

## Antigen Detection Tests for SARS-CoV-2: a systematic review and meta-analysis on real world data

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**Abstract.** *Background and aim* Rapid antigen detection (RAD) tests on nasopharyngeal specimens have been recently made available for SARS-CoV-2 infections, and early studies suggested their potential utilization as rapid screening and diagnostic testing. The present systematic review and meta-analysis was aimed to assess available evidence and to explore the reliability of antigenic tests in the management of the SARS-CoV-2 pandemic. *Materials and Methods.* We reported our meta-analysis according to the PRISMA statement. We searched Pubmed, Embase, and pre-print archive medRxiv.org for eligible studies published up to November 5<sup>th</sup>, 2020. Raw data included true/false positive and negative tests, and the total number of tests. Sensitivity and specificity data were calculated for every study, and then pooled in a random-effects model. Heterogeneity was assessed using the I<sup>2</sup> measure. Reporting bias was assessed by means of funnel plots and regression analysis. *Results.* Based on 25 studies, we computed a pooled sensitivity of 72.8% (95%CI 62.4–81.3), a specificity of 99.4% (95%CI 99.0–99.7), with high heterogeneity and risk of reporting bias. More precisely, RAD tests exhibited higher sensitivity on samples with high viral load (i.e. <25 Cycle Threshold; 97.6%; 95%CI 94.1–99.0), compared to those with low viral load (≥25 Cycle Threshold; 43.6%; 95% 27.6–61.1). *Discussion.* As the majority of collected reports were either cohort or case-control studies, deprived of preventive power analysis and often oversampling positive tests, overall performances may have been overestimated. Therefore, the massive referral to antigenic tests in place of RT-qPCR is currently questionable, and also their deployment as mass screening test may lead to intolerable share of missing diagnoses. On the other hand, RAD tests may find a significant role in primary care and in front-line settings (e.g. Emergency Departments). ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** COVID-19; SARS-CoV-2; point-of-care diagnostics; rapid testing; real-world data; systematic review and meta-analysis

## Introduction

The “*Severe Acute Respiratory Syndrome coronavirus type 2*” (SARS-CoV-2) is an enveloped, single-stranded, positive-sense RNA beta-coronavirus that was discovered in late December 2019. By November 2020, it caused a global pandemic with over 53 million confirmed cases, and almost 1.2 million deaths worldwide (<https://covid19.who.int>): the managing of the clinical syndrome elicited by SARS-CoV-2 infection (coronavirus disease 19 or COVID-19) has rapidly become a main stressor for all healthcare systems around the world (1–3).

Timely and accurate SARS-CoV-2 laboratory testing is an essential step in the management of the pandemic [4–8]. To date, both the “European Centre for Disease Prevention and Control” (ECDC) and the “World Health Organization” (WHO) have recommended the use of an assay based on the reverse-transcription polymerase chain reaction (RT-qPCR) in respiratory samples as gold standard for the COVID-19 diagnosis (4,5,7,9,10). Unfortunately, RT-qPCR is usually based on specimens collected through the use of nasopharyngeal swabs, which is affected by several practical limitations. First at all, it is a relatively invasive sampling technique, that requires specialized operators, and may elicit cough and bleeding, with an increasing risk of healthcare workers infection (11). Moreover, RT-qPCR is a time- and reagent-consuming procedure, needing specialized lab operators and certified laboratories; such requirements have limited the testing capacity of laboratories to provide results in a timely manner (10). Such limitations have elicited interest in alternative methodologies, including rapid antigen detection (RAD) tests. RAD tests, including immunochromatographic tests (ICT), chemiluminescence enzyme assays (CIA), and fluorescent immunoassays (FIA), detect viral antigen by the immobilized coated SARS-CoV-2 antibody on the device. By design, results of RAD tests are generally available within 30 minutes, and can be usually interpreted without specialized instruments. Therefore, appropriate use of RAD tests can relieve the workload in diagnostic hospitals and laboratories, ultimately improving the turnaround time. However, according to WHO, RAD tests for SARS-CoV-2 detection still need to be evalu-

ated in the clinical practice, and they are not extensively recommended for clinical diagnosis (12).

Despite several stakeholders are urging for their diffuse deployment, performance characteristics of rapid tests for SARS-CoV-2 remain largely uncertain (12,13). As some RAD have been licensed in both EU and USA for emergency use, early results are surfacing (14), being sometimes conflicting, suggesting the need for an updated synthesis of the literature to better inform health policies and guidelines. Reliance on modern diagnostic options made available by the scientific research should be supported by a synthesis of empirical evidence in which the heterogeneity of the different studies shows their efficacy. Therefore, the present systematic review and meta-analysis was undertaken to explore the deficiencies and contradictions found in the literature by analyzing the empirical evidence available (15), emphasizing the actual reliability of RAD tests for SARS-CoV-2 in the management of SARS-CoV-2 pandemic.

## Materials and Methods

This systematic review and meta-analysis of the literature has been conducted following the “*Preferred Reporting Items for Systematic Reviews and Meta-Analysis*” (PRISMA) guidelines (15). We searched different scholarly databases (namely, PubMed/MEDLINE and EMBASE) as well as pre-print servers including medrxiv.org for relevant studies from inception up to November 5<sup>th</sup>, 2020, without applying any backward chronological restrictions. The search strategy was a combination of the following keywords (free text and Medical Subject Heading [MeSH] terms, where appropriate): antigen\* AND (COVID-19 OR SARS-CoV-2 OR Coronavirus) AND (diagnostic OR diagnostic test OR screening).

Records were handled using references management software (Mendeley Desktop Version 1.19.5, Mendeley Ltd, London, UK, 2019), and duplicates were removed. Articles eligible for review were original research publications available online or through inter-library loan. A language filter was applied, by retaining articles written in Italian, English, German, French or Spanish, the languages spoken by the investigators.

Only articles (a) dealing with COVID-19 cases diagnosed by means of conventional RT-qPCR tests on nasopharyngeal swabs (5); (b) based on commercially available tests; and (c) reporting the raw number of true positive/true negative, and false positive/false negative results were eligible for the full review. Two independent reviewers (M.V. and S.R.) reviewed titles, abstracts, and the full text of articles. Titles were screened for relevance with respect to the subject under study.

Any article reporting original results, which met one or more of the inclusion criteria, was retained for the full-text review. The investigators independently read full-text versions of eligible articles. Disagreements were resolved by consensus between the two reviewers; when it was not possible to reach consensus, input from a third investigator (M.R.) was searched and obtained. Further studies were retrieved from reference lists of relevant articles and consultations with experts in the field. Data extracted included:

- 1) Settings of the study;
- 2) Characteristics of reference cases;
- 3) Characteristics of the commercially available point-of-care test employed (i.e. manufacturer; diagnostic kit; sampling technique; being a FIA, ICT or CIA test).
- 4) Total number of true positive, true negative, false positive, false negative cases.

We first performed a descriptive analysis to report the characteristics of the included studies. Each study was assessed by means of sensitivity (Se), specificity (Sp), positive and negative likelihood ratio (PLR and NLR), diagnostic odds ratio (DOR), accuracy and Cohen's "kappa". Sensitivity can be defined as the proportion of positive cases among people with a given disease, and specificity as the proportion of negative cases among people without that disease.

The likelihood ratios express the likelihood that a given test result would be expected in a patient with a certain disorder compared to the likelihood of that same result among subjects without that disorder; PLR was calculated by dividing the pooled Se by  $(1 - Sp)$ ; NLR was calculated by dividing  $(1 - Se)$  by Sp. The DOR expresses how much greater the odds of having the disorder are for the people with a positive test result than for the people with a negative test result. It

is a single measure of diagnostic test performance that combines both likelihood ratios by dividing PLR by NLR. The accuracy of any test is measured by comparing the results from a diagnostic test (positive or negative) with the true disease using a gold standard (presence or absence). Cohen's kappa coefficient is a statistical measure that is used to quantitatively assess inter-rater reliability (and also intra-rater reliability) for qualitative (i.e., categorical) items. In other words, it measures the agreement between two raters who each classify N items into C mutually exclusive categories. Cohen's kappa values  $< 0.600$  suggest "weak" to "none" agreement, while values 0.600 to 0.799 suggest a "moderate" agreement, 0.800 to 0.900 a "strong" agreement, and  $> 0.900$  an "almost perfect" agreement.

Pooled Se, Sp, PLR, NLR, accuracy, Cohen's kappa and DOR were calculated by combining each study's results, by means of a random-effects model. For DOR, a correction factor of one half was added to each cell to avoid calculation problems by having a value of zero in the  $2 \times 2$  table.

Potential heterogeneity for threshold effect (i.e., differences in Se and Sp occurring because of different cut-offs used in different diagnostic kits) was assessed by reporting accuracy estimates from each study in a summary receiver operating characteristic (sROC) space and subsequent visual inspection of the resulting curves: i.e., a substantial difference in curves suggested a possible heterogeneity for threshold effect, while the overlap of curves was reported as a sign of its absence (16).

$I^2$  statistics were then calculated to quantify the amount of inconsistency between included studies; it estimates the percentage of total variation across studies that is due to heterogeneity rather than to chance.  $I^2$  values ranging from 0% to 25% were considered to represent low heterogeneity, from 26% to 50% moderate heterogeneity and above 50% substantial heterogeneity, being pooled using a fixed-effects model because of the reduced number of samples eventually included.

To investigate publication bias, contour-enhanced funnel plots were initially generated: publication bias was evaluated by testing the null hypothesis that publication bias does not exist by means of the regression test for funnel plot asymmetry. The null hypothesis was rejected if the  $p$ -value was less than 0.10.

