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Meta-Analysis of Robustness of COVID-19 Diagnostic Kits During Early Pandemic

Chandrakumar Shanmugam, M.D., M.B.A.,[1#] Michael Behring, Ph.D.,[2#] Vishwas Luthra, M.B.B.S.,[3] Sixto M Leal Jr., M.D., Ph.D.,[2] Sameer Al Diffalha, M.D.,[2] Sooryanarayana Varambally,[2, 4] PhD, George J Netto, M.D.,[2, 4] and Upender Manne, M.S., Ph.D.[2, 4,*]

Department of Pathology[1], MNR Medical College & Hospital, MNR Nagar, Fasalwadi, Sangareddy, Telangana, India 502279

Department of Pathology[2], University of Alabama at Birmingham, Birmingham, Alabama, USA 35274

Department of General Medicine[3], Sri Guru Ram Das Institute of Medical Sciences and Research, Mehta Road, Vallah, Amritsar, Punjab, India 143501

O'Neal Comprehensive Cancer Center[4], University of Alabama at Birmingham, Birmingham, Alabama, USA 35274

# Authors contributed equally

Running title: Robustness of Diagnostic Kits During Early Phase COVID 19: Lessons Learned

[*]**Author for Correspondence:**

Dr. Upender Manne
Professor of Pathology, Epidemiology and Surgery
University of Alabama at Birmingham
Birmingham, Alabama, USA 35274
Tel: 001 2052493895
Email: upendermanne@uabmc.edu

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ABSTRACT:

Background

Accurate detection of severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is necessary to mitigate the coronavirus disease-19 (COVID-19) pandemic. However, the test reagents and assay platforms are varied and may not be sufficiently robust to diagnose COVID-19.

Methods

We reviewed 85 studies (21,530 patients), published from five regions of the world, to highlight issues involved in the diagnosis of COVID-19 in the early phase of the pandemic, following the standards outlined in the PRISMA statement. All relevant articles, published up to May 31, 2020, in PubMed, BioRiXv, MedRiXv, and Google Scholar, were included. We evaluated the qualitative (9749 patients) and quantitative (10,355 patients) performance of RT-PCR and serologic diagnostic tests for real-world samples, and assessed the concordance (5,538 patients) between methods in meta-analyses.

Results

The RT-PCR tests exhibited heterogeneity in the primers and reagents used. Of 1,957 positive RT-PCR COVID-19 participants, 1,585 had positive serum antibody (IgM +/- IgG) tests (sensitivity 0.81, 95%CI 0.66-.90). While 3,509 of 3581 participants RT-PCR negative for COVID-19 were found negative by serology testing (specificity 0.98, 95%CI 0.94-0.99). The chemiluminescent immunoassay exhibited the highest sensitivity, followed by ELISA and lateral flow immunoassays. Serology tests had higher sensitivity and specificity for laboratory-approval than for real-world reporting data.

Conclusions

The robustness of the assays/platforms is influenced by variability in sampling and reagents. Serological testing complements and may minimize false negative RT-PCR results. Lack of standardized assay protocols in the early phase of pandemic might have contributed to the spread of COVID-19.

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Strengths and limitations of this study:

- This study offers the first evaluation of COVID-19 test performance with consideration of the heterogeneity of RT-PCR primers.
- We compare the performance of manufacturer-based, laboratory/approval data to the performance of the same test kits in a real-world setting.
- We perform a qualitative analysis of RT-PCR assays using 85 studies (21,530 patients), and a quantitative meta-analysis of RT-PCR vs. serum antibody assays in a sub-set of 30 publications (10,355 patients).
- Our findings demonstrate the need for application of real-world, published results to the evaluation of valid test performance, as well as the importance of standardization of assay protocols and reporting criteria in COVID-19 research.

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INTRODUCTION

In December 2019, there was a cluster of unexplained pneumonia cases in Wuhan, China, and a novel coronavirus was identified as the causative agent.[1] The virus was named as severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), and the disease as corona virus disease-19 (COVID-19).[2] The clinical spectrum ranges from asymptomatic forms to acute respiratory failure and multi-organ dysfunction syndrome, coagulopathy, and death.[3,4] In February 2020, the World Health Organization described the spread of these infections as a pandemic, which persists as a global crisis. Robust diagnostic tests are required to mitigate the spread of this virus and thereby to minimize the impact of COVID-19 on the health, economy, and social well-being of mankind.

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The standard diagnosis of COVID-19 is based on clinical and radiologic evidence and viral genome detection by RT-PCR in respiratory samples.[5] Gene-specific primers are used in the RT-PCR assays; structural genes include *envelope* (*E*), *nucleocapsid* (*N*), and/or *spike (S)*-genes; non-structural genes include *RNA-dependent RNA polymerase* (*RdRp*) or *open reading frame1ab* (ORF1ab) [6,7] Some studies used only a single-gene specific primer, and others used multiple-gene primers. Since studies published in the early phase of the pandemic reported a 3%-41% range of false-negativity by RT-PCR, a repeat RT-PCR testing was suggested.[8,9] Furthermore, false negativity was attributed to either mutations in the regions to which the primers bind or to sampling and laboratory practices, including collection, transportation, and handling.[10] Timing of sample collection with respect to the course of infection and the sample type also influence test results.[11] Alternatively, the diagnosis can be made by detection of antigens (E, N, or S) and/or antibodies (IgM or IgG or both) in blood samples.[12] However, these tests have the potential for false positives owing to cross-reactivity with other human corona viruses.[13,14] Due to the unprecedented public health emergency, the FDA authorized, on June 1, 2020, EUA requests for more than 15 diagnostic and serologic tests. Though serology testing can detect the false positives of RT-PCR tests in clinically suspected patients, its value in COVID-19 diagnosis as a complementary assay in the

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mitigation of the pandemic is not well defined. However, given the complexities in COVID-19 testing, there is a need for a review of performance for tests commonly used.

In this systematic review and meta-analysis, we examine current tests for the diagnosis of COVID-19 and evaluate the sensitivity and specificity of serological tests relative to RT-PCR tests. Our objective was to identify reasons for variability in COVID-19 diagnostic tests in the early phase of the pandemic that might have contributed to the spread of COVID-19. In particular, we assessed the uniformity of primer usage in RT-PCR assays and evaluated whether primers used in gold-standard RT-PCR tests affect the validity of serological tests. Furthermore, we compared the performance of serological tests/platforms in approval contrived/laboratory *vs.* real-world data.

METHODS

Literature Search

This research was accomplished according to standards outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[15] To find relevant studies, international databases, including PubMed, MedRiXv, BioRiXv, and Google Scholar, were searched for articles published until May 31, 2020. The following search terms were used (selected using English MeSH keywords and Emtree terms): [SARS-CoV-2 AND diagnosis] OR [2019-nCoV AND diagnosis]" OR ["COVID-19 AND diagnosis] and [SARS-CoV-2 AND RT-PCR] OR, [2019-nCoV AND RT-PCR]" OR ["COVID-19 AND RT-PCR] and [SARS-CoV-2 AND serology] OR [2019-nCoV AND serology]" OR ["COVID-19 AND serology]. Additional searches were performed for references listed in the included studies.

Eligibility Criteria

Relevant articles that reported diagnostic information for infected patients were included in the analysis. Pre-print articles with non-peer review were considered for inclusion. Articles were excluded if appropriate information was not reported or if they were in the Chinese language. Population sample sizes of <5 participants were not included; reviews and editorials

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were not considered. For meta-analysis and approval *vs*. real-world performance, studies that reported percent sensitivity/specificity without including patient numbers were also excluded.

Data Extraction and Report Quality Evaluation

Two authors (CS and VL) screened and evaluated the literature independently. Discrepancies were resolved by consensus after evaluation by a third author (MB). The following were extracted for review and meta-analysis: journal name, authors, period of publication (end of May, 2020), location of study, total number of patients, tissue of origin for samples tested, whether samples were from upper or lower respiratory tract (or both), primers for RT-PCR, platforms for serology tests, and antibodies tested for serology. Counts of true positives, false negatives, true negatives, and false positives were used in the meta-analysis.

An author (MB) extracted and analyzed the approved testing kit performance data from the following sources: FDA EUA Authorized Serology Test Performance,[16] the Foundation for Innovative New Diagnostics (FIND) SARS-CoV-2 diagnostic pipeline,[17] and package inserts provided on company websites for each product. Real-world sample testing data from kits in meta-analyses were compared against the performance of the same kits, or platforms, reported in approval documentation. Variables abstracted were study authors/test developer, name of test, test platform, and true positives, false negatives, true negatives, and false positives for each antibody or antibody combination measured (IgM, IgG, IgA, combined, and Pan-Ig). Risk of bias within individual studies of meta-analysis was assessed using the QUADAS 2 tool for assessment of diagnostic studies.[18]

Patient and Public Involvement

Since we performed a meta-analysis and systematic review, it was not appropriate or possible to involve patients or the public in the design, conduct, reporting, or dissemination plans of our research.

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Statistical Analysis

Statistical analyses were performed with R version 6.3.2 (2019-12-12).[19] The package "meta" was used for meta-analyses.[20] Random effects models were used to measure sensitivity and specificity of outcomes across studies. Subgroup analysis was performed to evaluate the effect of assay, RT-PCR primer type, and setting (laboratory *vs*. real-world) upon serum test performance. Heterogeneity across studies and subgroups was evaluated using Cochrane's *Q* statistic, and residual heterogeneity was quantified as a percentage with the I^2 statistic. An I^2 measure of 0% shows no observed heterogeneity, with increasing values from 0%-100% indicating higher levels of heterogeneity. [21] An assumption of homogeneity was rejected for p-values < 0.1. Evaluation of publication bias was not possible in approval data.

RESULTS

Search Results and Population Characteristics

Our search generated 112 publications with potential relevance to the performance of COVID-19 diagnostic tests. After excluding duplicate publications, manuscripts that did not report numbers of patients used for sensitivity/specificity calculations and studies with a sample size of <5 patients, 85 studies were selected for qualitative synthesis of RT-PCR primer usage. From this set, a sub-set of 30 publications were selected for the quantitative meta-analysis of serologic *vs.* RT-PCR diagnostic testing for COVID-19 (**Table S1**). Ancillary analysis compared the performance of these 30 real-world studies to that reported in laboratory approval data from 47 diagnostic serum-based tests. In all, our qualitative synthesis of RT-PCR studies included 85 studies and 21,530 patients. From this synthesis, a group of 30 studies with 10,355 patients from 5 regions of the world were selected for meta-analysis and comparison to performance from laboratory approval data (**Fig S1**).

Uniformity of Primer Usage in RT-PCR Diagnostic Tests

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We reviewed use of single primer of structural genes as compared to use of both structural and non-structural gene primers in 56 population-based studies with 9,872 participants. Overall, high proportions of studies employed both structural and non-structural gene primers in RT-PCR testing (58% in studies and 56% of total participants). Additionally, 29 studies (11,658 patients) did not report RT-PCR primer data. Single markers were most frequently tested in China and North American studies (**Table 1**). In general, the most tested samples were from the upper respiratory tract, regardless of primer status. Sample source and location in the respiratory tract were not reported for 8-20% of patients, and this was more common for studies using single gene primer.

Meta-Analysis: RT-PCR *vs.* Serum Antibody Testing

In general, patient sera were tested for IgM and IgG antibodies. China was the region with the highest frequency of antibody testing, and lateral flow immunoassay (LFIA) and chemiluminescent immunoassay (CLIA) testing platforms were most often utilized. Of the 45 studies included in the qualitative synthesis, 30 manuscripts reported both serum antibody testing and RT-PCR testing for the same patients. Key characteristics of this population include: China as the regional location for research; lack of reporting of RT-PCR primer information for ~33% of all studies; most studies used IgM and IgG serum-based antibody tests; and LFIA, CLIA, and enzyme-linked immunosorbent assay (ELISA) platforms were common across studies (**Table 2**).

We used the IgM+/-IgG serum antibody test since it was most commonly utilized across studies. Of 1,957 participants (sensitivity 0.81, 95%CI 0.66-0.90) with a positive RT-PCR COVID-19 result, 1,585 were also detected as positive with serum antibody tests. Of 3,581 true negatives in RT-PCR, 3,509 negatives were also found by serum antibody testing (specificity 0.98, 95%CI 0.94-0.99). For both models, heterogeneity between studies was significant ($p<0.01$ for both, I^2=97% and I^2 =98% for sensitivity and specificity, respectively).

