall bias (I^2 = 96.5% for sensitivity and 98.0% for specificity); studies of low (I^2 = 96.9% for sensitivity and 96.5% for specificity) or moderate risk of bias (I^2 = 94.5% for sensitivity and 99.0% for specificity); preprint articles (I^2 = 96.0% for sensitivity and 96.4% for specificity) or published studies (I^2 = 96.3% for sensitivity and 95.5% for specificity); commercial tests (I^2 = 96.5% for sensitivity and 94.5% for specificity) or in-house assays (I^2 = 93.4% for sensitivity and 99.1% for specificity).

Recommendations from Other Groups

The **Infectious Diseases Society of America** (IDSA, 18 Aug 2020) [1] suggests testing for SARS-CoV-2 IgG or total antibody three to four weeks after symptom onset to detect past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence). Serosurveillance studies should use assays with high specificity (≥ 99.5%) especially when the expected SARS-CoV-2 community prevalence is low. The IDSA panel suggests against using (1) IgA antibodies and (2) IgM or IgG antibody combination tests, i.e., detecting either antibody class defines a positive result, with very low certainty of evidence. They make no recommendation on the use of IgM antibodies to detect past SARS-CoV-2 infection.

The US **Centers for Disease Control and Prevention** (CDC, 17 March 2021) [2] state that serologic testing can be used for public health purposes such as serologic surveys to differentiate natural infection from vaccination. Production of vaccine-induced antibodies is indicated by a positive result for antibodies against vaccine antigen targets, e.g., spike protein, and negative for other antigens. Seroprevalence studies can be used to estimate the cumulative incidence of infection or vaccination. The CDC however does not recommend using antibody testing to assess immunity after COVID-19 vaccination.

In the Philippines, the **Health Technology Assessment Council** (HTAC, 1 August 2020) [24] of the Department of Health did not recommend the use of RATs in seroprevalence surveys and disease surveillance activities. HTAC cited limited evidence on the accuracy of RATs for this use case as well as paucity of evidence linking SARS-CoV-2 antibodies and immunity to subsequent infection.

Research Gaps

There are 11 ongoing seroprevalence studies involving adults (n > 100) using SARS-CoV-2 antibody tests and PCR tests (**Appendix 6**). Study sites include Japan, Czech Republic, Israel, Germany, France, India, Pakistan, and the United States. Four studies have been completed and their results are awaited.

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Appendix 1: Characteristics of Included Studies

Study ID Study Design Country	Sample Size	Population	Index Test	Reference Standard	Outcome
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 2021 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

Study ID Study Design Country	Sample Size	Population	Index Test	Reference Standard	Outcome
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
Mortgat 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)	Self-reported previous PCR result via nasal and/or throat swab	Diagnostic accuracy

Study ID Study Design Country	Sample Size	Population	Index Test	Reference Standard	Outcome
			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		
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	RT PCR using nasopharyngeal swabs (device not specified)	Diagnostic accuracy

Study ID Study Design Country	Sample Size	Population	Index Test	Reference Standard	Outcome
			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)		
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

Study ID Study Design Country	Sample Size	Population	Index Test	Reference Standard	Outcome
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

Appendix 2: Summary of Diagnostic Test Performance: Antibody Tests for COVID-19 Seroprevalence