All calculations were performed in R (version 3.6.1; R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>) and RStudio (version 1.2.5019, RStudio PBC, 250 Northern Ave,

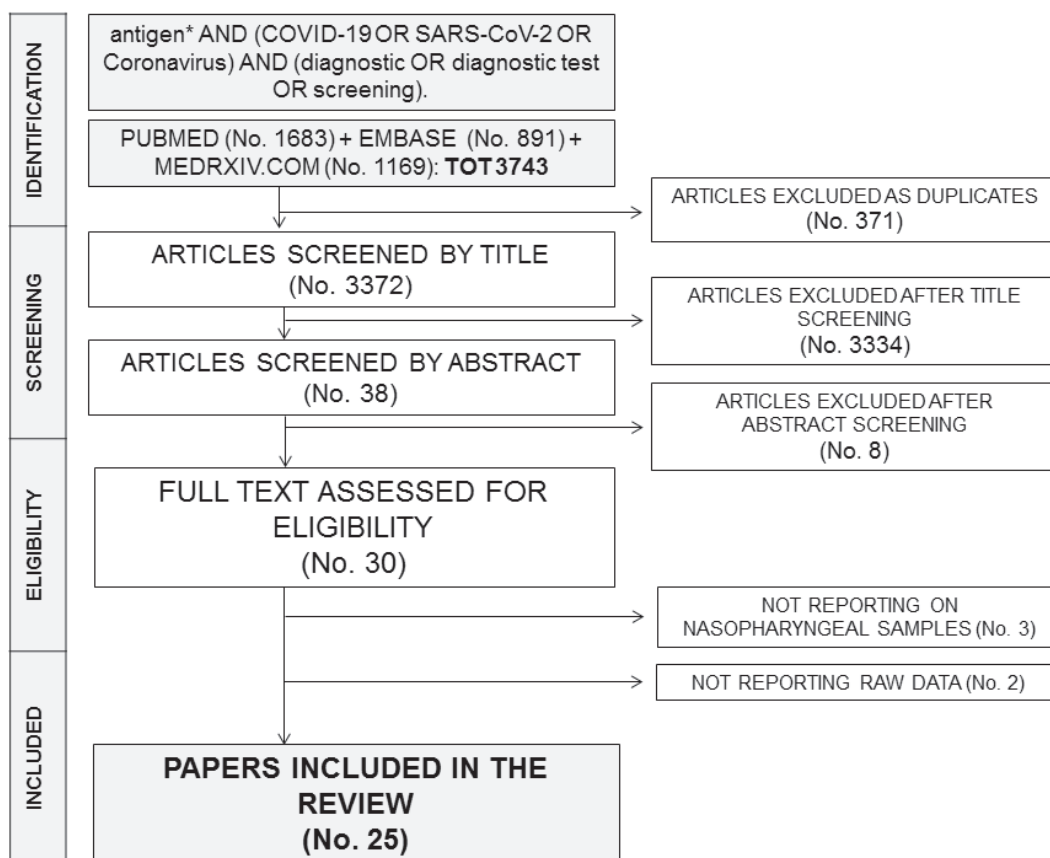
Boston, MA, USA) software by means of *meta* (version 4.9-9), *mada* (version 0.5-9), and *nsROC* (version 1.1) packages. All packages are open-source add-ons for conducting meta-analyses.

## Results

The initial literature search yielded a pool of 3749 entries. After removing duplicates ( $n = 371$ ), 3372 articles were evaluated by title and abstract. After title screening, 3334 articles were removed, and 38 were subsequently screened by abstract. Eight articles were

excluded based on abstract screening and, finally, 30 articles were assessed for eligibility, reviewed by full-text. As one article was performed on salivary fluids, while two further reports dealt on respiratory sample, and two more articles lacked raw number of reported cases, that were otherwise not reverse-calculable, those 5 articles were ultimately excluded. Twenty-five studies were therefore eventually retrieved (14,17-33). As three studies reported data on multiple diagnostic kits (23,27,33), the present systematic review and meta-analysis included a total of 29 estimates. The process of studies retrieval and inclusion is pictorially shown in Figure 1.

The main characteristics of the studies included in the present systematic review and meta-analysis are reported in Table 1. Briefly, 13 of the included reports were preprints (17,20,22-25,27,29,32-37), while remaining 12 studies had received a full peer-review process (14,18,19,21,26,28,30,31,38-41).



**Figure 1.** The process of studies retrieval and inclusion adopted in the present systematic review and meta-analysis. A total of 25 studies with 29 estimates were retrieved.

**Table 1.** Summary of studies included in the meta-analysis

Reference	Commercial test	Characteristics of the study	No. Of samples	TP	FP	FN	TN	Se.	Sp.	PPV	PNV	Accuracy	Cohen's Kappa
1) Albert et al. (22)	Abbot Diagnostics COVID-19 ICT Ag Rapid Test device	Prospective study, 412 patients (327 adults; 85 pediatrics) with clinical suspicion of COVID-19 in a primary care centre.	412	43	0	11	358	79.6%	100%	100%	97.0%	97.3%	0.872
2) Alemany et al. (35)	Abbot Diagnostics Panbio COVID-19 ICT Ag Rapid Test device	Retrospective study including a total of 1406 frozen samples (swabs) with a RT-qPCR result available (951 positive and 455 negative studies).	1406	872	5	79	450	91.7%	98.9%	99.4%	85.1%	94.0%	0.896
3) Blairon et al. (40)	Coris Bioconcept COVID-19 Ag Respi-Strip	Prospective study including a total of 774 either COVID-19 positive or negative cases from an original sample of 912.	774	60	0	159	555	27.4%	100%	100%	77.7%	79.5%	0.351
4) Cerutti et al. (21)	SD Biosensor STANDARD F COVID-19 Ag FIA	Prospective study including 330 patients: 185 randomly selected patients from the Emergency Department of Turin and Genoa, and 145 travelers returning from high risk countries	330	77	0	32	221	70.6%	100%	100%	87.4%	90.3%	0.591
5) Courtellemont et al. (36)	AAZ COVID-VIRO	Prospective study including 226 subjects voluntarily accessing to the COVID-19 Screening department and SARS-CoV-2 positive patients from two specialized hospitals in Paris and Orleans	226	113	0	4	109	96.6%	100%	100%	96.5%	98.2%	0.965

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6) Fenollar et al. (41)	Abbot Diagnostics Panbio COVID-19 ICT Ag Rapid Test device	Prospective study including a total of 341 consecutive patients from a COVID-19 testing Center in Marseilles	341	154	7	50	130	75.5%	94.9%	95.7%	72.2%	83.3%	0.669
7) Gremmels et al. (23)	Abbot Diagnostics Panbio COVID-19 ICT Ag Rapid Test device	Prospective study including all individuals (No. 1367; 139 Positive cases) visiting COVID-19 community testing centers, located at the University Medical Center Utrecht (UMCU),	1367	101	0	38	1228	72.7%	100%	100%	97.0%	97.2%	0.827
8) Gupta et al. (38)	SD Biosensor STANDARD Q COVID-19 Ag FIA	Prospective study; single-blinded, cross sectional, single-center study including all consecutive patients either symptomatic for COVID-19 or asymptomatic / presymptomatic contacts of laboratory confirmed COVID-19 cases	330	63	1	15	252	81.8%	99.6%	98.4%	94.7%	95.5%	0.865
9) Herrera et al. (17)	Quidel Corporation SOFIA SARS ANTIGEN FIA	Prospective study on all patients (No. 1172) presenting with COVID19 symptoms	1172	352	6	107	707	76.7%	99.2%	98.3%	86.9%	90.4%	0.789

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10) Hirotsu et al. (26)	Fuji Rebio inc Espline	CIA	313	32	1	26	254	55.2%	99.6%	97.0%	90.7%	91.4%	0.657
	Biocasy Biotechnology Co. Bioeasy 2019–Novel Coronavirus (2019– nCoV) Fluorescence Antigen Rapid Test Kit	FIA	727	10	5	49	663	16.9%	99.3%	66.7%	93.1%	92.6%	0.245
11) Krüger et al. (27)	Coris Bioconcept COVID-19 Ag Respi-Strip	ICT	417	4	4	17	392	19.0%	99.0%	50.0%	95.8%	95.0%	0.255
12) Lambert-Nielot et al. (28)	SD Biosensor STANDARD F COVID-19 Ag FIA	FIA	1263	36	11	9	1207	80.0%	99.1%	76.6%	99.3%	98.4%	0.774
12) Lambert-Nielot et al. (28)	Coris Bioconcept COVID-19 Ag Respi-Strip	ICT	138	47	0	47	44	50.0%	100%	100%	48.4%	65.9%	0.389
13) Linares et al. [29]	Abbot Diagnostics Panbio COVID-19 Ag Rapid Test device	ICT	316	68	0	16	232	81.0%	100%	100%	93.6%	94.9%	0.862

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Reference	Commercial test	Characteristics of the study	No. Of samples	TP	FP	FN	TN	Se.	Sp.	PPV	PNV	Accuracy	Cohen's Kappa
14) Lindner et al. (25)	SD Biosensor STANDARD F COVID-19 Ag FIA	Prospective study on 289 adults at high risk for SARS-CoV-2 infection according to clinical suspicion, 39 of them positive for SARS-COV-2	289	29	2	10	248	74.4%	99.2%	93.6%	96.1%	95.8%	0.805
15) Liotti et al. (30)	SD Biosensor STANDARD F COVID-19 Ag FIA	Retrospective study on 359 samples previously sampled and tested	359	49	4	55	251	47.1%	98.4%	92.5%	82.0%	83.6%	0.533
16) Mertens et al. (31)	Coris Bioconcept COVID-19 Ag ICT Respi-Strip	Retrospective study on specimens from 328 suspected cases	328	76	1	56	195	57.6%	99.5%	98.7%	77.7%	82.6%	0.612
17) Nalumansi et al. (39)	SD Biosensor STANDARD F COVID-19 Ag FIA	Prospective, cross-section, un-blinded study including 90 COVID-19 cases and 172 controls from regional referral hospitals in Uganda	267	68	13	27	159	71.6%	92.4%	84.0%	85.5%	85.0%	0.662
18) Pekosz et al. (32)	BD Life Sciences Veritor Plus System	Retrospective study on 38 RT-PCR positive samples and 176 RT-PCR negative samples from a total of 251 samples	251	27	3	1	220	96.4%	98.7%	90.0%	99.6%	98.4%	0.922
19) Pilarowski et al. (37)	Abbot Diagnostics BinaxNow COVID-19 Ag Rapid Test device	Prospective study including a total 878 volunteers (either symptomatic or not) who presented at a public testing site at community level; 214 randomly selected samples.	214	5	9	2	198	71.4%	95.7%	35.7%	99.0%	94.9%	0.452