Sub-analyses of differences based on the testing platform found that sensitivity between groups differed ($p <0.0001$), with CLIA tests performing best (0.99, 95%CI 0.97-0.99); ELISA

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as next-best (0.89, 95%CI 0.82-0.93); and LFIA as having the poorest sensitivity (0.67, 95%CI 0.50-0.81). LFIA test sensitivity also showed heterogeneity between studies ($p<0.01$, I^2 95%). For IgM/IgG tests, specificity did not differ significantly by platform (p= 0.06). However, a performance trend followed sensitivity, with LFIA underperforming (**Figure 1**).

Serum Antibody Testing Performance: Approval Data vs. Real-World Data

For manufacturer-based, laboratory approval data, IgM+/-IgG testing detected COVID-19 positivity for 1,045 of 1,068 RT-PCR-determined “true” positive patients (sensitivity 0.98, 95%CI 0.92-1.0). In the same group, serum testing correctly identified 1,928 of 1,967 (specificity 0.98, 95%CI 0.95-.099) true negatives by RT-PCR. For both models (sensitivity and specificity), there was evidence of heterogeneity (p <0.01 for both and I^2=93% and I^2=94% for sensitivity and specificity, respectively).

We evaluated IgM+/-IgG serum test performance in subgroup analyses comparing laboratory approval performance data to real-world performance in study data. In manufacturer data presented for approval, serum antibody testing detected 1,047 of 1,068 “true positive” cases of COVID-19 (sensitivity 0.98, 95%CI 0.92-1.0). Real-world use of serum IgM+/-IgG testing was evident for 2,450 of 3,025 participants diagnosed with COVID-19 by RT-PCR (sensitivity 0.81, 95% CI 0.66-0.90). For both groups, there was heterogeneity between studies (p <0.01 for both, I^2=93% and I^2 =97% for approval and real-world specificity, respectively) (**Figure 2**). In addition, the overall sensitivity between approval and real-world testing groups differed significantly (Q=8.37, p=0.004). An analysis of specificity by the same subgroups found no significant difference between laboratory approval and real-world data. Laboratory data identified 1,928 of 1,967 participants with true COVID-19 negative status (specificity 0.98, 95% CI 0.95-0.99). Real-world data found 5,437 of 5,548 true negatives (specificity 0.98, 95% CI, 0.96-0.99) (analysis not shown).

Since, in IgM+/-IgG tests, there were differences in sensitivity between platforms, we evaluated the effect of approval-based data *vs.* real-world data by the type of platform. In an

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analysis stratified for ELISA, CLIA, and LFIA, there was no significant difference in specificity between approval and real-world data (data not shown). However, for ELISA tests, real-world capacity to detect true positives was lower than in laboratory-based analyses. In real-world studies, the sensitivity of ELISA was 0.89 (95% CI, 0.82-0.93), different from laboratory sensitivity for the same platform (0.94, CI95% 0.91-0.96, Q =4.74, p=0.03). The LFIA platform also showed a trend of lower real-world sensitivity (0.67, 95% CI, 0.50-0.81) compared to laboratory approval sensitivity (0.99, CI95% 0.90-0.99, Q =8.56, p 0.003). Laboratory/real-world groups for CLIA platforms were too small to be tested reliably (1 and 2 groups, respectively).

Serum Antibody Testing Performance: Effect of Primer Choice on Test Validity

To evaluate the reliability of RT-PCR as a gold standard for serum-based test performance, we tested the consequences of using structural and non-structural primers in RT-PCR reference testing of serum. Analyses were divided into three subgroups based on antibody targets: IgM, IgG, and IgG+/-IgM combined. In IgM and combined IgG+/-IgM testing, the primer choice had no significant influence on sensitivity or specificity. However, for IgG antibody tests, use of both a structural and a non-structural gene-specific primers in RT-PCR resulted in reduced sensitivity for serum testing (**Figure 3**, Q=6.17, p=0.013). Furthermore, although not statistically significant, the sensitivity of both IgM and IgG+/-IgM combined data sets was lower when using a referent RT-PCR test with both primer types.

DISCUSSION

Because of the highly infectious nature of COVID-19, a prompt, accurate, and early diagnosis is necessary to deal with the ongoing pandemic, for such diagnoses can help reduce the spread of infection and its associated risk for mortality. Currently, the COVID-19 diagnosis is generally based on RT-PCR assays.[8] Alternative methods such as antigen- and antibody-based serology tests, although available, have uncertain value. The current systematic review and meta-analysis addresses the challenges encountered in the diagnosis of COVID-19 by

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various methods. It also analyzes differences between the FDA-approved EUA data and real-world data. There is worldwide non-uniformity in the performance of RT-PCR, including the number and types of primers and reagents used for COVID19 diagnosis, which raise questions about its generalized applicability. Similarly, the studies based on serological tests showed diagnostic inaccuracies owing to individual differences in mounting an immune response as well as dependency on the time duration after the onset of symptoms. Overall, the sensitivity between RT-PCR and serology tests was 0.81 (95% CI, 0.66-0.90), and specificity was 0.98 (95% CI, 0.94-0.99). Among the various platforms for serodiagnosis, the highest sensitivity was exhibited by ELISA, followed by CLIA and LFIA. Furthermore, use of primers (structural, non-structural, or both) had a variable effect on sensitivity based on antibody targets. Sensitivity was significantly higher for IgG serology tests using structural-primer-only RT-PCR tests as a referent. Serology tests had higher sensitivity for approval-based data than for real-world reporting. This difference was significant for ELISA-based platforms, and a non-significant trend towards inflated approval-based sensitivity was evident for both CLIA and LFIA platforms. These observations highlight the inconsistencies/challenges in the COVID-19 diagnosis by RT-PCR, which is the current gold standard, as well as in serologic testing.

For RT-PCR assays, the targets in SARS-CoV-2 include structural genes like *E, N* and *S*, and nonstructural genes, including that for *RdRp* or *ORF1ab.*[22] In the early phase of the pandemic, some studies used a two-step diagnosis that included an initial screening phase using structural genes followed by a confirmatory phase using nonstructural genes. [6,7,23] The test is considered positive when both structural and non-structural markers are positive.[24,25] However, currently both types of primers are used simultaneously to diagnose COVID-19. The viral load or copy number of the viral genome is expressed as a Ct-value, which when <37 is indicative of a positive test, and a value of ≥ 40 is considered negative. A Ct value between >37 and < 40 requires repetition of RT-PCR analysis to confirm the diagnosis.[24] However, the Ct value range varies widely according to assays and laboratory practices. A COVID-19-RdRp/Hel assay has a higher sensitivity than a conventional RdRp-P2 assay irrespective of

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the type of sample.[26] Overall, higher proportions of studies (58%) employed both structural and non-structural gene primers in RT-PCR testing. Single markers were used in some Chinese and North American studies. These findings are indicative of non-uniformity in the RT-PCR methodology. We note that half of the positive, symptomatic patients became negative by the second week, when they became asymptomatic. In contrast, the asymptomatic, positive patients became negative two days after hospital admission, indicating the importance of a temporal factor in COVID-19 diagnosis by RT-PCR.[27,28]

Published in the early phase of the pandemic, 11 of 85 studies had clinically suspected COVID-19 patients. In these studies, the average test positivity by RT-PCR, regardless of the sample source, was 44% (Supplementary Table 1), and test sensitivity was influenced by sample source (upper *vs*. lower respiratory *vs.* other samples), issues related to testing performance, and delay after onset of symptoms.[29] In the early phase of the COVID-19 pandemic, for studies evaluating suspected COVID-19 cases, the total positive RT-PCR for throat swabs was in the range of 30–60% at initial presentation.[8,30] One study reported a yield of 72-93% positive cases for lower respiratory samples (bronchioalveolar lavage and sputum) as compared to 32-63% positivity for upper respiratory samples (oral and nasopharyngeal swabs) and 29% for stool samples.[29] Hence, a negative COVID-19 test based only on an upper respiratory sample at a single time point is questionable. For most studies, the testing sample was from the upper respiratory tract, regardless of primer type used. However, the sample source was not reported for 8-20% of patients, which was more common for studies using only structural gene primers. For stool samples testing positive for COVID-19, 66.7% also tested positive on pharyngeal swabs. Of the stool samples, 64.3% remained positive after pharyngeal clearance of the virus.[31] In contrast, none of the patients showed a positive test on upper respiratory samples after the anal swabs tested negative.[31] These findings raise concerns about whether patients with negative respiratory swabs are truly virus-free, and sampling of additional body sites is needed. As determined by various studies, the performance of the RT-PCR depends on usage of comparable protocols, including primers

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and reagents.[32] Additionally, it is unknown whether the currently used RT-PCR primers detect all SARS-Cov-2 strains.

The specific immune response to SARS-CoV-2 can be measured by serological testing. Several rapid serological tests, including point-of-care tests, are being developed. Even though some of these tests have been approved by the FDA through EUA, their accuracy needs to be validated.[33] A minimum of 1–2 weeks after the onset of infection is needed for seroconversion. Hence, antibody testing is of no value in the early phase of infection. Additionally, its value is limited by its cross-reactivity with other coronaviruses.[34,35] The initial RT-PCR positivity during the early stages (<15 days) of SARS-CoV-2 infection declines to 66.7% in the later phase (15-39 days), during this period, the antibody test can supplement RT-PCR in the diagnosis of COVID-19.[34,35] Additionally, serology testing becomes valuable for clinically suspected and RT-PCR negative (false-negative) individuals.

This research has limitations. Due to the dynamic reporting of COVID-19 testing research and inconsistencies in reporting of predictive variables across studies, bias in sampling may have some effect on our results. Patient flow analysis suggests that lack of consistent RT-PCR reference standard given to patients in the same study, as well as the unclear reporting of patient selection methods could contribute to bias in these results (**Fig. S2**). In addition, the observed heterogeneity between studies in the meta-analysis suggests that we must consider the possibility that the differences in results may be due to chance. Lastly, it is questionable to compare two separate testing methods of RT-PCR and seroprevalence in sensitivity/specificity analysis. In particular, given the relationship between time since diagnosis and accuracy of serology testing, a contributor to the observed differences in performance is time.

The effective containment of COVID-19 involves accurate diagnoses and isolation of SARS-CoV-2-infected persons. Robustness of the assays/platforms is determined by variability of the samples, primers, and reagents used. Serological tests alone are of value only during the latter times of infection; however, they complement RT-PCR when used in

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conjunction and minimize false negative RT-PCR results. Additionally, some of the approved serological assays/platforms, particularly those developed using contrived/laboratory data, perform poorly when applied to real-world samples. We are currently in a new phase of the pandemic, and there is a need for a reliable/robust diagnostic test to mitigate the spread of COVID-19.

Our analyses of studies published in the early-phase of the pandemic have highlighted issues related to COVID-19 diagnosis that need to be addressed as follows: 1) The high mutational rate exhibited by the SARS-CoV-2 virus may lead to the generation of new strains. Therefore, like for influenza virus, the existing diagnostic kits need to be modified constantly to optimize the detection of new strains; 2) Though RT-PCR diagnosis of COVID-19 is the gold standard, its combination with a serologic test may increase the accuracy of SARS-CoV-2 detection; 3) Approval agencies must account for the type of data (contrived versus real world) presented by diagnostic kit developer; 4) Although agencies employed EUA processes for the approval of diagnostic kits, there is a need to monitor their performance and assess their robustness in real-world samples, to permit continued use of these kits; and 5) Standardized assay protocols need to be developed and continually updated to mitigate the COVID-19 pandemic.

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DATA SHARING

No additional data is available

AUTHOR CONTRIBUTIONS

CS, MB, and UM made substantial contributions to the conception and design of the work. CS, MB and VL contributed to the acquisition of the study data. Data were analyzed by CS, MB and UM. MB was responsible for the statistical analysis. CS, MB, SML, SAD, SV, GJN,

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and UM contributed to the interpretation of the data, and to drafting and revising of the manuscript. CS, MB and VL have accessed and verified the data. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work. All authors have read and approved the manuscript.