Study ID	n	Sensitivity, % (95% CI)	Specificity, % (95%CI)	Positive Predictive Value, % (95% CI)	Negative Predictive Value, % (95% CI)	Diagnostic Accuracy, % (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
Afzal 2020	426	89.5 (82.0-94.7)	99.1 (97.3-99.8)	96.9 (91.0-99.0)	96.7 (94.3-98.1)	96.7 (94.6-98.2)	95.8 (31.0-296)	0.11 (0.06-0.18)
Flannery 2020	1,109	71.9 (59.2-82.4)	97.5 (96.4-98.4)	63.9 (54.0-72.7)	98.3 (97.5-98.8)	96.0 (94.7-97.1)	28.89 (19.2-43.5)	0.29 (0.19-0.43)
Fong 2020	250	25.0 (0.63-80.6)	99.6 (97.8-100)	50.0 (6.98-93.0)	98.8 (97.9-99.3)	98.4 (96.0-99.6)	61.5 (4.61-819.6)	0.75 (0.43-1.33)
Gonzalez 2021	237	93.3 (68.1-99.8)	91.9 (87.5-95.1)	43.8 (32.9-55.3)	99.5 (96.9-99.9)	92.0 (87.8-95.1)	11.5 (7.24-18.3)	0.07 (0.01-0.48)
Ige 2020 – IgM	521	14.4 (9.43-20.6)	92.9 (89.8-95.4)	49.0 (36.1-62.0)	69.7 (68.2-71.1)	67.8 (63.6-71.8)	2.03 (1.20-3.45)	0.92 (0.86-0.99)
Ige 2020 - IgG	521	15.6 (10.4-22.0)	98.6 (96.7-99.5)	83.9 (67.0-93.0)	71.2 (69.9-72.6)	72.0 (67.9-75.8)	11.0 (4.31-28.2)	0.86 (0.80-0.92)
Jespersen 2020	4,797	98.2 (96.2-99.4)	97.8 (97.3-98.2)	77.1 (73.5-80.4)	99.9 (99.7-99.9)	97.8 (97.4-98.2)	44.2 (36.4-53.8)	0.02 (0.01-0.04)
Mortgat 2020	699	85.2 (66.3-95.8)	94.6 (92.7-96.2)	39.0 (31.0-47.7)	99.4 (98.5-99.8)	94.3 (92.3-95.9)	15.9 (11.1-22.7)	0.16 (0.06-0.39)
Mulchandani 2020	2,847	41.0 (35.1-47.19)	85.6 (84.2-86.9)	22.8 (19.9-26.0)	93.3 (92.7-93.9)	81.4 (79.9-82.8)	2.85 (2.40-3.38)	0.69 (0.62-0.76)
Percivalle 2020	390	15.0 (3.21-37.9)	76.2 (71.5-80.5)	3.30 (1.17-8.95)	94.3 (93.2-95.3)	73.08 (68.4-77.4)	0.63 (0.22-1.82)	1.12 (0.92-1.35)
Robinson 2021 – Abbott / Western Sussex	11,507	39.8 (35.7-44.0)	94.02 (93.6-94.5)	25.3 (23.0-27.8)	96.8 (96.6-97.0)	91.4 (90.9-91.9)	6.65 (5.86-7.55)	0.64 (0.60-0.69)
Robinson 2021 – Roche / Brighton & Sussex	133	81.0 (58.1-94.6)	70.5 (61.2-78.8)	34.0 (26.6-42.3)	95.2 (89.0-98.0)	72.2 (63.8-79.6)	2.75 (1.93-3.91)	0.27 (0.11-0.66)
Santarelli 2021	108	100 (83.2-100)	92.1 (84.3-96.7)	74.1 (58.4-85.3)	100	93.5 (87.1-97.4)	12.6 (6.18-25.6)	0
Shields 2020	216	93.2 (85.8-97.5)	59.4 (50.3-68.0)	61.2 (55.9-66.2)	92.7 (85.2-96.5)	73.2 (66.7-78.9)	2.29 (1.85-2.85)	0.11 (0.05-0.25)
Silva 2020	321	54.6 (38.9-69.6)	96.0 (93.0-98.0)	68.6 (53.5-80.5)	93.0 (90.6-94.9)	90.3 (86.6-93.3)	13.7 (7.25-26.0)	0.47 (0.34-0.65)

Note: Values for test performance were generated using MedCalc diagnostic test evaluation calculator (4 April 2021): https://www.medcalc.org/calc/diagnostic_test.php