Table 1. Summary of studies included in the meta-analysis

Reference	Commercial test	Characteristics of the study	No. Of samples	TP	FP	FN	TN	Se.	Sp.	PPV	PNV	Accuracy	Cohen's Kappa
20) Porte et al. (18)	Biocasy Biotechnology Co. Bioeasy 2019–Novel Coronavirus (2019– nCoV) Fluorescence Antigen Rapid Test Kit	Retrospective study including a total of 127 samples (randomly selected) from a total of 1453 respiratory samples	127	77	0	5	45	93.9%	100%	100%	90.0%	96.1%	0.916
21) Porte et al. (33)	SD Biosensor STANDARD F COVID-19 Ag FIA Quidel Corporation SOFIA SARS ANTIGEN FIA	Retrospective study on 32 RT-PCR positive samples and 32 RT-PCR negative samples, randomly selected from an undisclosed number of clinical specimens	64	29	1	3	31	90.6%	96.9%	96.7%	91.2%	93.8%	0.875
22) Scohy et al. (14)	Coris Bioconcept COVID-19 Ag Respi-Strip	Prospective random sample (No. 148) from all patients admitted to the parent hospital during April, 2020	148	32	0	74	42	30.2%	100%	100%	36.2%	50.0%	0.197
23) Van der Moeren et al. (24)	BD Life Sciences Veritor Plus System	Prospective study including a total of 354 adults individuals (age $\geq$ 18 years) presenting at the Breda test centre	351	16	2	1	332	94.1%	99.4%	88.9%	99.7%	99.1%	0.910
24) Veyrenche et al. (20)	Coris Bioconcept Veritor Plus System	Retrospective study on 45 patients with RT-PCR confirmed COVID-19 and 20 negative controls	65	13	0	32	20	28.9%	100%	100%	38.5%	50.8%	0.200
25) Young et al. (19)	BD Life Sciences Veritor Plus System	Prospective study including 251 specimens from 260 participants from various geographic areas	251	29	1	9	212	76.3%	99.5%	96.7%	95.9%	96.0%	0.830

(Note: CIA = chemiluminescence enzyme assay; FIA = fluorescence immunoassay; ICT = immunochromatographic tests; TP = No. of True Positive Cases; FP = No. of False Positive Cases; FN = No. of False Negative Cases; TP = No. of True Positive Cases; Se. = sensitivity; Sp. = specificity; PPV = predicted positive value; PNV = predicted negative value; Cohen's Kappa values should be interpreted as follows: 0.0–0.20 no agreement, 0.21–0.39 minimal agreement, 0.40–0.59 weak agreement, 0.60–0.79 moderate agreement, 0.80–0.90 strong agreement, >0.90 almost perfect agreement).

Overall, our meta-analysis included a total of 12518 samples: the size of every single estimate ranged from 64 to 1406, and only 3 studies had performed a preventive power analysis (27,35,36). Of the retrieved samples, 3510 were SARS-CoV-2 positive as confirmed by RT-qPCR (28.0%), with a positive rate ranging between 1.9% and 60.6%.

The retrieved estimates reported on a total of 9 different commercially available tests (1 of them was commercialized with two different brand names in North America and Western Europe), all of them targeting the nucleocapsid (N) protein [42], with 18 estimates on ICT (60.3% of the pooled sample) (14,19,20,22–24,27–29,31,32,35,36,38,40,41), 10 on FIA (37.2%) (17,18,21,25,27,30,33,39), while only one report dealt on CIA (2.5%) (26).

A summary of the diagnostic performances of the RAD tests that were included in this systematic review is reported in Table 2.

More precisely, the specificity of reported studies ranged between 94.9% to 100%, with a pooled estimate of 99.4% (95%CI 99.0–99.7). As shown in Figure 2, ICT tests exhibited a pooled specificity of 99.7% (95%CI 99.1 – 99.9), that was slightly but significantly greater than that reported for FIA i.e. (98.9% 95%CI 97.7 – 99.4; chi squared test  $p$  value 0.006). The only CIA based study we were able to retrieve scored a specificity of 99.6% (95%CI 97.8–100). The heterogeneity was substantial ( $I^2$  81.0%,  $p < 0.001$ ), for both ICT ( $I^2$  83%) and FIA based studies ( $I^2$  74%).

As shown in Figure 3, the value of sensitivity ranged from 16.9% to 96.4%, with a pooled sensitiv-

ity of 72.8% (95%CI 62.4–81.3), that was not significantly greater in FIA (75.3%; 95%CI 58.4–86.9) than in ICT based studies (72.3%; 95%CI 58.0–83.2;  $p = 0.150$ ), while the study on CIA scored a sensitivity of 55.2% (95%CI 41.5–68.3%). As observed for specificity, heterogeneity was substantial ( $I^2$  96%), for both ICT ( $I^2$  97%) and FIA based studies ( $I^2$  96%).

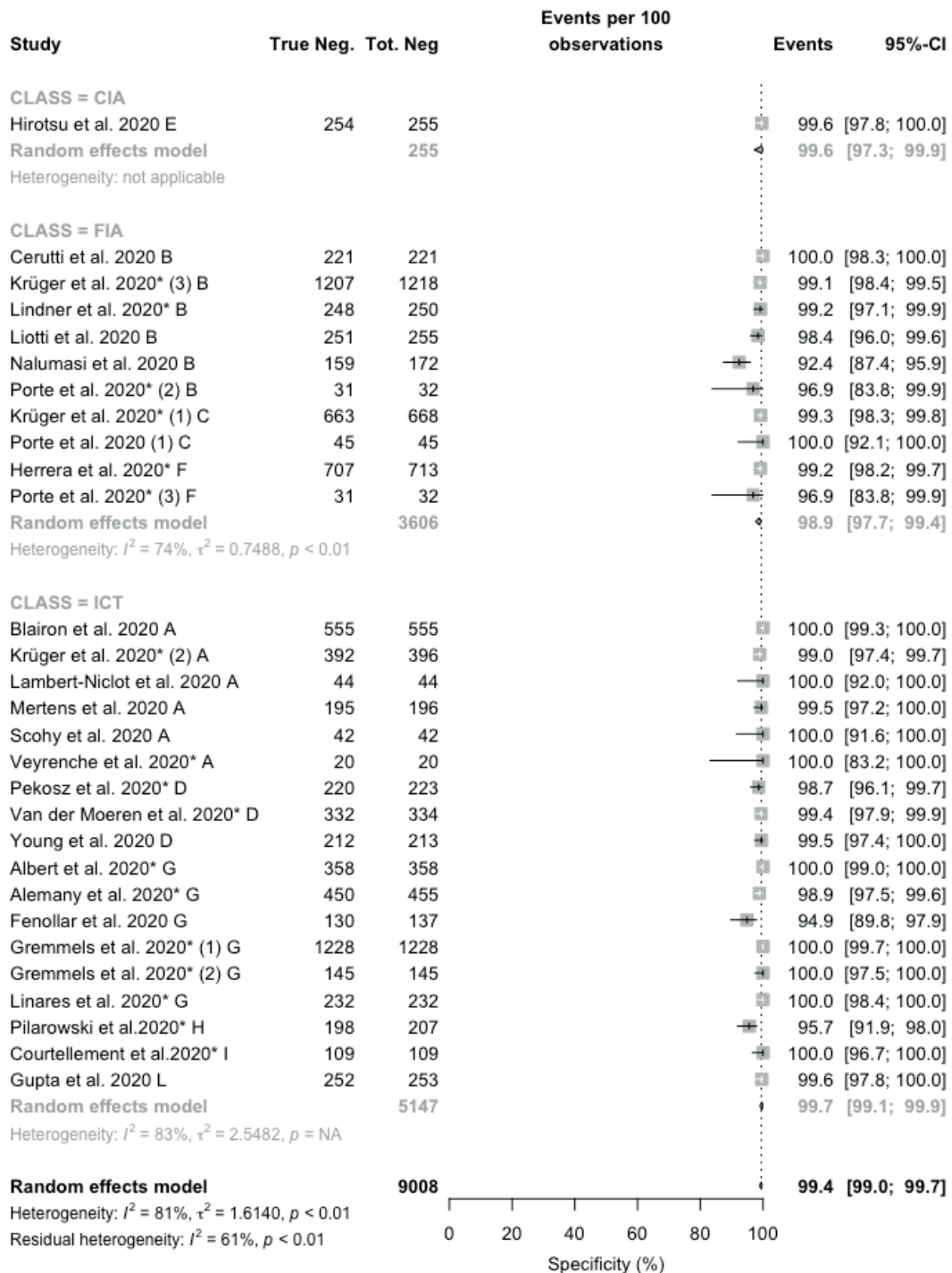
Assuming a cut-off value of 25 cycle threshold (Ct; i.e.  $1.8 \times 10^5$  viral copies/mL), and dichotomizing samples in those characterized by high (i.e.  $Ct < 25$ ) and low (i.e.  $Ct \geq 25$ ) viral load, a pooled sensitivity of 97.6% (95%CI 94.1–99.0), and 43.6% (95% 27.6–61.1) was identified, respectively (Figure 4).

In other words, RAD tests exhibited a considerably higher sensitivity in samples characterized by a higher viral load compared to samples with low viral load. Interestingly, performances of FIA and ICT tests were similar both with higher viral load (pooled sensitivity of 98.3%, 95%CI 95.0–99.5 vs. 96.6%, 95%CI 90.2–98.8, respectively  $p = 0.106$ ), while for samples having a  $Ct \geq 25$  the pooled sensitivity of ICT tests (44.7%, 95% 22.8–68.9) significantly outperformed that of FIA tests (41.0%; 95%CI 21.0–64.5;  $p < 0.001$ ). Residual heterogeneity was again substantial, for both sub-analyses (i.e.  $I^2$  89% for samples with high viral load vs.  $I^2$  77% in samples with low viral load).