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Table 1. Characteristics of the studies included in qualitative analysis

	Total Studies	Total pop.	Structural gene primers		Structural and non-Structural gene primers		Non-Structural gene primers		Not reported	
			N studies	N pop.	N studies	N pop.	N studies	N pop.	N studies	N pop.
Total	**85**	**21530**	**22**	**4265**	**31**	**5484**	**3**	**123**	**29**	**11658**
Location										
Asia (excl. China)	6	378	2	53	4	325				
China	28	12187	8	1802	17	3047	3	123	24	7215
Europe	12	5757	4	528	8	993			4	4236
North America	10	3001	8	1882	2	1119				
Global		207							1	207
Primers										
N -single	11	2016	11	2016						
E -single	4	759	4	759						
S -single	1	412	1	412						
N, E	2	226	2	226						
S, N	4	852	4	852						
ORF1Ab, single	2	59					2	59		
RdRp, single	1	64					1	64		
E+ORF1Ab	2	1119			2	1119				
E + RdRp	2	259			2	259				
M, E	1	48			1	48				
N+ORF1Ab	14	2703			14	2703				
N + E + RdRp	4	333			4	333				
S, N, E, RdRp, ORF1ab	1	13			1	13				
N, E, ORF1ab	1	33			1	33				
N, RNAse P	1	190			1	190				
S, N, RdRp, ORF1ab, E, M	1	52			1	52				
N, S, RdRp	1	273			1	273				
N, E, S, RdRp	2	349			2	349				
S, ORF1Ab	1	112			1	112				
Sample Source										
Upper Respiratory	23	6748	3	575	9	2633	1	64	10	3476
Upper & Lower Respiratory	1	52			1	52				
Upper Respiratory + Other*	9	751	3	368	2	44	1	38	3	301
Lower Respiratory + Other*	1	273			1	273				
Upper Respiratory + Serum	20	6407	7	1473	9	1432			4	3502
Upper Respiratory + Serum + Other*	4	941	2	840	1	80	1	21		
Upper & Lower Respiratory + Other*	4	678	1	280	3	398				
Upper & Lower Respiratory + Serum + Other*	2	518			1	132			1	386
Serum	18	2376	6	729	4	440			8	1207
Other*	1	199							1	199
Not reported	2	2587							2	2587

* Other = bronchioalveolar lavage, feces, urine, neonatal, amniotic fluid, and breast milk. N pop. = patient population

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Table 2. Characteristics of studies included in quantitative meta-analysis

	N studies	N pop.
Total	30	10355
Location		
Asia (excl. China)	2	261
China	19	6375
Europe	7	2900
North America	2	819
PCR primers		
Structural		
N, single	5	1084
E, single	1	49
N, E	1	201
N, S	2	408
Structural and Non-structural		
with ORF1Ab	8	1115
with RdRp	2	186
N, RNAse P	1	190
not reported	10	7122
Ab tested		
IgG	2	220
IgM + IgG	25	7828
IgA + IgG +IgM	1	208
IgA + IgG	1	37
not reported	1	2062
Serum Ab		
CLIA	8	3705
ELISA	8	1908
LFIA	10	3800
CLIA + ELISA	2	548
LFIA + ELISA	1	80
not reported	1	314

CLIA = chemiluminescent immunoassay, LFIA = lateral flow immunoassay, ELISA = enzyme-linked immunosorbent assay

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Figure Legends

Figure 1. **Comparison of Performance (Sensitivity and Specificity) of Serology Tests (IgM/IgG) Based on Assay Platforms**

Figure 2. **Comparison of Sensitivity of Laboratory setting versus Real World setting of RT-PCR and Serology (IgM/IgG) kits**

Figure 3. **The Effect of Primer Choice in RT-PCR Referent on Sensitivity of Tests based on Serum IgG**

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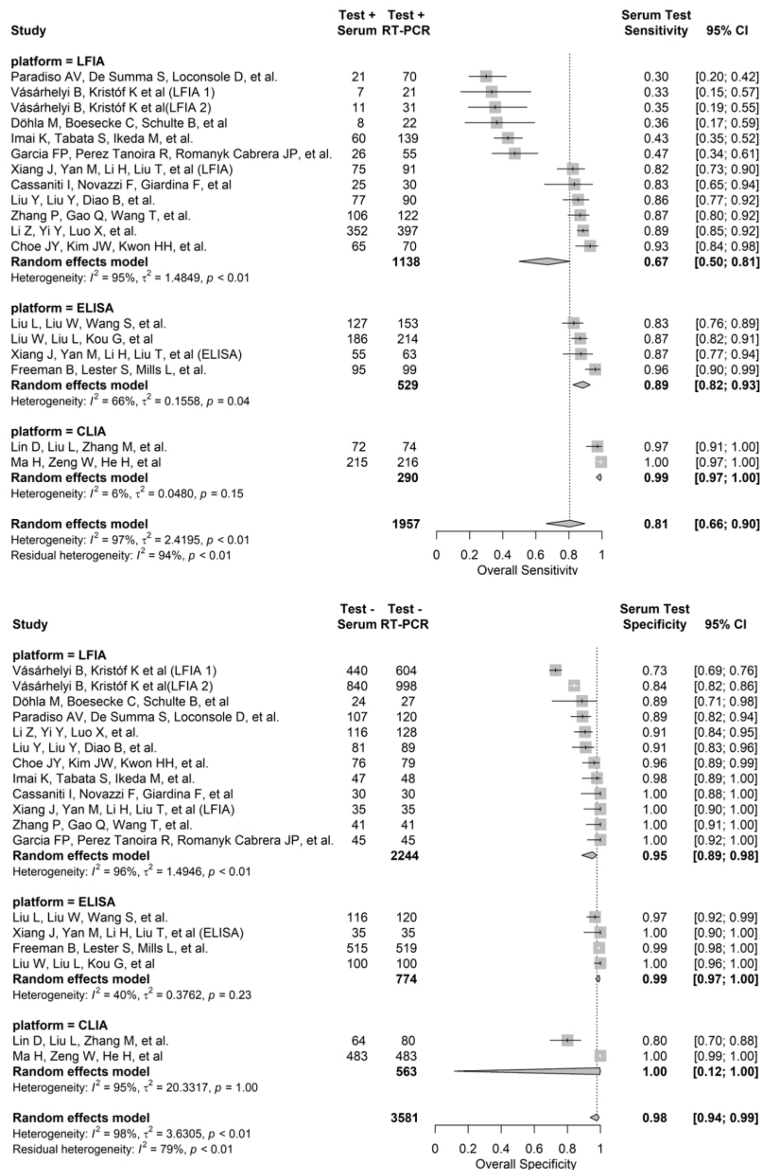

Figure 1. Comparison of Performance (Sensitivity and Specificity) of Serology Tests (IgM/IgG) Based on Assay Platforms

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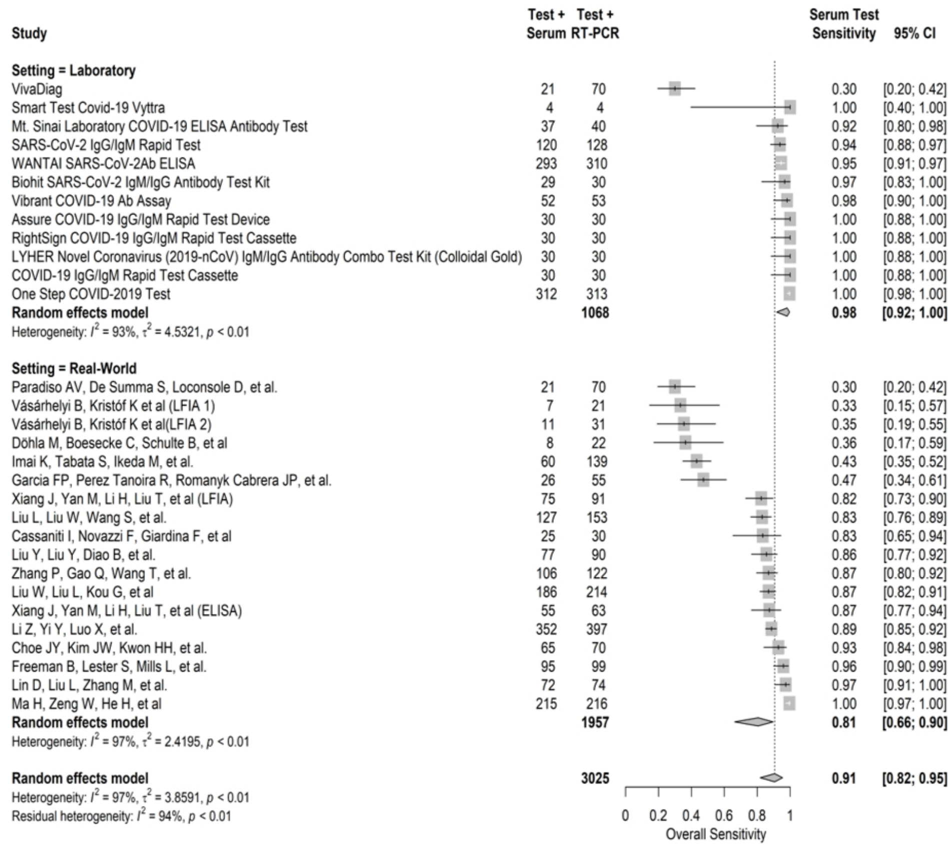

Figure 2. Comparison of Sensitivity of Laboratory setting versus Real World setting of RT-PCR and Serology (IgM/IgG) kits

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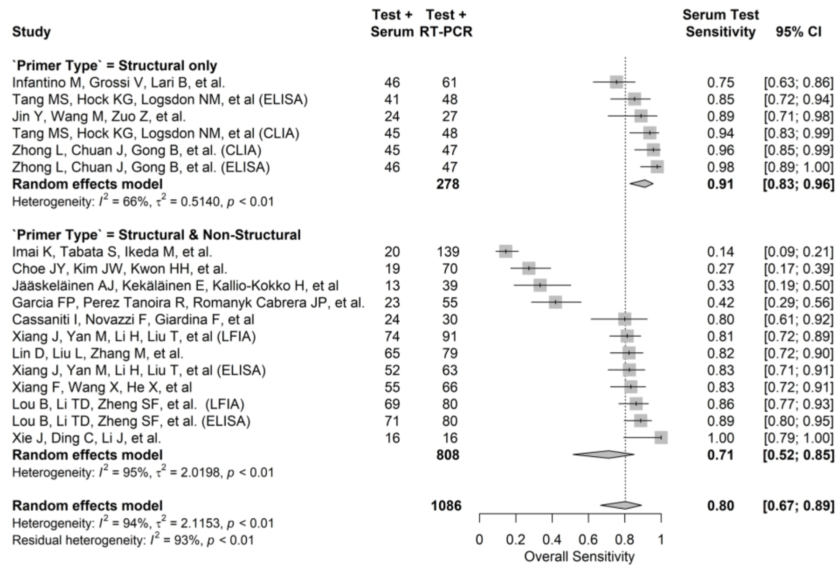

Figure 3. The Effect of Primer Choice in RT-PCR Referent on Sensitivity of Tests based on Serum IgG

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Meta-Analysis of Robustness of COVID-19 Diagnostic Kits During Early Pandemic. Supplemental information

Table S1. Description of 55 Studies Included for Qualitative Synthesis (gray) and 30 Studies Included in the Meta-Analysis (tan)