Appendix 3: Forest Plots

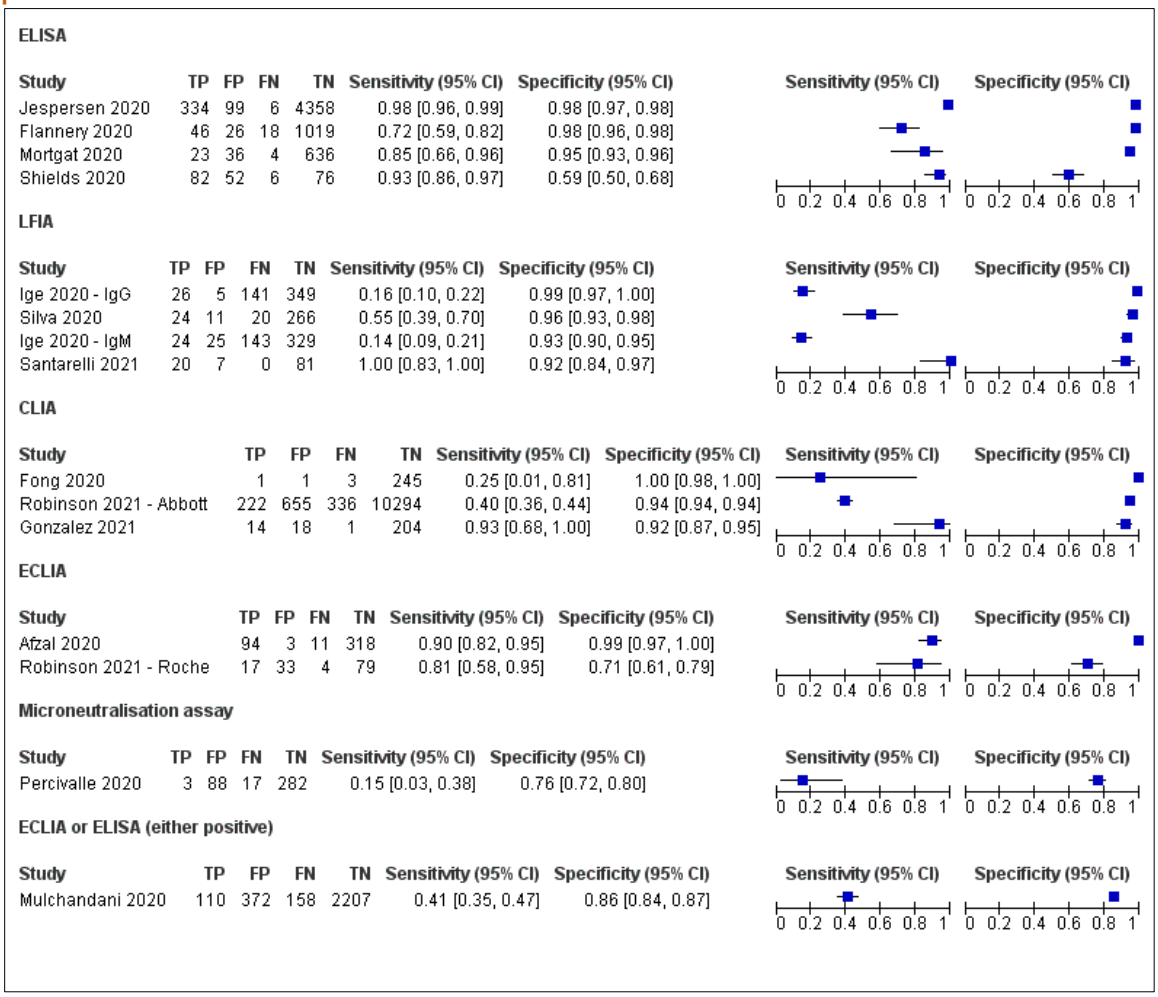

Figure 1. Diagnostic test performance of antibody tests in COVID-19 seroprevalence studies according to population