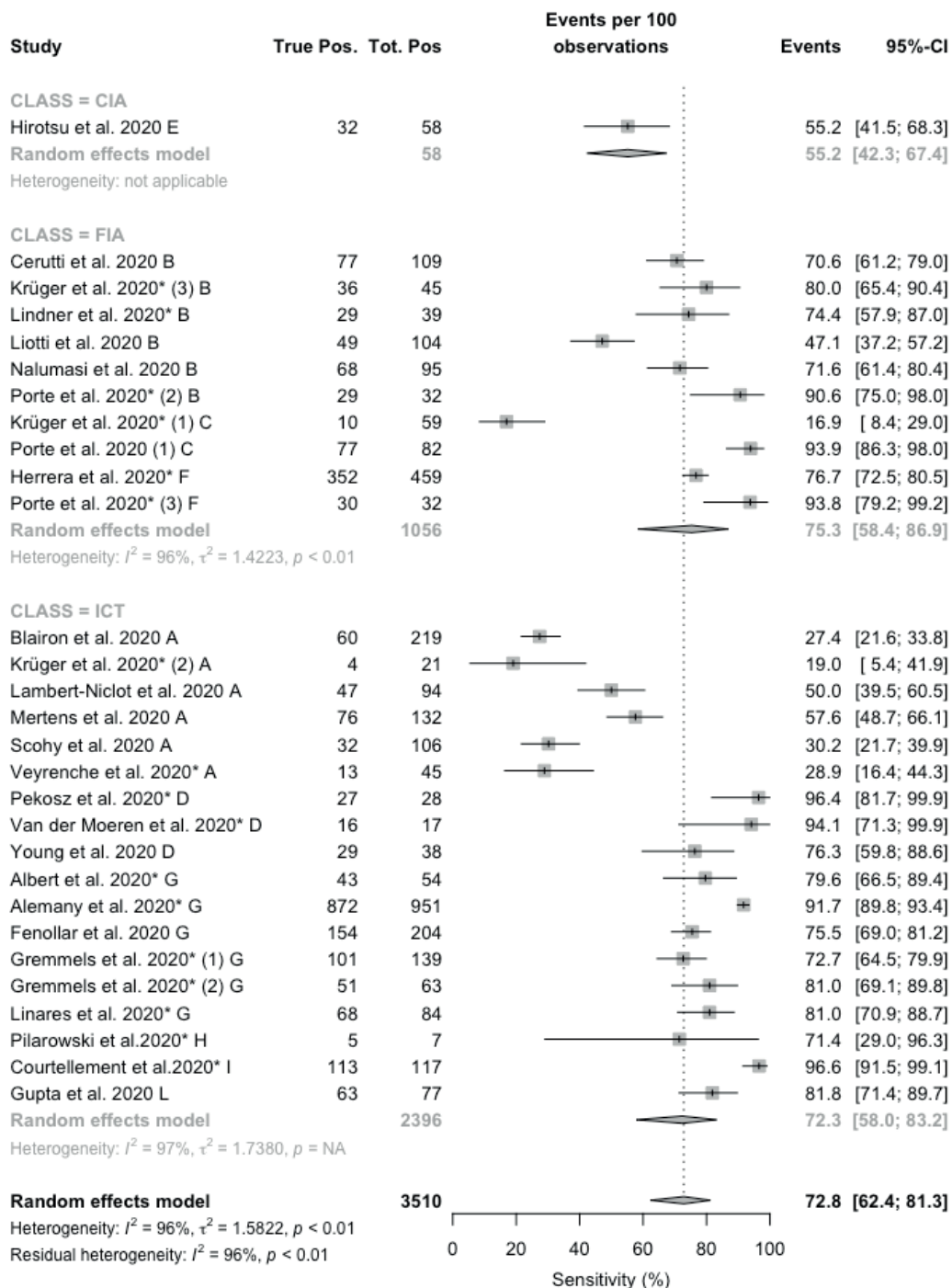
Visual inspection of contour-enhanced funnel plots (Figure 5), suggested a seemingly considerable asymmetry for all analyses, with subsequent reporting bias. However, regression analysis confirmed a significant reporting bias for overall analysis of specificity ( $t = 3.504$ ,  $df = 27$ ,  $p$ -value = 0.002), while it was sub-

**Table 2.** Summary of the diagnostic performances of the RAD tests included in the systematic review (note: DOR = diagnostic odds ratio; 95%CI = 95% confidence intervals; NLR = negative likelihood ratio; PLR = positive likelihood ratio; Se = sensitivity; Sp = specificity; Ct = cycle threshold)

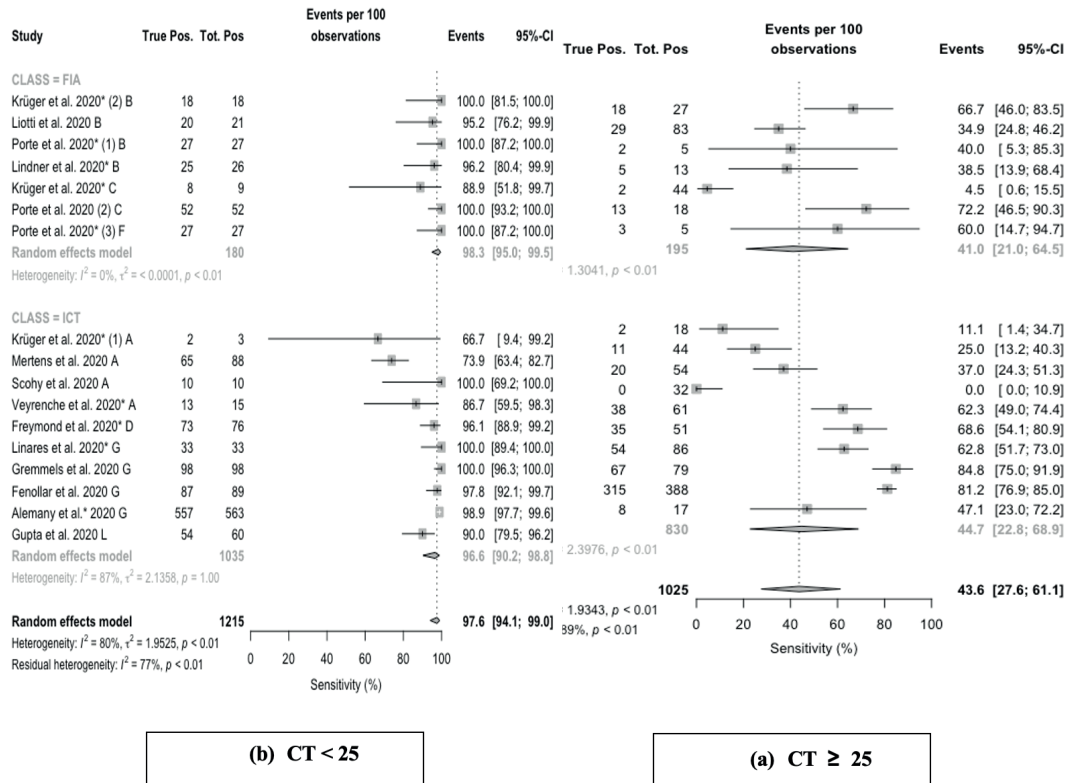
Variable	Estimate	95%CI	Tau <sup>2</sup>	P value	Q(df)	I <sup>2</sup>
<b>Se</b>	72.8	62.4; 81.3	1.582	< 0.001	831.62 (28)	96.5
< 25 Ct	97.6	94.1; 99.0	1.953	< 0.001	102.07 (16)	80.0
≥ 25 Ct	43.6	27.6; 61.1	1.934	< 0.001	306.61 (16)	94.8
<b>Sp</b>	99.4	99.0; 99.7	1.614	< 0.001	121.87 (28)	81.0
<b>Kappa</b>	0.684	0.635; 0.733	0.018	< 0.001	809931.373 (28)	99.9
<b>Accuracy</b>	0.890	0.873; 0.907	0.002	< 0.001	100310.901 (28)	99.9
<b>DOR</b>	288.394	153.206; 542.870	1.941	< 0.001	118.145 (28)	76.3
<b>NLR</b>	0.230	0.169; 0.315	0.190	0.051	41.283 (28)	32.2
<b>PLR</b>	61.687	38.586; 98.620	0.899	< 0.001	90.910 (28)	69.2



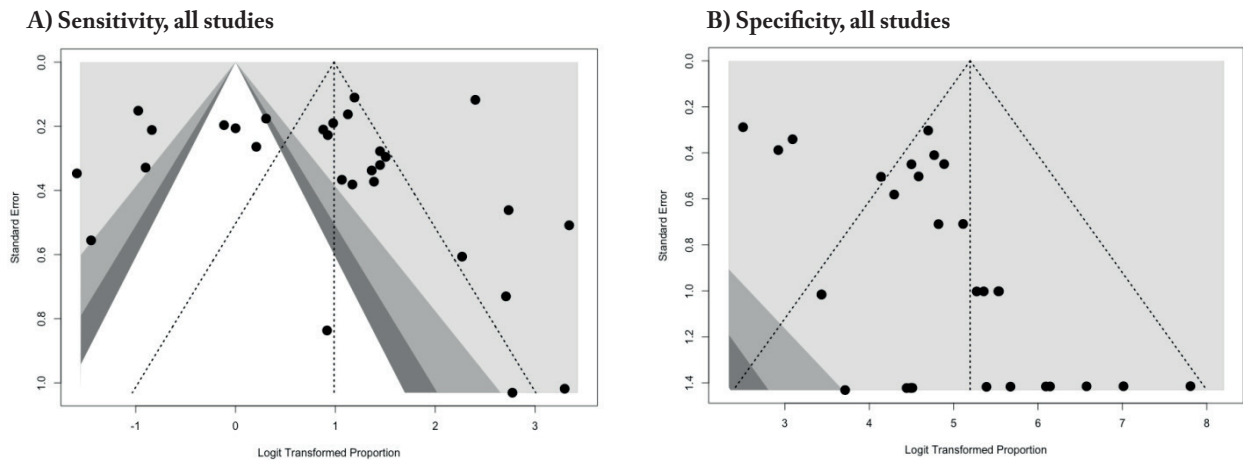
**Figure 2.** Forest plot representing the estimated specificity of SARS-CoV-2 antigen-based studies. Pooled specificity was 99.4% (95%CI 99.0–99.7), with considerable heterogeneity ( $I^2$  81%), and a significant difference between FIA (98.9%, 95%CI 97.7 – 99.4) and ICT based estimates (99.7%, 95%CI 99.1 – 99.9; chi squared test p value < 0.001). Note: A = Coris Bioconcept - COVID-19 Ag Respi-Strip; B = SD Biosensor - STANDARD F COVID-19 Ag FIA; C = Bioeasy Biotechnology Co. - Bioeasy 2019-Novel Coronavirus (2019-nCoV) Fluorescence Antigen Rapid Test Kit; D = BD Life Sciences - Veritor Plus System; E = Fujirebio Inc - Lumipulse G; F = Quidel Corporation - SOFIA SARS ANTIGEN FIA; G = Abbot Diagnostics - Panbio COVID-19 Ag Rapid Test device; H = Abbot Diagnostics - BinaxNow COVID-19 Ag Card; I = AAZ - COVID-Viro; L = SD Biosensor - STANDARD Q COVID-19 Ag.