Journal	Authors	Region	N (patients)	Percent COVID-19 positivity	Sample origin	RT-PCR primers	RT-PCR primer type	RT-PCR/Serology platform	Serology targeted antibody	Study included in meta-analysis
J Clin Virol	Rahman H, Carter I, Basile K, et al. [1]	Asia	52		UR+LR	S, N, E, RdRp, ORF1ab, M	Both	RT-PCR		No
Exp Neurobiol	Won J, Lee S, Park M, et al. [2]	Asia	12		UR	N, E, S, RdRp	Both	RT-PCR		No
Jpn J Infect Dis.	Okamaoto K, Shirato K, Nao N, et al. [3]	Asia	25		UR	N, E	Structural	RT-PCR		No
J Med Virol	Choe JY, Kim JW, Kwon HH, et al. [4]	Asia	149	0.47	Serum	E, RdRp	Both	RT-PCR /CLIA	IgM + IgG	Yes
Lancet Infect Dis	Yong SEF, Anderson DE, Wei WE, et al. [5]	Asia	28		UR + Serum	N, single	Structural	RT-PCR /ELISA	IgG	No
J Clin Virol	Imai K, Tabata S, Ikeda M, et al. [6]	Asia	112		UR + Serum	S, ORF1Ab	Both	RT-PCR /LFIA	IgM + IgG	Yes
Emerg Microbes Infect	Xu Y, Xiao M, Liu X, et al. [7]	China	6		Serum			ELISA + LFIA	IgM	No
Radiology	Ai T, Yang Z, Hou H, Zhan C, et al. [8]	China	1014	0.59	UR			RT-PCR		No
NEJM	Cao B, et al.[9]	China	199		Other			RT-PCR		No
Radiology	Bai HX, Hsieh B, Xiong Z, et al.[10]	China	256		UR			RT-PCR		No
Lancet	Chen H, Guo J, Wang C, et al. [11]	China	9		UR + Other			RT-PCR		No
AJR Am J Roentgenol	Liu D, Li L, Wu X, et al. [12]	China	15		UR			RT-PCR		No
Eur J Radiol	Long C, Xu H, Shen Q, et al. [13]	China	87		UR			RT-PCR		No
Pediatr Pulmonol	Xia W, Shao J, Guo Y, Peng X, Li Z, Hu D. [14]	China	20		UR			RT-PCR		No
Am J Obstetr Gynecol	Yan J, Guo J, Fan C, et al. [15]	China	116	0.56	Other			RT-PCR		No
J Hosp Infect	Ye G, Li Y, Lu M, et al. [16]	China	91	0.52	UR			RT-PCR		No
J Med Virol	Zhang J, Wang S, Xue Y. [17]	China	14		UR + Other			RT-PCR		No
Respir Res	Zhang G, Zhang J, Wang B, Zhu X, Wang Q, Qiu S. [18]	China	95		UR			RT-PCR		No
Lancet	Zhou F, Yu T, Du R, et al. [19]	China	191		UR			RT-PCR		No

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J Clin Microbiol	Liu W, Liu L, Kou G, et al.[20]	China	314		UR + Serum			RT-PCR	IgM + IgG	Yes
J Med Virol	Li, Y et al. [21]	China	610	0.40	UR	N, ORF1Ab	Both	RT-PCR		No
medRxiv	Diao B, Wen K, Chen J, et al.[22]	China	239		UR + Serum	N, ORF1Ab	Both	RT-PCR		No
J Clin Microbiol	Chan JF, Yip CC, To KK, et al. [23]	China	273		UR + Other	N, S, RdRp	Both	RT-PCR		No
Nature Microbiol	Kong WH, Li Y, Peng MW, et al. [24]	China	640		UR	N, ORF1Ab	Both	RT-PCR		No
Front Med	Liu W, Wang J, Li W, Zhou Z, Liu S, Rong Z. [25]	China	38	0.53	UR + Other	N, ORF1Ab	Both	RT-PCR		No
Int J Biol Sci	Lo IL, Lio CF, Cheong HH, et al. [26]	China	10		UR + LR + Other	N, ORF1Ab	Both	RT-PCR		No
Travel Med Infect Dis	Wu J, Liu J, Li S, Peng Z, et al. [27]	China	132		UR + LR + Serum + Other	N, E, RdRp	Both	RT-PCR		No
Int J Infect Dis	Xu T, Chen C, Zhu Z, et al. [28]	China	51		UR + LR + Other	N, ORF1Ab	Both	RT-PCR		No
J Med Virol	Yuan Y, Wang N, et al. [29]	China	6		UR + Other	N, E, RdRp	Both	RT-PCR		No
AJR Am J Roentgenol	Cheng Z, Lu Y, Cao Q, et al. [30]	China	33	0.33	UR	N, E, ORF1ab	Both	RT-PCR		No
Arch Pathol Lab Med	Schwartz, DA [31]	China	38		UR + Other	ORF1Ab, single	Non-structural	RT-PCR		No
Radiology	Wong HYF, Lam HYS, Fong AH, et al. [32]	China	64		UR	RdRp, single	Non-structural	RT-PCR		No
Chin Med J	Ling Y, Xu SB, Lin YX, et al. [33]	China	292		UR + Other	E, single	Structural	RT-PCR		No
Clin Infect Dis	Zhao R, Li M, Song H, et al. [34]	China	412		UR	S, single	Structural	RT-PCR		No
medRxiv	Ma H, Zeng W, He H, et al.[35]	China	699		UR + Serum			RT-PCR /CLIA	IgM + IgG	Yes
medRxiv	Cai X, Chen J, Hu J, et al.[36]	China	443		Serum			RT-PCR /CLIA	IgM + IgG	Yes
medRxiv	Qian C, Zhou M, Cheng F, et al. [37]	China	2062					RT-PCR /CLIA	IgM + IgG	Yes
J Infect Dis	Zhang G, Nie S, Zhang Z, Zhang Z. [38]	China	112		UR + Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	No
medRxiv	Lin D, Liu L, Zhang M, et al.[39]	China	159		UR + Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	Yes

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J Med Virol	Xie J, Ding C, Li J, et al. [40]	China	56		UR +Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	Yes
Nature Med	Long QX, Liu BZ, Deng HJ, et al. [41]	China	285		UR+ Serum	S, N	Structural	RT-PCR /CLIA	IgM + IgG	No
Int J Infect Dis	Jin Y, Wang M, Zuo Z, et al. [42]	China	76	0.57	Serum	N, single	Structural	RT-PCR /CLIA	IgM + IgG	Yes
Emerg Microbes Infect	Zhang W, Du RH, Li B, et al. [43]	China	278		UR + Other			RT-PCR /ELISA	IgM + IgG	No
Clin Infect Dis	Zhao J, Yuan Q, Wang H, et al.[44]	China	386		UR + LR + Serum			RT-PCR /ELISA	IgM + IgG	Yes
Euro Surveill	Perera RA, Mok CK, Tsang OT, et al. [45]	China	51		Serum			RT-PCR /ELISA	IgM + IgG	Yes
Clin Infect Dis	Xiang F, Wang X, He X, et al. [46]	China	216		UR + Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
medRxiv	Xiang J, Yan M, Li H, Liu T, et al. [47]	China	154		Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
medRxiv	Liu L, Liu W, Wang S, et al.[48]	China	238		UR + Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
Clin Infect Dis	Guo L, Ren L, Yang S, et al. [49]	China	208	0.39	Serum	N, single	Structural	RT-PCR /ELISA	IgM + IgA + IgG	Yes
Sci China Life Sci	Zhong L, Chuan J, Gong B, et al.[50]	China	347		UR NP/OP + Serum + Other	N, S	Structural	RT-PCR /ELISA + CLIA	IgM + IgG	Yes
Eur Respir J	Lou B, Li TD, Zheng SF, et al. [51]	China	80		UR + LR + Serum + Other	N, ORF1Ab	Both	RT-PCR /ELISA + LFIA + CLIA	IgM + IgG	Yes
J Med Virol	Du Z, Zhu F, Guo F, Yang B, Wang T. [52]	China	60		Serum			RT-PCR /LFIA	IgM + IgG	No
J Infect	Pan Y, Li X, Yang G, et al. [53]	China	105		Serum			RT-PCR /LFIA	IgM + IgG	No
J Med Virol	Li Z, Yi Y, Luo X, et al.[54]	China	525					RT-PCR /LFIA	IgM + IgG	Yes
medRxiv	Liu Y, Liu Y, Diao B, et al.[55]	China	179		UR + Serum			RT-PCR /LFIA	IgM + IgG	Yes
Emerg Microbes Infect	Yongchen Z, Shen H, Wang X, et al. [56]	China	21		UR + Serum + Other	ORF1Ab, single	Non-structural	RT-PCR /LFIA	IgM + IgG	No
Anal Chem	Chen Z, Zhang Z, Zhai X, et al.[57]	China	19		UR + Serum	N, single	Structural	RT-PCR /LFIA	IgG	Yes

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medRxiv	Zhang P, Gao Q, Wang T, et al.[58]	China	163		UR + Serum	N, single	Structural	RT-PCR /LFIA	IgM + IgG	Yes
JAMA	Grasselli G, Zangrillo A, Zanella A, et al. [59]	Europe	1591		UR			RT-PCR		No
Radiology	Caruso D, Zerunian M, Polici M, et al. [60]	Europe	158	0.39	UR	N, E, RdRp	Both	RT-PCR		No
Travel Med Infect Dis	Lagier JC, Colson P, Tissot Dupont H, et al. [61]	Europe	337		UR +LR+ Other	N, E, S, RdRp	Both	RT-PCR		No
J Clin Virol	van Kasteren PB, van der Veer B, van den Brink S, et al. [62]	Europe	13		UR	S, N, E, RdRp, ORF1ab	Both	RT-PCR		No
Int J Mol Sci	Toptan T, Hoehl S, Westhaus S, et al. [63]	Europe	48		UR	M, E	Both	RT-PCR		No
Trop Med Infect Dis	Amrane S, Tissot-Dupont H, Doudier, et al. [64]	Europe	280		UR + LR + Other	E, single	Structural	RT-PCR		No
J Clin Microbiol	Lambert-Niclot S, Cuffel A, Le Pape S, et al. [65]	Europe	138		UR	E, single	Structural	RT-PCR		No
J Med Virol	Infantino M, Grossi V, Lari B, et al. [66]	Europe	61		Serum	S, N	Structural	RT-PCR /CLIA	IgM + IgG	Yes
Euro Surveill	Jääskeläinen AJ, Kekäläinen E, Kallio-Kokko H, et al. [67]	Europe	37		Serum	N, E, RdRp	Both	RT-PCR /ELISA	IgA + IgG	Yes
J Infect	Tré-Hardy M, Blairon L, Wilmet A, et al. [68]	Europe	182		Serum			RT-PCR /ELISA + CLIA	IgA + IgG	No
Orvo Hetil	Vásárhelyi B, Kristóf K, Ostorházi E, Szabó D, Prohászka Z, Merkely B. [69]	Europe	2310	0.06	UR + Serum			RT-PCR /LFIA	IgM + IgG	Yes
Infect Ecol Epidemiol	Hoffman T, Nissen K, Krambrich J, et al.[70]	Europe	153		Serum			RT-PCR /LFIA	IgM + IgG	Yes
J Med Virol	Cassaniti I, Novazzi F, Giardina F, et al. [71]	Europe	110		UR + Serum	E, RdRp	Both	RT-PCR /LFIA	IgM + IgG	No
medRxiv	Garcia FP, Perez Tanoira R, Romanyk Cabrera JP, et al. [72]	Europe	100		Serum	N, ORF1Ab	Both	RT-PCR /LFIA	IgM + IgG	Yes
medRxiv	Paradiso AV, De Summa S, Loconsole D, et al.[73]	Europe	190		UR + Serum	N, RNAse P	Both	RT-PCR /LFIA	IgM + IgG	Yes
Public Health	Döhla M, Boesecke C, Schulte B, et al. [74]	Europe	49		Serum	E, single	Structural	RT-PCR /LFIA	IgM + IgG	Yes
J Emerg Infect Dis	Okba NMA, Muller MA, Li W, et al. [75]	Global	207		Serum			RT-PCR /ELISA	IgM + IgG	No

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J Clin Virol	Smithgall MC, Scherberkova I, Whittier S, Green DA. [76]	North America	113		UR	E, ORF1Ab	Both	RT-PCR		No
J Med Virol	Pujadas E, Ibeh N, Hernandez MM, et al. [77]	North America	1006		UR	E, ORF1Ab	Both	RT-PCR		No
J Infect Dis	Burbelo PD, Riedo FX, Morishima C, et al. [78]	North America	100		Serum	N, single	Structural	RT-PCR		No
Am J Obstet Gynecol MFM	Penfield CA, Brubaker SG, Limaye MA, et al. [79]	North America	32		UR + Other	N, single	Structural	RT-PCR		No
medRxiv	Wyllie AL, Fournier J, et al. [80]	North America	44		UR + Other	N, single	Structural	RT-PCR		No
J Appl Lab Med	Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. [81]	North America	235		Serum	N, single	Structural	RT-PCR /CLIA	IgM + IgG	No
Clin Chem	Tang MS, Hock KG, Logsdon NM, et al. [82]	North America	201		UR + LR + Serum	N, E	Both	RT-PCR /CLIA + ELISA	IgG	Yes
medRxiv	Randad PR, Pisanic N, Kruczynski K, et al. [83]	North America	493		UR + Serum + Other	N, single	Structural	RT-PCR /ELISA	IgM + IgA + IgG	No
JMIR Public Health Surveill	Sullivan PS, Sailey C, Guest JL, et al. [84]	North America	159		UR + Serum	S, N	Structural	RT-PCR /ELISA	IgM + IgA + IgG	No
bioRxiv	Freeman B, Lester S, Mills L, et al.[85]	North America	618		UR NP/OP + Serum	N, single	Structural	RT-PCR /ELISA	IgM + IgG	Yes