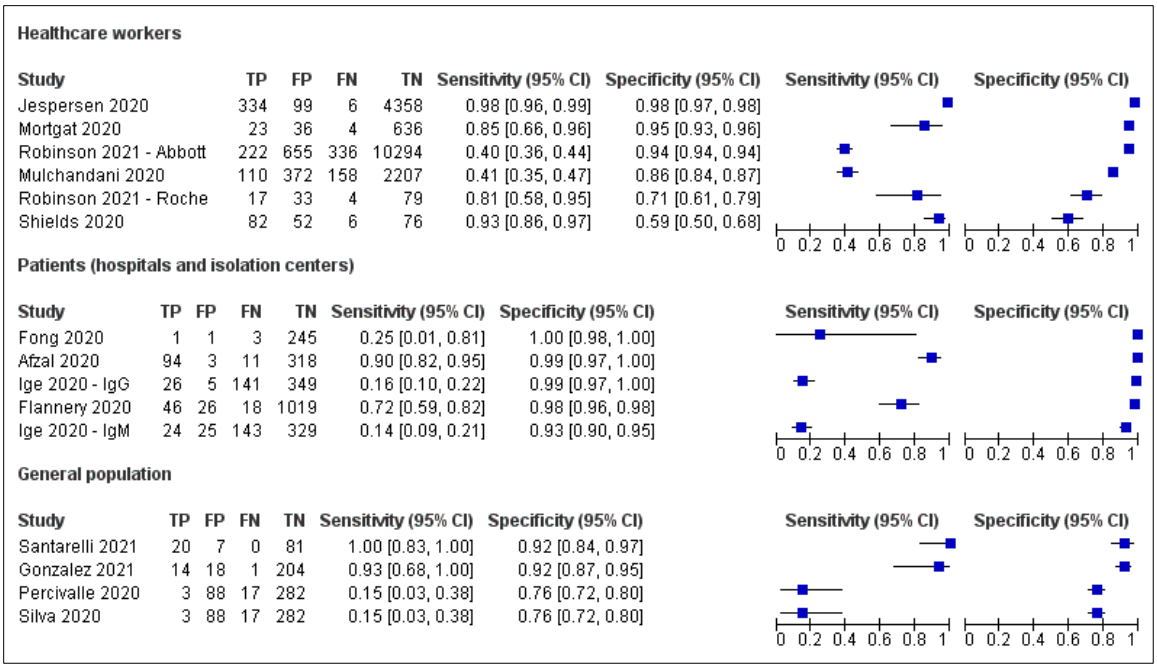

Figure 2. Diagnostic test performance of antibody tests in COVID-19 seroprevalence studies according to serology technique used