**Figure 3.** Forest plot representing the estimated sensitivity of SARS-CoV-2 antigen-based studies. Pooled sensitivity was 72.8% (95%CI 62.4–81.3), being not significantly greater in FIA based studies (i.e. 75.3%, 95%CI 58.4–86.9) than in ICT based studies (72.3%; 95%CI 58.6–83.2). Heterogeneity was substantial ( $I^2$ , 97%), for both groups (i.e.  $I^2$ , 95% in ICT studies,  $I^2 = 96\%$  in FIA studies). Note: A = Coris Bioconcept - COVID-19 Ag Respi-Strip; B = SD Biosensor - STANDARD F COVID-19 Ag FIA; C = Bioeasy Biotechnology Co. - Bioeasy 2019-Novel Coronavirus (2019-nCoV) Fluorescence Antigen Rapid Test Kit; D = BD Life Sciences – Veritor Plus System; E = Fujirebio Inc – Lumipulse G; F = Quidel Corporation – SOFIA SARS ANTIGEN FIA; G = Abbot Diagnostics - Panbio COVID-19 Ag Rapid Test device; H = Abbot Diagnostics - BinaxNow COVID-19 Ag Card; I = AAZ – COVID-Viro; L = SD Biosensor - STANDARD Q COVID-19 Ag.

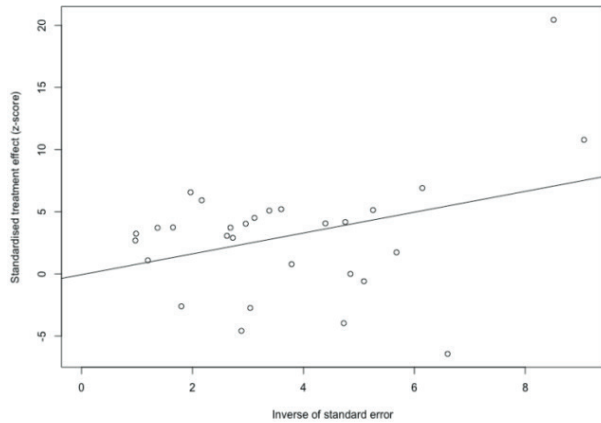


**Figure 4.** Forest plot representing the estimated sensitivity of SARS-CoV-2 antigen-based studies by cycle threshold value, i.e. CT < 25 (left) vs. CT ≥ 25 (right). Pooled sensitivity was 97.6% (95%CI 94.1–99.0) for studies with higher viral load (CT < 25), compared to 43.6% (95% 27.6–61.1) in studies with lower viral load (CT ≥ 25). Heterogeneity was substantial, for both sub-analyses (i.e. I2 80% for samples with high viral load vs. I2 95% in samples with low viral load). Note: Note: A = Coris Bioconcept - COVID-19 Ag Respi-Strip; B = SD Biosensor - STANDARD F COVID-19 Ag FIA; C = Bioeasy Biotechnology Co. - Bioeasy 2019-Novel Coronavirus (2019-nCoV) Fluorescence Antigen Rapid Test Kit; D = BD Life Sciences – Veritor Plus System; F = Quidel Corporation – SOFIA SARS ANTIGEN FIA; G = Abbot Diagnostics - Panbio COVID-19 Ag Rapid Test device; L = SD Biosensor - STANDARD Q COVID-19 Ag.

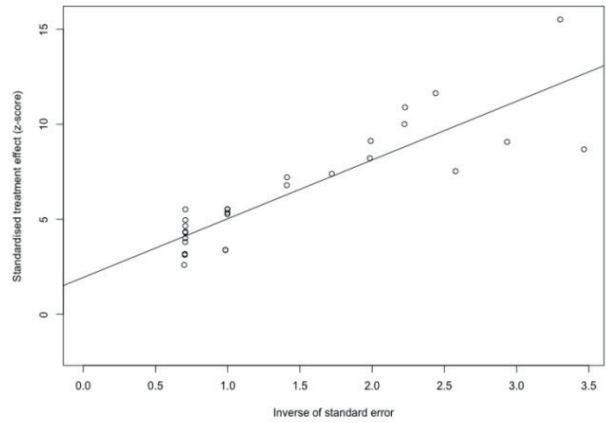


**Figure 5.** Funnel plots for Sensitivity (a), and Specificity (b) of studies included in the meta-analysis. Visual inspection suggested a significant asymmetry for all analyses, with subsequent reporting bias. However, regression analysis dismissed a significant reporting bias for overall analysis of sensitivity ( $t = -0.0329$ ,  $df = 27$ ,  $p\text{-value} = 0.974$ ), but not for specificity ( $t = 3.504$ ,  $df = 27$ ,  $p\text{-value} = 0.002$ ).

A) Sensitivity, all studies



B) Specificity, all studies



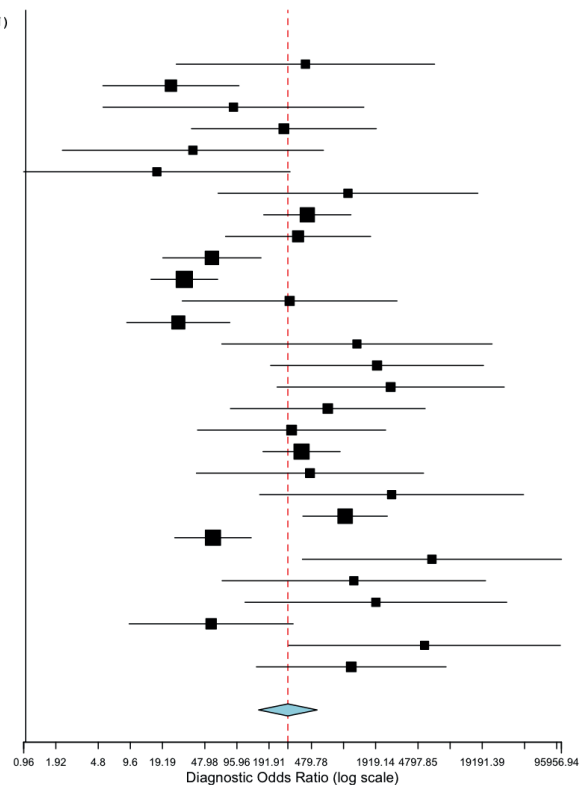
**Figure 6.** Radial plots for Sensitivity (a), and Specificity (b) of studies included in the metaanalysis. Visual inspection suggested the seemingly random distribution of included studies on both sides of the regression line for sensitivity, but not for specificity.

stantially dismissed for sensitivity ( $t = -0.0329$ ,  $df = 27$ ,  $p$ -value = 0.974).

Not coincidentally, corresponding radial plot for sensitivity was characterized by a seemingly random distribu-

tion of the studies across the regression line for sensitivity, while the radial plot for specificity was characterized by a non-random scattering of studies, suggesting a possible small-study effect on the overall estimates (Figure 6).

Studies	Estimate (95% C.I.)	(TP * TN)/(FP * FN)
Blairon et al. 2020	421.414 (25.915, 6852.827)	33300/0
Kr.ger et al. * (2) 2020	23.059 (5.310, 100.138)	1568/68
Lambert-Niclot et al. 2020	89.000 (5.325, 1487.478)	2068/0
Mertens et al. 2020	264.643 (35.993, 1945.843)	14820/56
Scohy et al. 2020	37.081 (2.214, 621.066)	1344/0
Veyrenche et al. * 2020	17.031 (0.960, 302.268)	260/0
Cerutti et al. 2020	1056.385 (63.918, 17458.953)	17017/0
Kr.ger et al. * (3) 2020	438.909 (171.237, 1124.999)	43452/99
Lindner et al. * 2020	359.600 (75.102, 1721.831)	7192/20
Liotti et al. 2020	55.905 (19.366, 161.385)	12299/220
Nalumasi et al. 2020	30.803 (14.993, 63.285)	10812/351
Porte et al. * (2) 2020	299.667 (29.475, 3046.635)	899/3
Kr.ger et al. * (1) 2020	27.061 (8.900, 82.281)	6630/245
Porte et al. (1) 2020	1282.273 (69.289, 23730.069)	3465/0
Pekosz et al. * 2020	1980.000 (198.872, 19713.191)	5940/3
Van der Moeren et al. * 2020	2656.000 (228.666, 30849.992)	5312/2
Young et al. 2020	683.111 (83.475, 5590.191)	6148/9
Hirotsu et al. 2020	312.615 (41.022, 2382.319)	8128/26
Herrera et al. * 2020	387.639 (168.665, 890.902)	248864/642
Porte et al. * (3) 2020	465.000 (40.031, 5401.407)	930/2
Albert et al. * 2020	2712.130 (157.061, 46833.126)	15394/0
Alemay et al. * 2020	993.418 (399.488, 2470.356)	392400/395
Fenollar et al. 2020	57.200 (25.075, 130.482)	20020/350
Gremmels et al. * (1) 2020	6477.545 (395.051, 106210.608)	124028/0
Gremmels et al. * (2) 2020	1198.920 (69.733, 20613.155)	7395/0
Linares et al. * 2020	1930.455 (114.337, 32593.727)	15776/0
Pilarowski et al. * 2020	55.000 (9.362, 323.111)	990/18
Courtellement et al. * 2020	5523.667 (293.913, 103809.299)	12317/0
Gupta et al. 2020	1134.000 (146.358, 8786.402)	15876/14
<b>Overall (I<sup>2</sup>=7630 %, P&lt; 0.001)</b>	<b>288.394 (153.206, 542.870)</b>	<b>1034644/2523</b>



**Figure 7.** Forrest plot representing the pooled diagnostic odds ratio (DOR) of antigen-based tests for SARS-CoV-2. A substantial heterogeneity in reported studies was identified (I<sup>2</sup> 76.3%, Cochran's Q: 118.145 (df=28, p < 0.001)).