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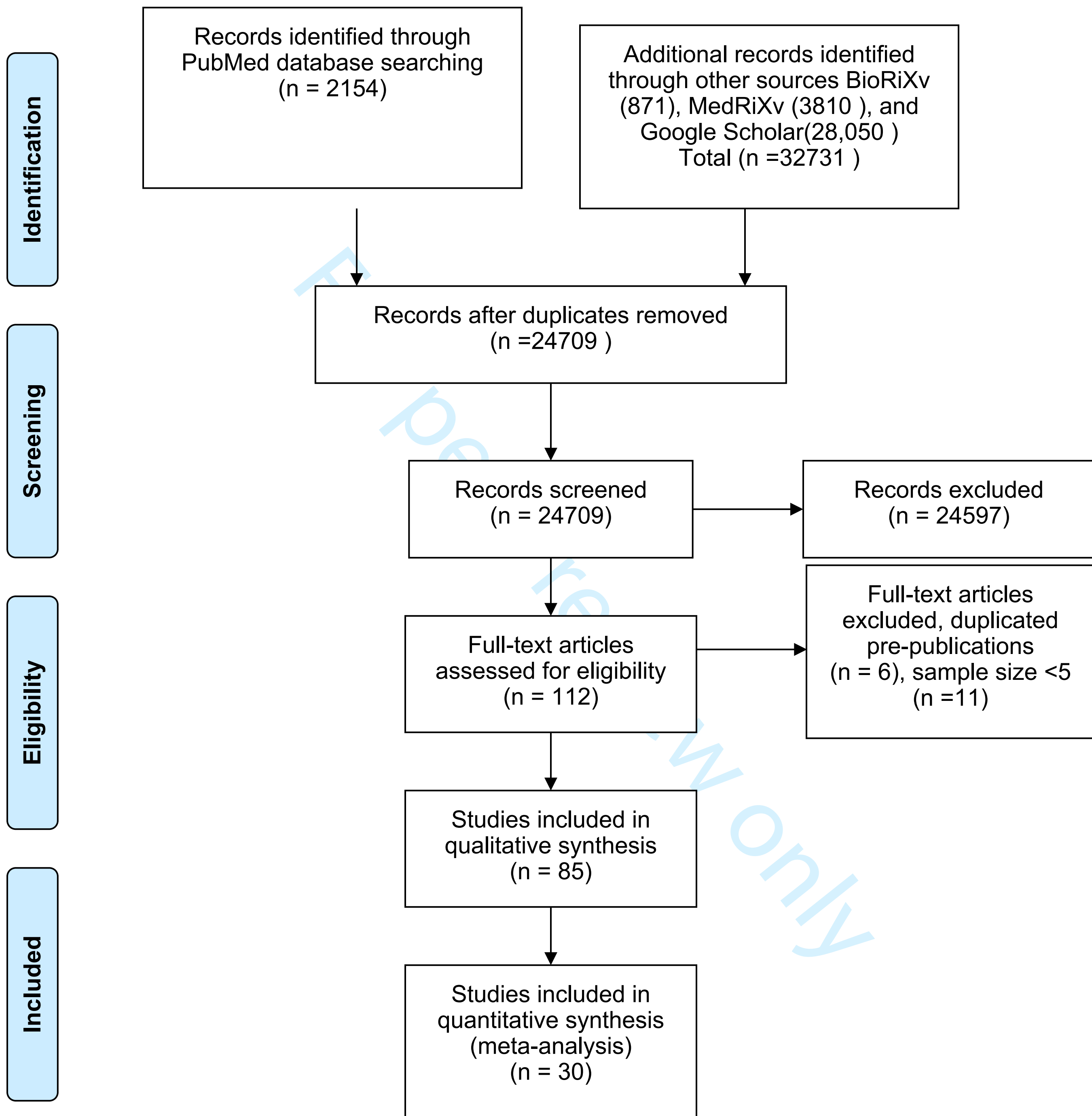

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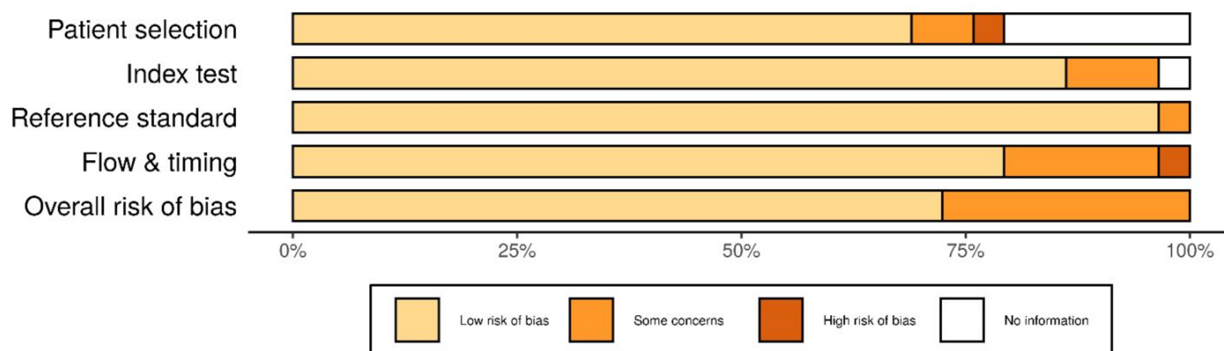
Suppl. Figure S2. Summary plot of risk of bias for each study included in meta-analysis according to QUADAS-2 domain.



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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6-7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Suppl
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Supp table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Supp Fig 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	figures
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figures
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	figures
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	figures
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7-10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10-14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	14

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Meta-Analysis of Robustness of COVID-19 Diagnostic Kits During Early Pandemic

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Meta-Analysis of Robustness of COVID-19 Diagnostic Kits During Early Pandemic

Chandrakumar Shanmugam, M.D., M.B.A.,[1#] Michael Behring, Ph.D.,[2#] Vishwas Luthra, M.B.B.S.,[3] Sixto M Leal Jr., M.D., Ph.D.,[2] Sooryanarayana Varambally,[2, 4] PhD, George J Netto, M.D.,[2, 4] and Upender Manne, M.S., Ph.D.[2, 4,*]

Department of Pathology[1], MNR Medical College & Hospital, MNR Nagar, Fasalwadi, Sangareddy, Telangana, India 502279

Department of Pathology[2], University of Alabama at Birmingham, Birmingham, Alabama, USA 35274

Department of General Medicine[3], Sri Guru Ram Das Institute of Medical Sciences and Research, Mehta Road, Vallah, Amritsar, Punjab, India 143501

O'Neal Comprehensive Cancer Center[4], University of Alabama at Birmingham, Birmingham, Alabama, USA 35274

# Authors contributed equally

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[*]**Author for Correspondence:**

Dr. Upender Manne
Professor of Pathology, Epidemiology and Surgery
University of Alabama at Birmingham
Birmingham, Alabama, USA 35274
Tel: 001 2052493895
Email: upendermanne@uabmc.edu

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ABSTRACT:

Background

Accurate detection of severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is necessary to mitigate the coronavirus disease-19 (COVID-19) pandemic. However, the test reagents and assay platforms are varied and may not be sufficiently robust to diagnose COVID-19.

Methods

We reviewed 85 studies (21,530 patients), published from five regions of the world, to highlight issues involved in the diagnosis of COVID-19 in the early phase of the pandemic. All relevant articles, published up to May 31, 2020, in PubMed, BioRiXv, MedRiXv, and Google Scholar, were included. We evaluated the qualitative (9749 patients) and quantitative (10,355 patients) performance of RT-PCR and serologic diagnostic tests for real-world samples, and assessed the concordance (5,538 patients) between test performance in meta-analyses. Synthesis of results was done using random effects modelling and bias was evaluated according to QUADAS-2 guidelines.

Results

The RT-PCR tests exhibited heterogeneity in the primers and reagents used. Of 1,957 positive RT-PCR COVID-19 participants, 1,585 had positive serum antibody (IgM +/- IgG) tests (sensitivity 0.81, 95%CI 0.66-.90). While 3,509 of 3581 participants RT-PCR negative for COVID-19 were found negative by serology testing (specificity 0.98, 95%CI 0.94-0.99). The chemiluminescent immunoassay exhibited the highest sensitivity, followed by ELISA and lateral flow immunoassays. Serology tests had higher sensitivity and specificity for laboratory-approval than for real-world reporting data.

Discussion

The robustness of the assays/platforms is influenced by variability in sampling and reagents. Serological testing complements and may minimize false negative RT-PCR results. Lack of

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standardized assay protocols in the early phase of pandemic might have contributed to the spread of COVID-19.

Strengths and limitations of this study:

- This study offers the first evaluation of COVID-19 test performance with consideration of the heterogeneity of RT-PCR primers.
- We compare the performance of manufacturer-based, laboratory/approval data to the performance of the same test kits in a real-world setting in the early phase of the pandemic.
- We perform a qualitative analysis of RT-PCR assays using 85 studies (21,530 patients), and a quantitative meta-analysis of RT-PCR vs. serum antibody assays in a sub-set of 30 publications (10,355 patients).
- Much of the information in the early pandemic was reported from China, and often from non-peer reviewed, preprint sources.
- Data measuring duration of the infection was not available in majority of included studies.

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INTRODUCTION

In December 2019, there was a cluster of unexplained pneumonia cases in Wuhan, China, and a novel coronavirus was identified as the causative agent.[1] The virus was named as severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), and the disease as corona virus disease-19 (COVID-19).[2] The clinical spectrum ranges from asymptomatic forms to acute respiratory failure and multi-organ dysfunction syndrome, coagulopathy, and death.[3,4] On March 11th 2020, the World Health Organization described the spread of these infections as a pandemic, which persists as a global crisis. Robust diagnostic tests are required to mitigate the spread of this virus and thereby to minimize the impact of COVID-19 on the health, economy, and social well-being of mankind.

The standard diagnosis of COVID-19 is based on clinical and radiologic evidence and viral genome detection by RT-PCR in respiratory samples.[5] Gene-specific primers are used in the RT-PCR assays; structural genes include *envelope* (*E*), *nucleocapsid* (*N*), and/or *spike (S)*-genes; non-structural genes include *RNA-dependent RNA polymerase* (*RdRp*) or *open reading frame1ab* (ORF1ab) [6,7] Some studies used only a single-gene specific primer, and others used multiple-gene primers. Since studies published in the early phase of the pandemic reported a 3%-41% range of false-negativity by RT-PCR, a repeat RT-PCR testing was suggested.[8,9] Furthermore, false negativity was attributed to either mutations in the regions to which the primers bind or to sampling and laboratory practices, including collection, transportation, and handling.[10] Timing of sample collection with respect to the course of infection and the sample type also influence test results.[11] Alternatively, the diagnosis can be made by detection of antigens (E, N, or S) and/or antibodies (IgM or IgG or both) in blood samples.[12] However, these tests have the potential for false positives owing to cross-reactivity with other human corona viruses.[13,14] Due to the unprecedented public health emergency, the FDA authorized, on June 1, 2020, EUA requests for more than 15 diagnostic and serologic tests. Though serology testing can detect the false positives of RT-PCR tests in clinically suspected patients, its value in COVID-19 diagnosis as a complementary assay in the

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mitigation of the pandemic is not well defined. However, given the complexities in COVID-19 testing, there is a need for a review of performance for tests commonly used.

In this systematic review and meta-analysis, we examine testing for the diagnosis of COVID-19 in the early pandemic and evaluate the sensitivity and specificity of serological tests relative to RT-PCR tests. Our objectives were to assess the uniformity of primer usage in RT-PCR assays and evaluate whether primers used in gold-standard RT-PCR tests affect the validity of serological tests. Furthermore, we compared the performance of serological tests/platforms in approval contrived/laboratory *vs.* real-world data.

METHODS

Literature Search

This research was accomplished according to standards outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[15] To find relevant studies, international databases, including PubMed, Embase, MedRiXv, BioRiXv, and Google Scholar, were searched for articles published until May 31, 2020. The following search terms were used (selected using English MeSH keywords and Emtree terms): [SARS-CoV-2 AND diagnosis] OR [2019-nCoV AND diagnosis]" OR ["COVID-19 AND diagnosis] and [SARS-CoV-2 AND RT-PCR] OR, [2019-nCoV AND RT-PCR]" OR ["COVID-19 AND RT-PCR] and [SARS-CoV-2 AND serology] OR [2019-nCoV AND serology]" OR ["COVID-19 AND serology]. Additional searches were performed for references listed in the included studies.