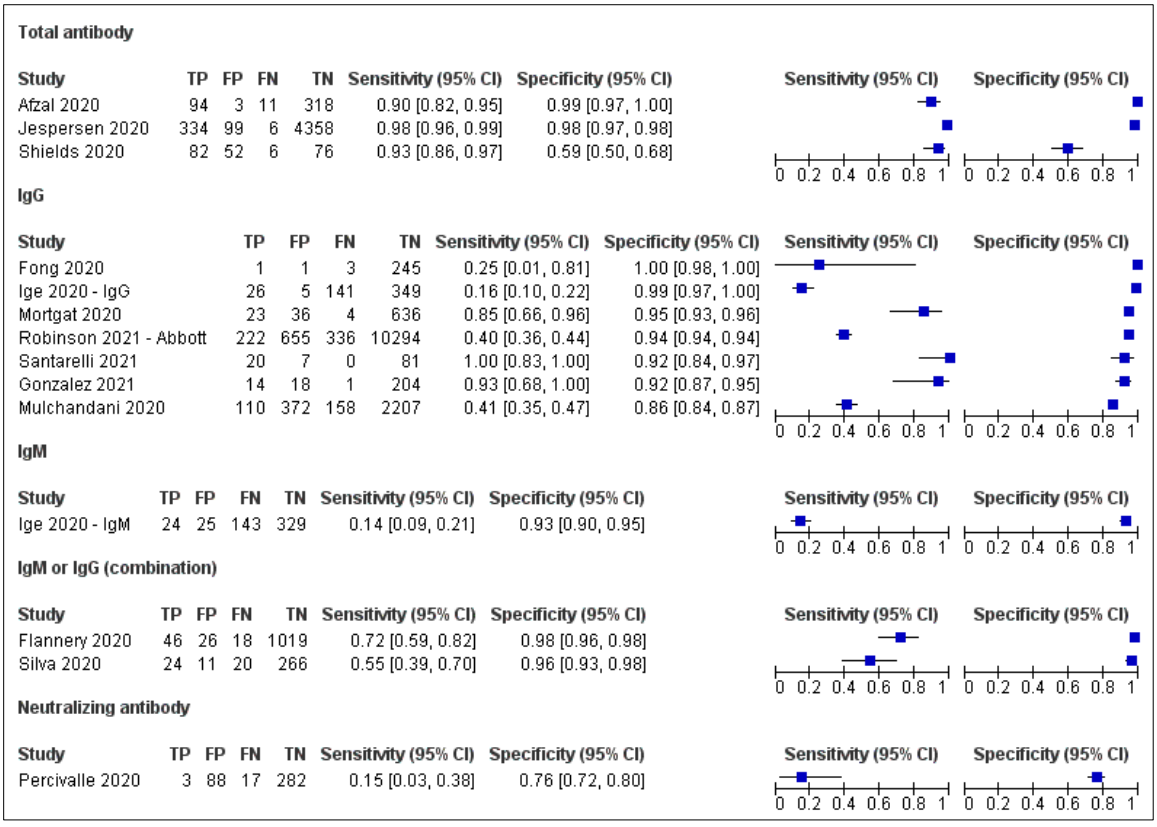

Figure 3. Diagnostic test performance of antibody tests in COVID-19 seroprevalence studies according to type of antibody detected

Appendix 4: Additional Subgroup Analyses

Subgroup	No. of studies	Sensitivity (95% CI)	I^2	Specificity (95% CI)	I^2
Risk of Bias					
Low	9	68.9% (33.3-90.8)	96.9%	96.5% (93.0-98.3)	97.2%
Moderate	4	72.6% (46.3-89.0)	94.5%	85.2% (70.7- 93.2)	99.0%
Recall Bias					
Yes (self-reported RT PCR)	3	78.9% (45.0-94.5)	96.5%	84.4% (63.1-94.4)	98.0%
No	10	67.5% (37.7-87.7)	96.4%	95.5% (91.3-97.8)	97.3%
In-house Assay					
Yes	3	41.8% (9.5-83.1)	93.4%	91.8% (66.2-98.5)	99.1%
No (commercial)	10	74.2% (48.3-89.8)	96.5%	94.5% (89.2-97.3)	98.0%
Publication Status					
Preprint	7	60.5% (28.9-85.3)	96.0%	92.3% (84.0-96.5)	96.4%
Published	6	77.5% (43.3-93.9)	96.3%	95.5% (88.4-98.4)	98.2%
Rapid Tests	4	42.9% (9.1-84.9)	87.7%	93.8% (85.9-97.4)	95.7%

Appendix 5: GRADE Evidence Profile

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

Sensitivity	0.70 (95% CI: 0.46 to 0.87)
Specificity	0.94 (95% CI: 0.89 to 0.97)