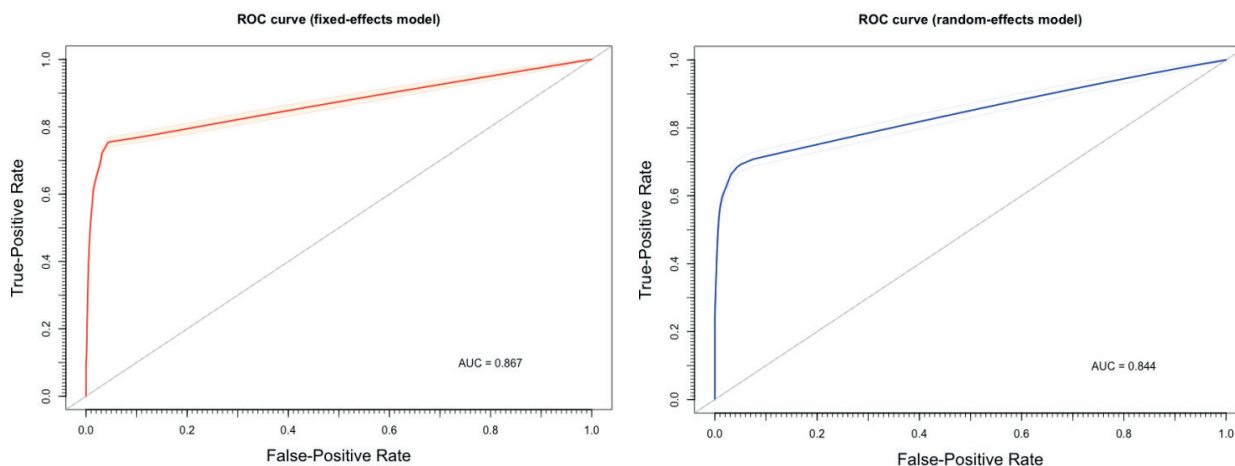
Effectiveness of the diagnostic tests was eventually summarized by calculation of PLR, NLR, DOR and Cohen's Kappa values. Pooled PLR estimate was 61.687 (95%CI 38.586–98.620), with a correspondent NLR of 0.230 (95%CI 0.169–0.315), i.e., while a positive test was associated with relatively strong evidence of infection, a negative one was associated with a reduced chance of being actually affected by SARS-CoV-2 infection. Correspondent pooled DOR (i.e. 288.394; 95%CI 153.206–542.870) from the retrieved studies is shown in Figure 7. Eventually, the pooled accuracy was 0.890 (95%CI 0.873–0.907) while Cohen's kappa was estimated in 0.684 (95%CI 0.629; 0.735). In other words, despite a substantial heterogeneity ( $I^2$  99.9% for both estimates), the agreement between RAD tests and RT-PCR may be assumed to be moderate.

In order to exclude that the differences in overall performance may have been associated with a different threshold level among studies, sROC of two distinctive models were determined, either using the overall DOR by combining each study's diagnostic odds ratio (random-effects model, AUC = 0.844), or a fixed (unweighted) method (AUC = 0.867). The small but noticeable difference between the symmetric and asymmetric sROC curves indicates that a threshold effect is actually present (Figure 8).

## Discussion

### Summary of Findings

In the current meta-analysis of 25 studies (29 estimates) on the diagnostic performances of RAD tests, we found a moderate agreement with RT-qPCR tests. Albeit quite specific (pooled estimates 99.4%; 95%CI 99.0–99.7), commercially available diagnostic kits were affected by relatively low sensitivity (i.e. 71.9%, 95%CI 59.8–81.5). In other words, available tests are at risk to incorrectly diagnose around 30% of all SARS-CoV-2 infection cases, being substantially inadequate for an extensive use as screening test. Actually, pooled performances were particularly poor for infections characterized by a very low content of viral copies in the collected specimens, which is often the case of asymptomatic patients suffering of COVID-19 (14,52,53), as substantially confirmed by the comparison of ROC curves. Moreover, estimates for specificity were affected by substantial heterogeneity, with a possible reporting bias (i.e. oversampling of studies characterized by “socially desirable” features), and a noticeable small study effect (i.e. smaller studies showing different performances than larger ones). In other terms, even though our data may hint that false positive to RAD tests are quite rare, the actual performance of antigenic tests in mass screening procedures could be significantly lower than expected.



**Figure 8.** Summary Receiver Operating Characteristics (sROC) curves for antigen tests for SARS-CoV-2 RNA. The small but noticeable differences between estimates from a random-effect model (AUC = 0.844) and a fixed-effect model (AUC = 0.867) are consistent with the hypothesis of a threshold effect in diagnostic performances of assessed tests.

### *Pros and Cons of RAD tests in SARS-CoV-2 management*

The ongoing SARS-CoV-2 pandemic, and particularly its resurgence during European Winter Season, has urged international health authorities to assess the extensive implementation of RAD tests in the diagnostic procedures for SARS-CoV-2 (13,31,41,43–45). Because of the increasing surge of intensive care during the later stages of COVID-19 syndrome, earlier diagnoses of SARS-CoV-2 infections would spare available medical resources for at-risk patients, improving their chances for an immediate, more intensive assistance, since the early stages of the clinical syndrome. RAD tests may fit very accurately such requirements, finding a number of potential locations and targets for on-site testing, including but not limitedly: Emergency Departments interacting with subjects affected with influenza-like illness in order to rapidly discern COVID-19 vs. non-COVID-19 cases; primary care professionals; nursing homes for staff, resident and visitor screening; occupational settings in order to allow safe return of key workers (45,49). On the other hand, as RAD tests are relatively inexpensive, being also quicker to perform and to analyze, as well as easier and somewhat less invasive than conventional molecular assays on nasal swabs. In other words, they may represent the highly demanded mass “screening test” for SARS-CoV-2 infection that will allow the detection of those paucisymptomatic / asymptomatic patients that, eluding tracing and tracking activities, and therefore avoiding the confinement measures, are critical in maintaining and increasing viral circulation at community level (13,31,41,43–45).

### *Usability of RAD tests in mass screening procedures*

It must be stressed that RAD tests are not a novelty in the diagnosis of respiratory diseases of viral etiology (46–48), and also their shortcomings have been diffusely acknowledged. More precisely, the analytical performances of RAD tests depend on factors such as the viral load; the quality of the specimen and how it is processed; the testing settings (14,45–48). Our results suggest that antigenic tests for SARS-CoV-2 share all such weaknesses, failing to achieve the high level of accuracy, in terms of both specificity and sensitivity

otherwise required to be considered a reliable screening tests (45,50,51).

The poor sensitivity we identified, particularly among cases with a  $Ct \geq 25$ , means that an extensive referral to RADs will miss a significant number of cases (50,51), i.e. persons not only potentially at risk to develop the dire consequences of the infection, but also still able to spread SARS-CoV-2 infection, eventually maintaining its endemic state. Furthermore, a large number of false negative results - true cases incorrectly told that they are free of disease - might lead a not negligible amount of people to indulge in at-risk habits and behaviors, contributing to a further spread of the infection at the community level. In other words, from an epidemiological point, RAD tests are not suitable for screening campaigns, unless we could rule out a significant role of asymptomatic patients in the spreading of the SARS-COV-2 virus, scenario which has already been excluded by scientific evidence (54,55). Conversely, it is possible to affirm that this unsatisfactory overall performance becomes somewhat satisfactory when limiting the use of RAD tests to high-viral load patients, which are generally those with a more symptomatic disease course (14,20,23,27,29,35,36,38,49,52–55). As a consequence, from a clinical point of view, RAD tests may be game-changing when reserved to the sole symptomatic patients, and particularly during the “exponential” stages of the epidemics (i.e. when the share of SARS-CoV-2 infected patients with higher viral load is reasonably high), in order to achieve a quick and reliable differential diagnosis with other respiratory infectious diseases. A focused referral to such instruments may therefore spare available diagnostic and clinical resources, eventually guaranteeing an early treatment start for positive cases, possibly at the home of the patient, avoiding delays which often result in hospitalization (31,43,45,49).

### *Reliability of the estimates*

Substantially unsatisfying as they are as screening test when employed in a general population having higher share of patients with low viral load, the diagnostic accuracy of RAD tests we examined may actually be a substantial overestimation of the real one (56,57).

Firstly, we assessed sensitivity and specificity of



RADs compared to RT-qPCR analysis on nasopharyngeal specimens. Despite their well acknowledged status, to date we are in the paradoxical situation where “gold standard” testing (i.e. nasopharyngeal swab with RT-qPCR processing) has a sensitivity which is far from satisfactory. In a recent study on 205 patients with confirmed SARS-CoV-2 infection, RT-qPCR positivity rate in nasal swabs accounted to only 63%, well below the 93% in bronchoalveolar lavage specimens, and 72% in sputum, with only a 32% positivity in pharyngeal swabs (58). As ACE2 receptor is reputed to be nearly ubiquitous in human body (59), such differences are reasonably due to an inappropriate timing of sample collection in relation to illness onset and/or last effective contact with an infected person, as well as to the proficiency in sampling technique (14,45–48,58). More precisely, not only oral and respiratory tissues seem to have a different conversion times (60–62), but secretory IgA, either specific for SARS-CoV-2 or maybe targeting other Coronaviruses, might also be able to interfere with the detection of viral antigens (63). Therefore, persistence of detectable viral components may be quite diachronous, with unclear clinical and epidemiological significance. As a consequence, on the one hand, the very same referral of RT-qPCR tests on nasal swabs may fail to recognize the whole of incident cases, particularly for very low viral loads, suggesting an even lower estimate for the “real-world” sensitivity of the assessed RADs. On the other hand, the heterogeneous representation of viral replication in respiratory and oral tissues may impair from their roots the comparisons of RADs with RT-qPCR tests on nasopharyngeal swabs, and particularly the eventual estimation of their actual accuracy (45,58,62).

Second, the large majority of reported studies were performed in clinical and/or laboratory settings, with intrinsic oversampling of positive SARS-CoV-2 compared to the overall sampled population, and eventual overestimation of actual sensitivity. As recently stated by Woloshin et al. (56,57), use of either known positive or contrived samples may lead to overestimates of the actual sensitivity of the assessed test, since the sample collection may miss infected material in the daily practice. In other words, the eventual pooled estimate for sensitivity in cohort studies may be an overestimate of the actual diagnostic performances. Not coincidentally,

even though the field study of Pilarowski et al. reported on a diagnostic kit that was industrially analogous to other RADs that had performed particularly well, assessed sensitivity and specificity were far from being satisfying (i.e. 71.4% and 95.7%, respectively) (37).