Eligibility Criteria

Relevant articles that reported diagnostic information for infected patients were included in the analysis. Pre-print articles with non-peer review were considered for inclusion. Articles were excluded if appropriate information was not reported or if they were in the Chinese language. Population sample sizes of <5 participants were not included; reviews and editorials were not considered. For meta-analysis and approval *vs*. real-world performance, studies that reported percent sensitivity/specificity without including patient numbers were also excluded.

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Data Extraction and Report Quality Evaluation

Two authors (CS and VL) screened and evaluated the literature independently. Discrepancies were resolved by consensus after evaluation by a third author (MB). The following were extracted for review and meta-analysis: journal name, authors, period of publication (end of May, 2020), location of study, total number of patients, tissue of origin for samples tested, whether samples were from upper or lower respiratory tract (or both), primers for RT-PCR, platforms for serology tests, and antibodies tested for serology. Counts of true positives, false negatives, true negatives, and false positives were used in the meta-analysis.

An author (MB) extracted and analyzed the approved testing kit performance data from the following sources: FDA EUA Authorized Serology Test Performance,[16] the Foundation for Innovative New Diagnostics (FIND) SARS-CoV-2 diagnostic pipeline,[17] and package inserts provided on company websites for each product. Real-world sample testing data from kits in meta-analyses were compared against the performance of the same kits, or platforms, reported in approval documentation. Variables abstracted were study authors/test developer, name of test, test platform, and true positives, false negatives, true negatives, and false positives for each antibody or antibody combination measured (IgM, IgG, IgA, combined, and Pan-Ig). Risk of bias within individual studies of meta-analysis was assessed using the QUADAS 2 tool for assessment of diagnostic studies.[18] QUADAS 2 has been developed specifically for evaluating bias in the meta-analyses of diagnostic test accuracy.

Patient and Public Involvement

Since we performed a meta-analysis and systematic review, it was not appropriate or possible to involve patients or the public in the design, conduct, reporting, or dissemination plans of our research.

Statistical Analysis

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Statistical analyses were performed with R version 6.3.2 (2019-12-12).[19] The package "meta" was used for meta-analyses.[20] Random effects models were used to measure sensitivity and specificity of outcomes across studies. Subgroup analysis was performed to evaluate the effect of assay, RT-PCR primer type, and setting (laboratory *vs*. real-world) upon serum test performance. Heterogeneity across studies and subgroups was evaluated using Cochrane's *Q* statistic, and residual heterogeneity was quantified as a percentage with the I^2 statistic. An I^2 measure of 0% shows no observed heterogeneity, with increasing values from 0%-100% indicating higher levels of heterogeneity. [21] An assumption of homogeneity was rejected for p-values < 0.1. The evaluation of publication bias was not possible using FDA and EU reported approval data.

RESULTS

Search Results and Population Characteristics

Our search generated 112 publications with potential relevance to the performance of COVID-19 diagnostic tests. After excluding duplicate publications, manuscripts that did not report numbers of patients used for sensitivity/specificity calculations and studies with a sample size of <5 patients, 85 studies were selected for qualitative synthesis of RT-PCR primer usage. From this set, a sub-set of 30 publications were selected for the quantitative meta-analysis of serologic *vs.* RT-PCR diagnostic testing for COVID-19 (**Table S1**). Ancillary analysis compared the performance of these 30 real-world studies to that reported in laboratory approval data from 47 diagnostic serum-based tests. In all, our qualitative synthesis of RT-PCR studies included 85 studies and 21,530 patients. From this synthesis, a group of 30 studies with 10,355 patients from 5 regions of the world were selected for meta-analysis and comparison to performance from laboratory approval data (**Fig S1**).

Uniformity of Primer Usage in RT-PCR Diagnostic Tests

We reviewed use of single primer of structural genes as compared to use of both structural and non-structural gene primers in 56 population-based studies with 9,872 participants.

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Overall, high proportions of studies employed both structural and non-structural gene primers in RT-PCR testing [55% (31 in 56) in studies and 56% (5484 in 9872) of total participants]. Additionally, 29 studies (11,658 patients) did not report RT-PCR primer data. Single markers were most frequently tested in China and North American studies (**Table 1**). In general, the most tested samples were from the upper respiratory tract, regardless of primer status. Sample source and location in the respiratory tract were not reported for 8-20% of patients, and this was more common for studies using single gene primer.

Meta-Analysis: RT-PCR *vs.* Serum Antibody Testing

In general, patient sera were tested for IgM and IgG antibodies. China was the region with the highest frequency of antibody testing, and lateral flow immunoassay (LFIA) and chemiluminescent immunoassay (CLIA) testing platforms were most often utilized. Of the 45 studies included in the qualitative synthesis, 30 manuscripts reported both serum antibody testing and RT-PCR testing for the same patients. Key characteristics of this population include: China as the regional location for research; lack of reporting of RT-PCR primer information for ~33% (10/30) of all studies; most studies used IgM and IgG serum-based antibody tests; and LFIA, CLIA, and enzyme-linked immunosorbent assay (ELISA) platforms were common across studies (**Table 2**).

We used the IgM+/-IgG serum antibody test since it was most commonly utilized across studies. Of 1,957 participants (pooled sensitivity 0.81, 95%CI 0.66-0.90) with a positive RT-PCR COVID-19 result, 1,585 were also detected as positive with serum antibody tests. Of 3,581 true negatives in RT-PCR, 3,509 negatives were also found by serum antibody testing (pooled specificity 0.98, 95%CI 0.94-0.99). For both models, heterogeneity between studies was significant (p<0.01 for both, I^2=97% and I^2 =98% for sensitivity and specificity, respectively).

Sub-analyses of differences based on the testing platform found that sensitivity between groups differed (p <0.0001), with CLIA tests performing best (0.99, 95%CI 0.97-0.99); ELISA as next-best (0.89, 95%CI 0.82-0.93); and LFIA as having the poorest sensitivity (0.67, 95%CI

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0.50-0.81). LFIA test sensitivity also showed heterogeneity between studies ($p<0.01$, I^2 95%). For IgM/IgG tests, specificity did not differ significantly by platform (p= 0.06). However, a performance trend followed sensitivity, with LFIA underperforming (**Figure 1 and Figure S2.1**).

Serum Antibody Testing Performance: Approval Data vs. Real-World Data

For manufacturer-based, laboratory approval data, IgM+/-IgG testing detected COVID-19 positivity for 1,045 of 1,068 RT-PCR-determined "true" positive patients (sensitivity 0.98, 95%CI 0.92-1.0). In the same group, serum testing correctly identified 1,928 of 1,967 (specificity 0.98, 95%CI 0.95-.099) true negatives by RT-PCR. For both models (sensitivity and specificity), there was evidence of heterogeneity (p <0.01 for both and I^2=93% and I^2=94% for sensitivity and specificity, respectively).

We evaluated IgM+/-IgG serum test performance in subgroup analyses comparing laboratory approval performance data to real-world performance in study data. In manufacturer data presented for approval, serum antibody testing detected 1,047 of 1,068 "true positive" cases of COVID-19 (sensitivity 0.98, 95%CI 0.92-1.0). Real-world use of serum IgM+/-IgG testing was evident for 2,450 of 3,025 participants diagnosed with COVID-19 by RT-PCR (sensitivity 0.81, 95% CI 0.66-0.90). For both groups, there was heterogeneity between studies (p <0.01 for both, I^2=93% and I^2 =97% for approval and real-world specificity, respectively) (**Figure 2 and Figure S2.2**). In addition, the overall sensitivity between approval and real-world testing groups differed significantly (Q=8.37, p=0.004). An analysis of specificity by the same subgroups found no significant difference between laboratory approval and real-world data. Laboratory data identified 1,928 of 1,967 participants with true COVID-19 negative status (specificity 0.98, 95% CI 0.95-0.99). Real-world data found 5,437 of 5,548 true negatives (specificity 0.98, 95% CI, 0.96-0.99) (analysis not shown).

Since, in IgM+/-IgG tests, there were differences in sensitivity between platforms, we evaluated the effect of approval-based data *vs.* real-world data by the type of platform. In an analysis stratified for ELISA, CLIA, and LFIA, there was no significant difference in specificity

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between approval and real-world data (data not shown). However, for ELISA tests, real-world capacity to detect true positives was lower than in laboratory-based analyses. In real-world studies, the sensitivity of ELISA was 0.89 (95% CI, 0.82-0.93), different from laboratory sensitivity for the same platform (0.94, CI95% 0.91-0.96, Q =4.74, p=0.03). The LFIA platform also showed a trend of lower real-world sensitivity (0.67, 95% CI, 0.50-0.81) compared to laboratory approval sensitivity (0.99, CI95% 0.90-0.99, Q =8.56, p 0.003). Laboratory/real-world groups for CLIA platforms were too small to be tested reliably (1 and 2 groups, respectively).

Serum Antibody Testing Performance: Effect of Primer Choice on Test Validity

To evaluate the reliability of RT-PCR as a gold standard for serum-based test performance, we tested the consequences of using structural and non-structural primers in RT-PCR reference testing of serum. Analyses were divided into three subgroups based on antibody targets: IgM, IgG, and IgG+/-IgM combined. In IgM and combined IgG+/-IgM testing, the primer choice had no significant influence on sensitivity or specificity. However, for IgG antibody tests, use of both a structural and a non-structural gene-specific primers in RT-PCR resulted in reduced sensitivity for serum testing (**Figure 3 and Figure S2.3**, Q=6.17, p=0.013). Furthermore, although not statistically significant, the sensitivity of both IgM and IgG+/-IgM combined data sets was lower when using a referent RT-PCR test with both primer types.

DISCUSSION

Because of the highly infectious nature of COVID-19, a prompt, accurate, and early diagnosis is necessary to deal with the ongoing pandemic, for such diagnoses can help reduce the spread of infection and its associated risk for mortality. Currently, the COVID-19 diagnosis is generally based on RT-PCR assays.[8] Alternative methods such as antigen- and antibody-based serology tests, although available, have uncertain value. The current systematic review and meta-analysis addresses the challenges encountered in the diagnosis of COVID-19 by various methods. It also analyzes differences between the FDA-approved EUA data and real-

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world data. There is worldwide non-uniformity in the performance of RT-PCR, including the number and types of primers and reagents used for COVID19 diagnosis, which raise questions about its generalized applicability. Similarly, the studies based on serological tests showed diagnostic inaccuracies owing to individual differences in mounting an immune response as well as dependency on the time duration after the onset of symptoms. Overall, the sensitivity between RT-PCR and serology tests was 0.81 (95% CI, 0.66-0.90), and specificity was 0.98 (95% CI, 0.94-0.99). Among the various platforms for serodiagnosis, the highest sensitivity was exhibited by ELISA, followed by CLIA and LFIA. Furthermore, use of primers (structural, non-structural, or both) had a variable effect on sensitivity based on antibody targets. Sensitivity was significantly higher for IgG serology tests using structural-primer-only RT-PCR tests as a referent. Serology tests had higher sensitivity for approval-based data than for real-world reporting. This difference was significant for ELISA-based platforms, and a non-significant trend towards inflated approval-based sensitivity was evident for both CLIA and LFIA platforms. These observations highlight the inconsistencies/challenges in the COVID-19 diagnosis by RT-PCR, which is the current gold standard, as well as in serologic testing.