Prevalences	1%	10%	40%

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 1%	pre-test probability of 10%	pre-test probability of 40%	
True positives (patients with COVID-19)	13 studies 1908 patients	cohort & case-control type studies	serious [a]	not serious	serious [b]	serious [c]	none	7 (5 to 9)	70 (46 to 87)	280 (183 to 347)	⨁◯◯◯ VERY LOW
False negatives (patients incorrectly classified as not having COVID-19)								3 (1 to 5)	30 (13 to 54)	120 (53 to 217)	
True negatives (patients without COVID-19)	13 studies 22174 patients	cohort & case-control type studies	serious [a]	not serious	serious [d]	not serious	none	933 (883 to 959)	848 (803 to 872)	565 (535 to 581)	⨁⨁◯◯ LOW
False positives (patients incorrectly classified as having COVID-19)								57 (31 to 107)	52 (28 to 97)	35 (19 to 65)	

Explanations

a. Downgraded once for risk of bias (several studies may be vulnerable to recall bias, and one study assumed those not tested with RT PCR as being negative for the reference standard)
b. Downgraded once for inconsistency (substantial heterogeneity on visual inspection and I^2 = 96.2%)
c. Downgraded once for imprecision (wide confidence interval)
d. Downgraded once for inconsistency (I^2 = 98.2%)

Appendix 6: Characteristics of Ongoing Studies

Study ID Country	Sample Size	Population	Index Test	Reference Standard	Outcomes
JPRN-UMIN000040733 A study on the prevalence of infection in dental institutions by measuring the antibody titer to COVID-19 of the staff members of Kanagawa Dental College Medical Center Japan	300	Staff of a dental college	IgG and IgM test by immunochromatography using a small blood sample collected from the fingertip IgA antibody level is analyzed by ELISA on saliva collected with a collection tube	COVID-19 PCR using saliva	Prevalence of IgG, IgM, IgA antibodies against COVID-19
NCT04453280 *Antibody Detection in COVID-19 Cured Patients (SARS-CoV-2-CZ-Immunity)* *Czechia* *Completed*	695	Cured COVID-19 patients determined by RT PCR	SARS-CoV-2 diagnostic rapid test	-	Determination of the concentration of anti-SARS-CoV-2 antibodies in relation to the categories of cured patients.
NCT04490837 Rapid Diagnostic Test for COVID-19 Based on Antibody Detection Israel *Completed*	400	Professionals from Parc Taul University Hospital Patients with clinical, radiological and/or PCR COVID-19 positive	Diagnostic Test: ELISA and Rapid test to detect antibodies against COVID-19	-	IgG anti-COVID-19 IgM anti-COVID-19 IgA anti-COVID-19
NCT04355533 **Seroprevalence and Antibody Profiling Against SARS-CoV2 in Children and Their Parents** **France**	1,000	Hospitalized children or consulting at hospital Physician in a participating centre Parent's agreement for blood, saliva and stool samples agreement for follow-up if PCR+	Serology test	Optional parent's agreement for nasopharynx swab Optional parent's	Seroconversion against SARS-CoV2 in children
NCT04699903 *Clinical Evaluation of a Point-of-Care (POC), SARS-CoV-2 IgG Antibody Test in Fingerstick Whole Blood*	215	Patients with high-sensitive EUA PCR results (positive result) Patients with high-sensitive EUA PCR results (negative result)	Diagnostic Test: POC SARS-Cov-2 IgG Antibody test	PCR	PPA (positive percent agreement) and NPA (negative percent agreement) of POC compared to SARS-CoV-2 reference