#### *Limits of our study*

Some significant limitations of our study should be then stressed. First and foremost, more than half of sampled studies were retrieved from a pre-print platform (17,20,22–25,27,29,32–37), and corresponding results will require a further validation before being acknowledged as biomedical evidence.

Second, even though we performed a specific sub-analysis of RAD tests sensitivity where the viral load was available, our meta-analysis did not take in account the presumptive delay between test performing and the reported onset of the symptoms, as not regularly available from selected studies. As a consequence, it is possible that most of commercial kits that did not perform particularly well may have been impaired from the source, being employed in an inappropriate timeframe, underestimating their actual sensitivity in optimal settings (64–67). However, as such items have been designed to be deployed as a point-of-care diagnostics, including primary care units and Emergency Departments, it is reasonable that their potential users will refer to RADs in settings quite heterogeneous, forcibly far from optimal sampling windows.

Third, the studies included in the analyses were quite heterogeneous, not only in terms of sample size, but also when dealing with the reference populations, and only a subgroup of estimates reported the actual viral load of the sampled cases (14,18,36,38,20,23,25, 27,29,30,33,35). As viral load is instrumental in influencing the estimated sensitivity of the majority of RAD tests for viral infection (47,48,68), we cannot rule out that the pooled sensitivity we assessed may have been either inflated or flattened by the characteristics of the original population.

Fourth, in the majority of the studies we retrieved, sampled were collected by convenience (27,35,36): therefore, not only the eventual estimates are affected by the aforementioned oversampling of positive cases, failing to be representative of the general population,

but also the lack of a preventive power analysis impairs the proper assessment of the tests' performances. As a consequence, we suggest that our results should be retained cautiously and not as a comparative assessment of commercially available kits.

## Conclusions

In conclusion, albeit promising, and potentially useful in certain, well-defined settings (69–70), the use of available RAD tests on suspected SARS-CoV-2 infection cases is currently questionable for clinical purposes. In other words, RAD tests not represent a suitable candidate for mass screening procedures, and they even cannot substitute the conventional collection of nasopharyngeal samples in daily practice. However, given the limitations of the present review, such as the small number of studies, the small sample sizes and the high, statistically significant amount of heterogeneity among studies, further high-quality research in the field is absolutely needed.

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## References

1. Guan W, Liang W, Zhao Y et al. Comorbidity and its impact on 1590 patients with Covid-19 in China: A Nationwide Analysis. *Eur. Respir. J.* 2020;55:2000547. doi: 10.1183/13993003.00547-2020
2. Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497-506. doi: 10.1016/S0140-6736(20)30183-5
3. Li Q, Guan X, Wu P, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N. Engl. J. Med.* 2020;382:1199-1207. doi: 10.1056/NEJMoa2001316.
4. Riccò M; Ferraro P, Gualerzi G, et al. Point-of-Care diagnostic of SARS-CoV-2: knowledge, attitudes, and beliefs (KAP) of medical workforce in Italy. *Acta Biomed* 2020;91:57–67. doi: 10.23750/abm.v91i2.9573
5. European Centre for Diseases Prevention and Control (ECDC) An overview of the rapid test situation for COVID-19 diagnosis in the EU / EEA; Stockholm, 2020; available from: <https://www.ecdc.europa.eu/en/publications-data/overview-rapid-test-situation-covid-19-diagnosis-eueea> (accessed on November 12th, 2020)
6. Lippi G, Mattiuzzi C, Bovo C, Plebani M. Current laboratory diagnostics of coronavirus disease 2019 ( COVID-19). *Acta Biomed.* 2019;91:137–145. doi: 10.23750/abm.v91i2.9548
7. Tang YW, Schmitz JE, Persing DH, Stratton CW. The Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges. *J. Clin. Microbiol.* 2020;58:e00512-20. doi: 10.1128/JCM.00512-20
8. Riccò M, Ferraro P, Gualerzi G et al. Point-of-Care Diagnostic Tests for Detecting SARS-CoV-2 Antibodies : A Systematic Review and Meta-Analysis of Real-World Data. *J. Clin. Med.* 2020;9:1515. doi: 10.3390/jcm9051515
9. Vashist SK. In Vitro Diagnostic Assays for COVID-19: Recent Advances and Emerging Trends. *Diagnostics (Basel, Switzerland)* 2020;10:202. doi: 10.3390/diagnostics10040202..
10. Cassaniti I, Novazzi F, Giardina F et al. Performance of VivaDiagTM COVID-19 IgM/IgG Rapid Test is inadequate for diagnosis of COVID-19 in acute patients referring to emergency room department. *J. Med. Virol.* 2020;92:1724–1727. doi: 10.1002/jmv.25800.
11. Xu R, Cui B, Duan X, Zhang P, Zhou X, Yuan Q. Saliva: potential diagnostic value and transmission of 2019-nCoV. *Int. J. Oral Sci.* 2020;12:11. doi: 10.1038/s41368-020-0080-z..
12. World Health Organization (WHO) Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays; 2020; Available from: <https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays>; accessed on November 12th, 2020
13. World Health Organization (WHO) Laboratory testing strategy recommendations for COVID-19: interim guidance, 22 March 2020; 2020; World Health Organization. available from <https://apps.who.int/iris/handle/10665/331509>, accessed on November 12th, 2020

14. Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *J. Clin. Virol.* 2020;129:104455. doi: 10.1016/j.jcv.2020.104455.
15. Moher D, Liberati A, Tetzlaff J et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.* 2009;6:e1000097. doi: 10.1371/journal.pmed.1000100
16. Bizzaro N, Villalta D, Giavarina D, Tozzoli R. Are anti-nucleosome antibodies a better diagnostic marker than anti-dsDNA antibodies for systemic lupus erythematosus? A systematic review and a study of metanalysis. *Autoimmun. Rev.* 2012;12:97–106. doi: 10.1016/j.autrev.2012.07.002
17. Herrera V, Hsu V, Adewale A et al. Testing Healthcare Workers Exposed to COVID19 using Rapid Author 's Affiliation : medRxiv 2020;2020.0812.20172726. doi: 10.1101/2020.08.12.20172726
18. Porte L, Legarraga P, Vollrath V, Aguilera X. Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. *Int J Infect Dis* 2020;99:328-333. doi: 10.1016/j.ijid.2020.05.098
19. Young S, Taylor SN, Cammarata CL, et al. Clinical evaluation of BD Veritor SARS-CoV-2 point-of-care test performance compared to PCR-based testing and versus the Sofia 2 SARS Antigen point-of-care test. *J Clin Microbiol* 2020;59:e02338-20. doi: 10.1128/JCM.02338-20
20. Veyrenche N, Bollore K, Pisoni A, et al. Diagnosis value of SARS-CoV-2 antigen/antibody combined testing using rapid diagnostic tests at hospital admission. medRxiv 2020; 2020.09.19.20197855. doi: 10.1101/2020.09.19.20197855
21. Cerutti F, Burdino E, Grazia M et al. Urgent need of rapid tests for SARS CoV-2 antigen detection: Evaluation of the SD-Biosensor antigen test for SARS-CoV-2. *J. Clin. Virol.* 2020;132: 104654. doi: 10.1016/j.jcv.2020.104654
22. Albert E, Torres I, Bueno F, et al. Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test 2 Device) for the diagnosis of COVID-19 in primary health-care centers. medRxiv 2020;2020.10.16.20213850. doi: 10.1101/2020.10.16.20213850
23. Gremmels H, Winkel BMF, Schuurman R et al. Real-life validation of the Panbio COVID-19 Antigen Rapid Test (Abbott) in community-dwelling subjects with symptoms of potential SARS-CoV-2 infection. medRxiv 2020; 2020.10.16.20214189. doi: 10.1101/2020.10.16.20214189
24. Van der Moeren N, Zwart VF, Lodder EB et al. Performance evaluation of a sars-cov-2 rapid antigen test: test performance in the community in the netherlands. medRxiv 2020; 2020.10.19.20215202. doi: 10.1101/2020.10.19.20215202
25. Lindner AK, Nikolai O, Kausch F et al. Head-to-head comparison of SARS-CoV-2 antigen-detecting rapid test with self-collected anterior nasal swab versus professional-collected nasopharyngeal swab. medRxiv 2020; 2020.10.26.20219600. doi: 10.1101/2020.10.26.20219600
26. Hirotsu Y, Maejima M, Shibusawa M, Nagakubo Y. Comparison of automated SARS-CoV-2 antigen test for COVID-19 infection with quantitative RT-PCR using 313 nasopharyngeal swabs, including from seven serially followed patients. *Int J Infect Dis* 2020;99:397–402. doi: 10.1016/j.ijid.2020.08.029
27. Krüger LJ, Gaeddert M, Köppel L et al. Evaluation of the accuracy, ease of use and limit of detection of novel, rapid, antigen-detecting point-of-care diagnostics for SARS-CoV-2. medRxiv 2020;2020.10.01.20203836. doi: 10.1101/2020.10.01.20203836
28. Lambert-Niclot S, Cuffel A, Le Pape S et al. Evaluation of a Rapid Diagnostic Assay for Detection of SARS-CoV-2 Antigen in Nasopharyngeal Swabs. *J. Clin. Microbiol.* 2020;58, e00977-20. doi: 10.1128/JCM.00977-20
29. Linares M, Pérez Tanoira R, Romanyk J et al. Panbio antigen rapid test is reliable to diagnose SARS-CoV-2 infection in the first 7 days after the onset of symptoms. medRxiv 2020, 2020.09.20.20198192. doi: 10.1101/2020.09.20.20198192
30. Liotti FM, Menchinelli G, Lalle E et al. Performance of a novel diagnostic assay for rapid SARS-CoV-2 antigen detection in nasopharynx samples. *Clin. Microbiol. Infect.* 2021;27:487-488. doi: 10.1016/j.cmi.2020.09.030.
31. Mertens P, De Vos N, Martiny D et al. Development and Potential Usefulness of the COVID-19 Ag Respi-Strip Diagnostic Assay in a Pandemic Context. *Front. Med.* 2020;7:225. doi: 10.3389/fmed.2020.00225.
32. Pekosz A, Cooper CK, Parvu V et al. Antigen-based testing but not real-time PCR correlates with SARS-CoV-2 virus culture. medRxiv 2020;2020.10.02.20205708. doi: 10.1101/2020.10.02.20205708
33. Porte L, Legarraga P, Iruretagoyena M et al. Rapid SARS-CoV-2 antigen detection by immunofluorescence – a new tool to detect infectivity. medRxiv 2020;2020.10.04.20206466. doi: 10.1101/2020.10.04.20206466
34. Freymond A, Segura M. Performance evaluation of the Simtomax® CoronaCheck rapid diagnostic test. medRxiv 2020; 2020.10.28.20219667. doi: 10.1101/2020.10.28.20219667
35. Alemany A, Baro B, Ouchi D et al. Analytical and Clinical Performance of the Panbio COVID-19 Antigen-Detecting Rapid Diagnostic Test Background. medRxiv 2020; 2020.10.30.20223198. doi: 10.1101/2020.10.30.20223198
36. Courtellement L, Guinard J, Guillaume C et al. Real-life performance of a novel antigen detection test on nasopharyngeal specimens for SARS-CoV-2 infection diagnosis: a prospective study. medRxiv 2020;2020.10.28.20220657. doi: 10.1101/2020.10.28.20220657
37. Pilarowski G, Lebel P, Sunshine S et al. Performance characteristics of a rapid SARS-CoV-2 antigen detection assay at a public plaza testing site in San Francisco. medRxiv 2020; 2020.11.02.20223891. doi: 10.1101/2020.11.02.20223891
38. Gupta A, Khurana S, Das R et al. Rapid chromatographic immunoassay-based evaluation of COVID-19: A cross-sectional, diagnostic test accuracy study & its implications for COVID-19 management in India. *Indian J Med Res Epub* 2021;153:126-131. doi: 10.4103/ijmr.IJMR\_3305\_20
39. Nalumansi A, Lutalo T, Kayiwa J et al. Field Evaluation of the Performance of a SARS-CoV-2 Antigen Rapid