For RT-PCR assays, the targets in SARS-CoV-2 include structural genes like *E, N* and *S*, and nonstructural genes, including that for *RdRp* or *ORF1ab.*[22] In the early phase of the pandemic, some studies used a two-step diagnosis that included an initial screening phase using structural genes followed by a confirmatory phase using nonstructural genes. [6,7,23] The test is considered positive when both structural and non-structural markers are positive.[24,25] However, currently both types of primers are used simultaneously to diagnose COVID-19. The viral load or copy number of the viral genome is expressed as a Ct-value, which when <37 is indicative of a positive test, and a value of ≥ 40 is considered negative. A Ct value between >37 and < 40 requires repetition of RT-PCR analysis to confirm the diagnosis.[24] However, the Ct value range varies widely according to assays and laboratory practices. A COVID-19-RdRp/Hel assay has a higher sensitivity than a conventional RdRp-P2 assay irrespective of the type of sample.[26] Overall, higher proportions of studies (58%) employed both structural

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and non-structural gene primers in RT-PCR testing. Single markers were used in some Chinese and North American studies. These findings are indicative of non-uniformity in the RT-PCR methodology. We note that half of the positive, symptomatic patients became negative by the second week, when they became asymptomatic. In contrast, the asymptomatic, positive patients became negative two days after hospital admission, indicating the importance of a temporal factor in COVID-19 diagnosis by RT-PCR.[27,28]

Published in the early phase of the pandemic, 11 of 85 studies had clinically suspected COVID-19 patients. In these studies, the average test positivity by RT-PCR, regardless of the sample source, was 44% (Supplementary Table 1), and test sensitivity was influenced by sample source (upper *vs*. lower respiratory *vs.* other samples), issues related to testing performance, and delay after onset of symptoms.[29] In the early phase of the COVID-19 pandemic, for studies evaluating suspected COVID-19 cases, the total positive RT-PCR for throat swabs was in the range of 30–60% at initial presentation.[8,30] One study reported a yield of 72-93% positive cases for lower respiratory samples (bronchioalveolar lavage and sputum) as compared to 32-63% positivity for upper respiratory samples (oral and nasopharyngeal swabs) and 29% for stool samples.[29] Hence, a negative COVID-19 test based only on an upper respiratory sample at a single time point is questionable. For most studies, the testing sample was from the upper respiratory tract, regardless of primer type used. However, the sample source was not reported for 8-20% of patients, which was more common for studies using only structural gene primers. For stool samples testing positive for COVID-19, 66.7% also tested positive on pharyngeal swabs. Of the stool samples, 64.3% remained positive after pharyngeal clearance of the virus.[31] In contrast, none of the patients showed a positive test on upper respiratory samples after the anal swabs tested negative.[31] These findings raise concerns about whether patients with negative respiratory swabs are truly virus-free, and sampling of additional body sites is needed. As determined by various studies, the performance of the RT-PCR depends on usage of comparable protocols, including primers

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and reagents.[32] Additionally, it is unknown whether the currently used RT-PCR primers detect all SARS-Cov-2 strains.

The specific immune response to SARS-CoV-2 can be measured by serological testing. Several rapid serological tests, including point-of-care tests, are being developed. Even though some of these tests have been approved by the FDA through EUA, their accuracy needs to be validated.[33] A minimum of 1–2 weeks after the onset of infection is needed for seroconversion. Hence, antibody testing is of no value in the early phase of infection. Additionally, its value is limited by its cross-reactivity with other coronaviruses.[34,35] The initial RT-PCR positivity during the early stages (<15 days) of SARS-CoV-2 infection declines to 66.7% in the later phase (15-39 days), during this period, the antibody test can supplement RT-PCR in the diagnosis of COVID-19.[34,35] Additionally, serology testing becomes valuable for clinically suspected and RT-PCR negative (false-negative) individuals.

This research has limitations. Due to the dynamic reporting of COVID-19 testing research and inconsistencies in reporting of predictive variables across studies, bias in sampling may have some effect on our results. Patient flow analysis suggests that lack of consistent RT-PCR reference standard given to patients in the same study, as well as the unclear reporting of patient selection methods could contribute to bias in these results (**Fig. S3**). In addition, the observed heterogeneity between studies in the meta-analysis suggests that we must consider the possibility that the differences in results may be due to chance. Lastly, it is questionable to compare two separate testing methods of RT-PCR and seroprevalence in sensitivity/specificity analysis. In particular, given the relationship between time since diagnosis and accuracy of serology testing, a contributor to the observed differences in performance is time. Furthermore, because of each diagnostic kit having differing cut points for positive/negative, threshold effect as a source of heterogeneity in sensitivity and specificity cannot be ruled out.

The effective containment of COVID-19 involves accurate diagnoses and isolation of SARS-CoV-2-infected persons. Robustness of the assays/platforms is determined by

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variability of the samples, primers, and reagents used. Serological tests alone are of value only during the latter times of infection; however, they complement RT-PCR when used in conjunction and minimize false negative RT-PCR results. Additionally, some of the approved serological assays/platforms, particularly those developed using contrived/laboratory data, perform poorly when applied to real-world samples. We are currently in a new phase of the pandemic, and there is a need for a reliable/robust diagnostic test to mitigate the spread of COVID-19.

Our analyses of studies published in the early-phase of the pandemic have highlighted issues related to COVID-19 diagnosis that need to be addressed as follows: 1) The high mutational rate exhibited by the SARS-CoV-2 virus may lead to the generation of new strains. Therefore, like for influenza virus, the existing diagnostic kits need to be modified constantly to optimize the detection of new strains; 2) Though RT-PCR diagnosis of COVID-19 is the gold standard, its combination with a serologic test may increase the accuracy of SARS-CoV-2 detection; 3) Approval agencies must account for the type of data (contrived versus real world) presented by diagnostic kit developer; 4) Although agencies employed EUA processes for the approval of diagnostic kits, there is a need to monitor their performance and assess their robustness in real-world samples, to permit continued use of these kits; and 5) Standardized assay protocols need to be developed and continually updated to mitigate the COVID-19 pandemic.

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DATA SHARING

Data is available upon request

ETHICAL APPROVAL STATEMENT

Not applicable

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AUTHOR CONTRIBUTIONS

CS, MB, and UM made substantial contributions to the conception and design of the work. CS, MB and VL contributed to the acquisition of the study data. Data were analyzed by CS, MB and UM. MB was responsible for the statistical analysis. CS, MB, SML, SV, GJN, and UM contributed to the interpretation of the data, and to drafting and revising of the manuscript. CS, MB and VL have accessed and verified the data. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work. All authors have read and approved the manuscript. All authors declare no competing interests.

REGISTRATION AND PROTOCOL

The work in this review was not registered and has no available review protocol.

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Table 1. Characteristics of the studies included in qualitative analysis

	Total Studies	Total pop.	Structural gene primers		Structural and non-Structural gene primers		Non-Structural gene primers		Not reported	
			N studies	N pop.	N studies	N pop.	N studies	N pop.	N studies	N pop.
Total	**85**	**21530**	**22**	**4265**	**31**	**5484**	**3**	**123**	**29**	**11658**
Location										
Asia (excl. China)	6	378	2	53	4	325				
China	28	12187	8	1802	17	3047	3	123	24	7215
Europe	12	5757	4	528	8	993			4	4236
North America	10	3001	8	1882	2	1119				
Global		207							1	207
Primers										
N -single	11	2016	11	2016						
E -single	4	759	4	759						
S -single	1	412	1	412						
N, E	2	226	2	226						
S, N	4	852	4	852						
ORF1Ab, single	2	59					2	59		
RdRp, single	1	64					1	64		
E+ORF1Ab	2	1119			2	1119				
E + RdRp	2	259			2	259				
M, E	1	48			1	48				
N+ORF1Ab	14	2703			14	2703				
N + E + RdRp	4	333			4	333				
S, N, E, RdRp, ORF1ab	1	13			1	13				
N, E, ORF1ab	1	33			1	33				
N, RNAse P	1	190			1	190				
S, N, RdRp, ORF1ab, E, M	1	52			1	52				
N, S, RdRp	1	273			1	273				
N, E, S, RdRp	2	349			2	349				
S, ORF1Ab	1	112			1	112				
Sample Source										
Upper Respiratory	23	6748	3	575	9	2633	1	64	10	3476
Upper & Lower Respiratory	1	52			1	52				
Upper Respiratory + Other*	9	751	3	368	2	44	1	38	3	301
Lower Respiratory + Other*	1	273			1	273				
Upper Respiratory + Serum	20	6407	7	1473	9	1432			4	3502
Upper Respiratory + Serum + Other*	4	941	2	840	1	80	1	21		
Upper & Lower Respiratory + Other*	4	678	1	280	3	398				
Upper & Lower Respiratory + Serum + Other*	2	518			1	132			1	386
Serum	18	2376	6	729	4	440			8	1207
Other*	1	199							1	199
Not reported	2	2587							2	2587

* Other = bronchioalveolar lavage, feces, urine, neonatal, amniotic fluid, and breast milk. N pop. = patient population

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Table 2. Characteristics of studies included in quantitative meta-analysis

	N studies	N pop.
Total	30	10355
Location		
Asia (excl. China)	2	261
China	19	6375
Europe	7	2900
North America	2	819
PCR primers		
Structural		
N, single	5	1084
E, single	1	49
N, E	1	201
N, S	2	408
Structural and Non-structural		
with ORF1Ab	8	1115
with RdRp	2	186
N, RNAse P	1	190
not reported	10	7122
Ab tested		
IgG	2	220
IgM + IgG	25	7828
IgA + IgG +IgM	1	208
IgA + IgG	1	37
not reported	1	2062
Serum Ab		
CLIA	8	3705
ELISA	8	1908
LFIA	10	3800
CLIA + ELISA	2	548
LFIA + ELISA	1	80
not reported	1	314

CLIA = chemiluminescent immunoassay, LFIA = lateral flow immunoassay, ELISA = enzyme-linked immunosorbent assay

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Figure Legends

Figure 1. Comparison of Performance (Sensitivity and Specificity) of Serology Tests (IgM/IgG) Based on Assay Platforms

Figure 2. Comparison of Sensitivity of Laboratory setting versus Real World setting of RT-PCR and Serology (IgM/IgG) kits

Figure 3. The Effect of Primer Choice in RT-PCR Referent on Sensitivity of Tests based on Serum IgG

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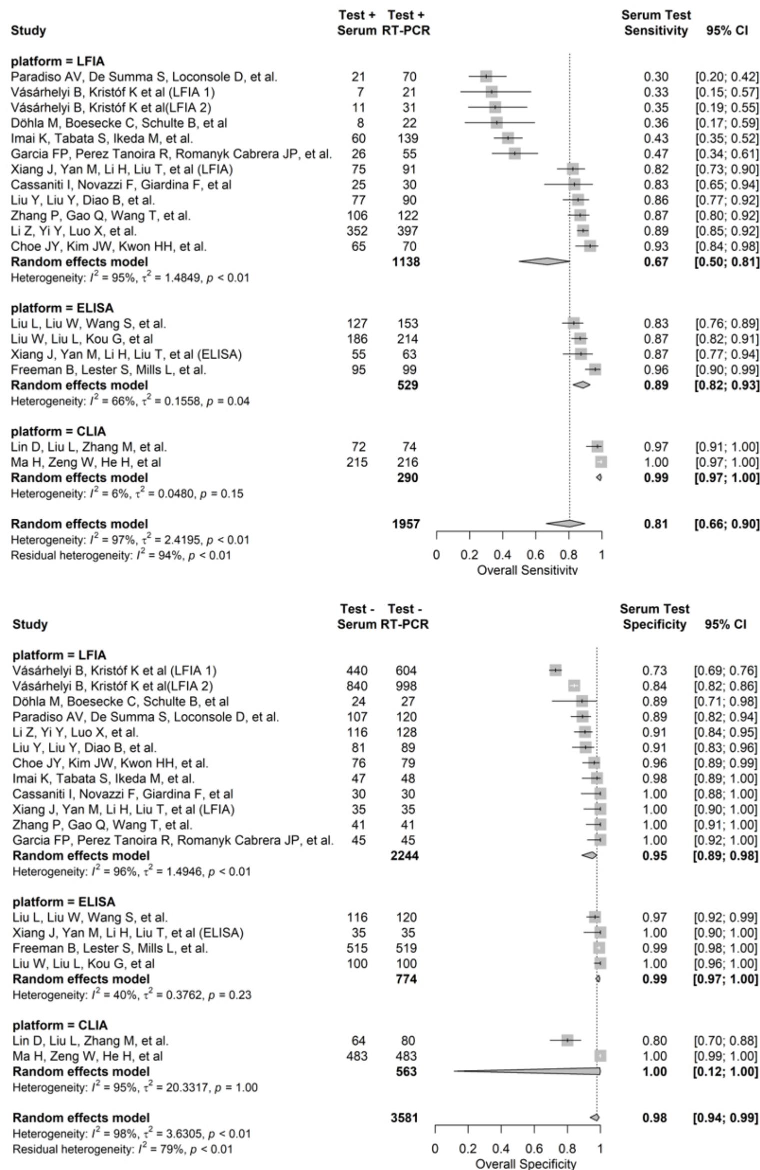