Study ID Country	Sample Size	Population	Index Test	Reference Standard	Outcomes
United States					PCR;PPA (positive percent agreement) of POC compared to SARS-CoV-2 reference PCR;PPA (positive percent agreement) of POC compared to SARS-CoV-2 reference PCR
CTRI/2020/07/026370 *Clinical performance validation of Recombinant Immunogenic Marker (India Health Foundation-IHFs COVIDAB-SP) based on single-chain fragment variable (scFv) Antigen specific to Spike S I & S II regions of SARS CoV2 for Rapid detection of Antibody* India	2,000	Symptomatic group - confirmed RT-PCR testing, admitted and treated in isolation wards that are declared good-to-discharge by a RT-PCR retest or Clinician judgment. Asymptomatic group Healthcare Workers / relatives of RT PCR positives Asymptomatic group with no links or contractable connects of infected (Random) Negative PCR	COVIDAB-SP diagnostic kit	PCR	Evaluation of the consistency of Positive COVID AB-SP for Positive PCR and Consistent Negative COVIDAB-SP for Negative PCR Change in status of asymptomatic as a result of multiple testing involving antigens (RT PCR) and COVIDAB-SP for antibody detection
DRKS00022564 *Registry of SARS-CoV2-seroprevalence and Transmission of Covid-19 infections in common households - FamilyCoviDD19* Germany	250	Common households with at least one SARS-CoV-2-PCR positive person or at least one person with SARS-CoV-2-antibodies	Antibodies against SARS-CoV-2 and the T-cell response	-	Registry of SARS-CoV2 prevalence, t-cell-response and questionnaire in common households.
DRKS00023561 *Covid19 Antibody prevalence and sustainability. A retrospective longitudinal monocentered observational study in Covid 19 PCR positive hospitalworkers.* *Germany*	207	Ernst von Bergmann staff. Conducted Covid 19 PCR test(s). Especially positive Covid 19 PCR positive tests.	Covid 19 IgG and IgM antibodies	PCR	Covid 19 IgG and IgM antibodies in PCR Covid 19 positive staff members, measured in Serum between April 1st and 15th of december 2020.

Study ID Country	Sample Size	Population	Index Test	Reference Standard	Outcomes
NCT04563247 *Seroprevalence of SARS-CoV 2 Among Asymptomatic Frontline Healthcare Workers During COVID 19: A Cross Sectional Study* *Pakistan* *Completed*	970	All HCWs who had been working in high exposure areas of COVID 19 Exclusion Criteria: Any HCW of high exposure areas of COVID 19 who suffered from COVID 19 themselves diagnosed on PCR or clinically	Diagnostic Test: IgG SARS CoV 2 antibodies	PCR	SARS CoV 2 IgG antibodies
NCT04784403 **SCREENING AND SEROEPIDEMIOLOGY OF SARS-CoV-2 INFECTION AT THE UNIVERSITY OF BARCELONA: A CROSS-SECTIONAL STUDY** Spain Completed	3,356	Students from the different centers and type of studies (undergraduate / graduate). Administrative and service personnel. Teaching and Research Staff.	Serology test	PCR	Number of people with a positive SARS-CoV-2 PCR. Incidence of people with a positive SARS-CoV-2 PCR Number of people with a positive total immunoglobulin titer and positive IgG for SARS-CoV-2. Prevalence of people with a positive total immunoglobulin titer and positive IgG for SARS-CoV-2. Secondary Outcome Measures : Number of people with a positive SARS-CoV-2 Ig total titer and a negative IgG
NCT04619407 **Screening for COVID-19 in Teachers, Childcare Educators, Pupils and Preschoolers (COKITS)** Germany	300	Teacher, pupils, preschoolers, childcare educators in Mecklenburg-Vorpommern	Serum testing Anti-SARS-CoV-2 antibody testing (IgA and IgG ELISA) for teachers and childcare educators	Nasopharyngeal swab SARS-CoV-2 PCR	Share of participants with SARS-CoV-2 detectable in PCR Percentage of Anti-SARS-COV2 S protein IgA and IgA ELISA positive participants (educational staff) Percentage of Anti-SARS-COV2 S protein IgA and IgA ELISA positive participants (educational staff)

Study ID Country	Sample Size	Population	Index Test	Reference Standard	Outcomes
					SARS-CoV-2 risk factors, perceived risk of infection, and impact of the pandemic on quality of life SARS-CoV-2 risk factors, perceived risk of infection, and impact of the pandemic on quality of life