- Diagnostic Test in Uganda using Nasopharyngeal Samples. *Int. J. Infect. Dis.* 2020;104:282-286 doi: 10.1016/j.ijid.2020.10.073
40. Blairon L, Wilmet A, Beukinga I, Tré-hardy M. Implementation of rapid SARS-CoV-2 antigenic testing in a laboratory without access to molecular methods: Experiences of a general hospital. *J Clin Virol.* 2020;129:104472. doi: 10.1016/j.jcv.2020.104472.
  41. Fenollar F, Bouam A, Ballouche M et al. Evaluation of the Panbio Covid-19 rapid antigen detection test device for the screening of patients with Covid-19. *J. Clin. Virol.* 2020, JCM.02589-20. doi: 10.1128/JCM.02589-20.
  42. Dutta NK, Mazumdar K, Gordy JT. The Nucleocapsid Protein of SARS-CoV-2: a Target for Vaccine Development. *J. Virol.* 2020;94:e00647-20. doi: 10.1128/JVI.00647-20
  43. World Health Organization (WHO) Assessment tool for laboratories implementing COVID-19 virus testing; 2020; Available from: <https://www.who.int/publications/i/item/assessment-tool-for-laboratories-implementing-covid-19-virus-testing>; accessed on November 12th, 2020.
  44. Harrington A, Cox B, Snowdon J, et al. Comparison of Abbott ID Now and Abbott m2000 methods for the detection of SARS-CoV-2 from nasopharyngeal and nasal swabs from symptomatic patients. *J. Clin. Microbiol.* 2020;58:e00798-20. doi: 10.1128/JCM.00798-20.
  45. Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z. Considerations for diagnostic COVID-19 tests. *Nat. Rev. Microbiol.* 2021;19:171-183. doi: 10.1038/s41579-020-00461-z..
  46. Lee CK, Cho CH, Woo MK, Nyeck AE, Lim CS, Kim WJ. Evaluation of Sofia fluorescent immunoassay analyzer for influenza A/B virus. *J. Clin. Virol.* 2012;55:239-243. doi: 10.1016/j.jcv.2012.07.008
  47. Hurt AC, Alexander R, Hibbert J, Deed N, Barr IG. Performance of six influenza rapid tests in detecting human influenza in clinical specimens. *J. Clin. Virol.* 2007;39:132-135. doi: 10.1016/j.jcv.2007.03.002
  48. Uyeki TM, Prasad R, Vukotich C et al. Low sensitivity of rapid diagnostic test for influenza. *Clin. Infect. Dis.* 2009; 48:e89-92. doi: 10.1086/597828.
  49. Cui Z, Chang H, Wang H et al. Development of a rapid test kit for SARS-CoV-2: an example of product design. *Bio-Design Manuf.* 2020, epub ahead of print. doi: 10.1007/s42242-020-00075-7.
  50. Miller AB. Fundamental issues in screening for cancer. In *Cancer epidemiology and prevention*. Second edition; Schottenfeld, D., Fraumeni, J., Eds.; Oxford University Press: New York, 1996.
  51. Wilson J, Jungne G. Principles and practice of screening for disease; 1st ed.; World Health Organization: Geneva, 1968; Vol. 34.
  52. Salvatore P, Dawson P, Wadhwa A et al. Epidemiological Correlates of PCR Cycle Threshold Values in the Detection of SARS-CoV-2. *Clin Infect Dis* 2020;ciaa1469. doi: 10.1093/cid/ciaa1469.
  53. Riva E, Sainaghi PP, Turriziani O, Antonelli G, Patti G. SARS-CoV-2 infection: diagnostic testing results occasionally require special attention. *Emerg. Microbes Infect.* 2020;9:1955-1957. doi: 10.1080/22221751.2020.1814165
  54. Yanes-Lane M, Winters N, Fregonese F et al. Proportion of asymptomatic infection among COVID-19 positive persons and their transmission potential: A systematic review and meta-analysis. *PLoS One* 2020;15:e0241536. doi: 10.1371/journal.pone.0241536
  55. Bellato V, Konishi T, Pellino G et al. Screening policies, preventive measures and in-hospital infection of COVID-19 in global surgical practices. *J. Glob. Health* 2020;10:020507. doi: 10.7189/jogh.10.020507
  56. Mutti A. Occupational Medicine in the time of COVID-19. *Med Lav* 2020;111:83-86. doi: 10.23749/mdl.v111i2.9546
  57. Woloshin S, Patel N; Kesselheim AS. False Negative Tests for SARS-CoV-2 Infection. *N Eng J Med* 2020, 383(6):e38. doi: 10.1056/NEJMp2015897
  58. Patel U, Malik P, Mehta D, et al. Early epidemiological indicators, outcomes, and interventions of COVID-19 pandemic: A systematic review. *J Glob Health* 2020;10:020506. doi: 10.7189/jogh.10.020506.
  59. Bourgonje, A.R.; Abdulle, A.E.; Timens, W.; Hillebrands, J.; Navis, G.J.; Gordijn, S.J.; et al. Angiotensin-converting enzyme-2 ( ACE2 ), SARS-CoV -2 and pathophysiology of coronavirus disease 2019 ( COVID -19 ) . *J. Pathol.* 2020;251:228-248. doi: 10.1002/path.5471.
  60. Fang Z, Zhang Y, Hang C, Ai J, Li S, Zhang W. Comparisons of viral shedding time of SARS-CoV-2 of different samples in ICU and non-ICU patients. *J. Infect.* 2020;81:147-178. doi: 10.1016/j.jinf.2020.03.013.
  61. Azzi L, Carcano G, Gianfagna F et al. Saliva is a reliable tool to detect SARS-CoV-2. *J. Infect.* 2020;81:e45-e50. doi: 10.1016/j.jinf.2020.04.005.
  62. Riccò M, Ranzieri S, Peruzzi S. et al. RT-qPCR assays based on saliva rather than on nasopharyngeal swabs are possible but should be interpreted with caution: Results from a systematic review and metaanalysis. *Acta Biomed.* 2020;91:e2020025. doi: 10.23750/abm.v91i3.10020.
  63. Padoan A, Zuin S, Cosma C, Basso D, Plebani M, Bonfante F. Clinical performances of an ELISA for SARS-CoV-2 antibody assay and correlation with neutralization activity. *Clin. Chim. Acta* 2020;510:654-655. doi: 10.1016/j.cca.2020.08.024
  64. Reusken CB, Buiting A, Bleeker-Rovers C et al. Rapid assessment of regional SARS-CoV-2 community transmission through a convenience sample of health-care workers, the Netherlands, March 2020. *Eurosurveillance* 2020;25:2000334. doi: 10.2807/1560-7917.ES.2020.25.12.2000334.
  65. To KKW, Tsang OTY, Leung WS et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect. Dis.* 2020;20:565-574. doi: 10.1016/S1473-3099(20)30196-1.
  66. Ceron JJ, Lamy E, Martinez-Subiela S et al. Use of Saliva for Diagnosis and Monitoring the SARS-CoV-2: A Gen-

- eral Perspective. *J. Clin. Med.* 2020;9:1491. doi: 10.3390/jcm9051491
67. Hirotsu Y, Maejima M, Shibusawa M et al. Pooling RT-PCR test of SARS-CoV-2 for large cohort of “healthy” and infection-suspected patients: A prospective and consecutive study on 1,000 individuals. *medRxiv* 2020;2020.05.04.20088146. doi: 10.1101/2020.05.04.20088146
68. Chartrand C, Leeflang MMG, Minion J, Brewer T, Pai M. Review Accuracy of Rapid Influenza Diagnostic Tests. *Ann. Intern. Med.* 2012;156:500–511. doi: 10.7326/0003-4819-156-7-201204030-00403
69. Riccò M, Ranzieri S, Marchesi F. Rapid antigen tests for large-scale diagnostic campaigns: A case study from North-Eastern Italy. *J Infect.* 2021;82:e39–e40. doi: 10.1016/j.jinf.2021.01.011
70. Riccò M. A systematic review on rapid antigen test devices for SARS-CoV-2 in nursing homes: Useful, but handle with care. *Enferm Infecc Microbiol Clin* 2022; epub ahead of print. doi: 10.1016/j.eimc.2022.02.012

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