Figure 1. Comparison of Performance (Sensitivity and Specificity) of Serology Tests (IgM/IgG) Based on Assay Platforms

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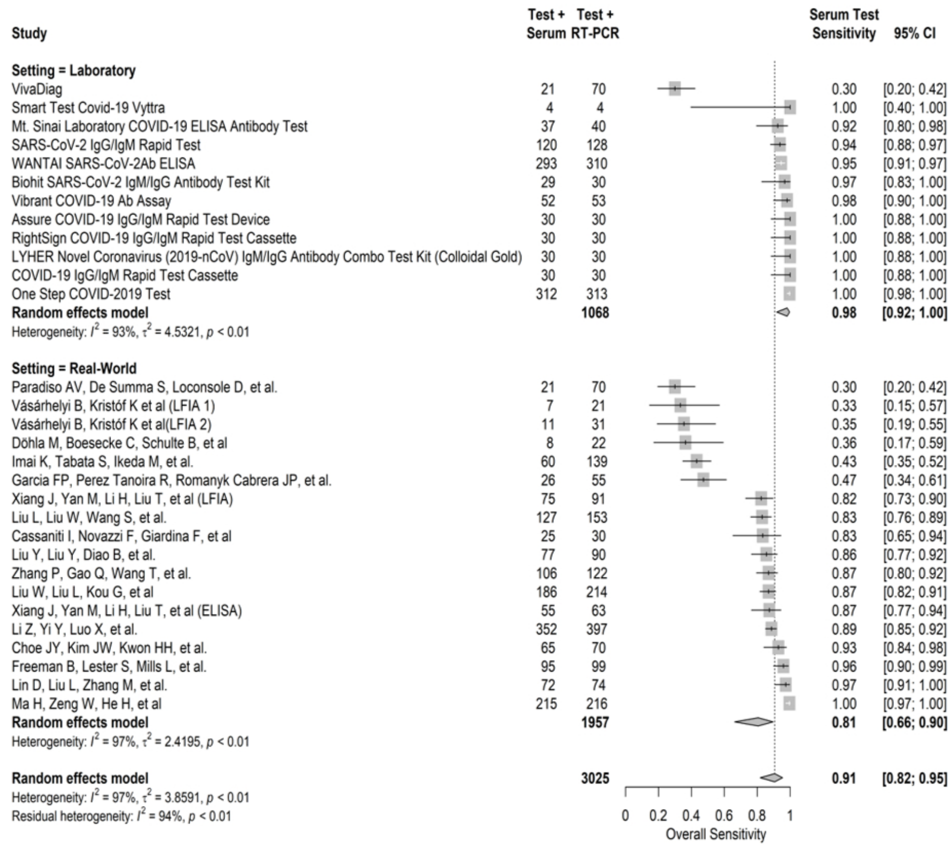

Figure 2. Comparison of Sensitivity of Laboratory setting versus Real World setting of RT-PCR and Serology (IgM/IgG) kits

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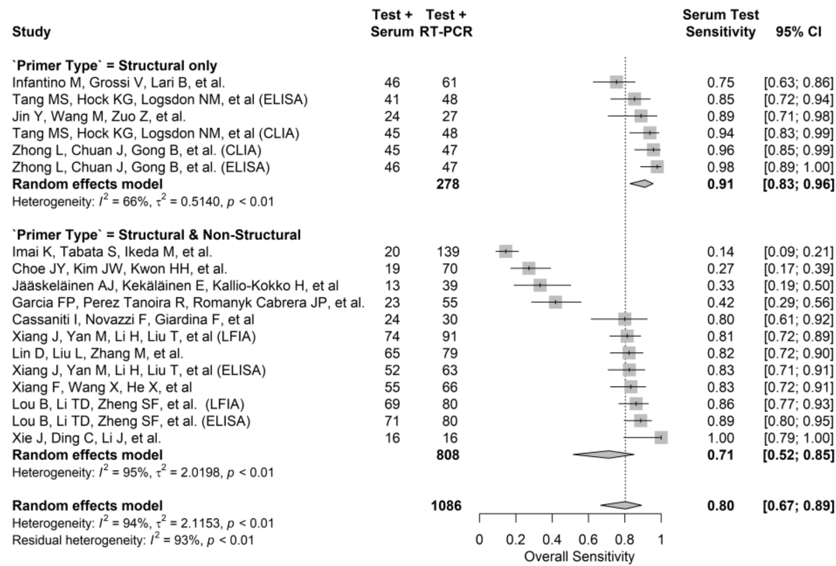

Figure 3. The Effect of Primer Choice in RT-PCR Referent on Sensitivity of Tests based on Serum IgG

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Meta-Analysis of Robustness of COVID-19 Diagnostic Kits During Early Pandemic. Supplemental information

Table S1. Description of 55 Studies Included for Qualitative Synthesis (gray) and 30 Studies Included in the Meta-Analysis (tan)

Journal	Authors	Region	N (patients)	Percent COVID-19 positivity	Sample origin	RT-PCR primers	RT-PCR primer type	RT-PCR/Serology platform	Serology targeted antibody	Study included in meta-analysis
J Clin Virol	Rahman H, Carter I, Basile K, et al. [36]	Asia	52		UR+LR	S, N, E, RdRp, ORF1ab, M	Both	RT-PCR		No
Exp Neurobiol	Won J, Lee S, Park M, et al. [37]	Asia	12		UR	N, E, S, RdRp	Both	RT-PCR		No
Jpn J Infect Dis.	Okamaoto K, Shirato K, Nao N, et al. [38]	Asia	25		UR	N, E	Structural	RT-PCR		No
J Med Virol	Choe JY, Kim JW, Kwon HH, et al. [39]	Asia	149	0.47	Serum	E, RdRp	Both	RT-PCR /CLIA	IgM + IgG	Yes
Lancet Infect Dis	Yong SEF, Anderson DE, Wei WE, et al. [40]	Asia	28		UR + Serum	N, single	Structural	RT-PCR /ELISA	IgG	No
J Clin Virol	Imai K, Tabata S, Ikeda M, et al. [41]	Asia	112		UR + Serum	S, ORF1Ab	Both	RT-PCR /LFIA	IgM + IgG	Yes
Emerg Microbes Infect	Xu Y, Xiao M, Liu X, et al. [42]	China	6		Serum			ELISA + LFIA	IgM	No
Radiology	Ai T, Yang Z, Hou H, Zhan C, et al. [8]	China	1014	0.59	UR			RT-PCR		No
NEJM	Cao B, et al.[43]	China	199		Other			RT-PCR		No
Radiology	Bai HX, Hsieh B, Xiong Z, et al.[44]	China	256		UR			RT-PCR		No
Lancet	Chen H, Guo J, Wang C, et al. [45]	China	9		UR + Other			RT-PCR		No
AJR Am J Roentgenol	Liu D, Li L, Wu X, et al. [46]	China	15		UR			RT-PCR		No
Eur J Radiol	Long C, Xu H, Shen Q, et al. [47]	China	87		UR			RT-PCR		No
Pediatr Pulmonol	Xia W, Shao J, Guo Y, Peng X, Li Z, Hu D. [48]	China	20		UR			RT-PCR		No
Am J Obstetr Gynecol	Yan J, Guo J, Fan C, et al. [49]	China	116	0.56	Other			RT-PCR		No
J Hosp Infect	Ye G, Li Y, Lu M, et al. [50]	China	91	0.52	UR			RT-PCR		No
J Med Virol	Zhang J, Wang S, Xue Y. [51]	China	14		UR + Other			RT-PCR		No
Respir Res	Zhang G, Zhang J, Wang B, Zhu X, Wang Q, Qiu S. [52]	China	95		UR			RT-PCR		No

Lancet	Zhou F, Yu T, Du R, et al. [53]	China	191		UR			RT-PCR		No
J Clin Microbiol	Liu W, Liu L, Kou G, et al.[54]	China	314		UR + Serum			RT-PCR	IgM + IgG	Yes
J Med Virol	Li, Y et al. [55]	China	610	0.40	UR	N, ORF1Ab	Both	RT-PCR		No
medRxiv	Diao B, Wen K, Chen J, et al.[56]	China	239		UR + Serum	N, ORF1Ab	Both	RT-PCR		No
J Clin Microbiol	Chan JF, Yip CC, To KK, et al. [26]	China	273		UR + Other	N, S, RdRp	Both	RT-PCR		No
Nature Microbiol	Kong WH, Li Y, Peng MW, et al. [57]	China	640		UR	N, ORF1Ab	Both	RT-PCR		No
Front Med	Liu W, Wang J, Li W, Zhou Z, Liu S, Rong Z. [58]	China	38	0.53	UR + Other	N, ORF1Ab	Both	RT-PCR		No
Int J Biol Sci	Lo IL, Lio CF, Cheong HH, et al. [59]	China	10		UR + LR + Other	N, ORF1Ab	Both	RT-PCR		No
Travel Med Infect Dis	Wu J, Liu J, Li S, Peng Z, et al. [60]	China	132		UR + LR + Serum + Other	N, E, RdRp	Both	RT-PCR		No
Int J Infect Dis	Xu T, Chen C, Zhu Z, et al. [61]	China	51		UR + LR + Other	N, ORF1Ab	Both	RT-PCR		No
J Med Virol	Yuan Y, Wang N, et al. [62]	China	6		UR + Other	N, E, RdRp	Both	RT-PCR		No
AJR Am J Roentgenol	Cheng Z, Lu Y, Cao Q, et al. [63]	China	33	0.33	UR	N, E, ORF1ab	Both	RT-PCR		No
Arch Pathol Lab Med	Schwartz, DA [64]	China	38		UR + Other	ORF1Ab, single	Non-structural	RT-PCR		No
Radiology	Wong HYF, Lam HYS, Fong AH, et al. [65]	China	64		UR	RdRp, single	Non-structural	RT-PCR		No
Chin Med J	Ling Y, Xu SB, Lin YX, et al. [66]	China	292		UR + Other	E, single	Structural	RT-PCR		No
Clin Infect Dis	Zhao R, Li M, Song H, et al. [67]	China	412		UR	S, single	Structural	RT-PCR		No
medRxiv	Ma H, Zeng W, He H, et al.[68]	China	699		UR + Serum			RT-PCR /CLIA	IgM + IgG	Yes
medRxiv	Cai X, Chen J, Hu J, et al.[69]	China	443		Serum			RT-PCR /CLIA	IgM + IgG	Yes
medRxiv	Qian C, Zhou M, Cheng F, et al. [70]	China	2062					RT-PCR /CLIA	IgM + IgG	Yes
J Infect Dis	Zhang G, Nie S, Zhang Z, Zhang Z. [71]	China	112		UR + Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	No

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medRxiv	Lin D, Liu L, Zhang M, et al.[72]	China	159		UR + Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	Yes
J Med Virol	Xie J, Ding C, Li J, et al. [73]	China	56		UR +Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	Yes
Nature Med	Long QX, Liu BZ, Deng HJ, et al. [74]	China	285		UR+ Serum	S, N	Structural	RT-PCR /CLIA	IgM + IgG	No
Int J Infect Dis	Jin Y, Wang M, Zuo Z, et al. [75]	China	76	0.57	Serum	N, single	Structural	RT-PCR /CLIA	IgM + IgG	Yes
Emerg Microbes Infect	Zhang W, Du RH, Li B, et al. [76]	China	278		UR + Other			RT-PCR /ELISA	IgM + IgG	No
Clin Infect Dis	Zhao J, Yuan Q, Wang H, et al.[34]	China	386		UR + LR + Serum			RT-PCR /ELISA	IgM + IgG	Yes
Euro Surveill	Perera RA, Mok CK, Tsang OT, et al. [77]	China	51		Serum			RT-PCR /ELISA	IgM + IgG	Yes
Clin Infect Dis	Xiang F, Wang X, He X, et al. [78]	China	216		UR + Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
medRxiv	Xiang J, Yan M, Li H, Liu T, et al. [79]	China	154		Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
medRxiv	Liu L, Liu W, Wang S, et al.[80]	China	238		UR + Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
Clin Infect Dis	Guo L, Ren L, Yang S, et al. [12]	China	208	0.39	Serum	N, single	Structural	RT-PCR /ELISA	IgM + IgA + IgG	Yes
Sci China Life Sci	Zhong L, Chuan J, Gong B, et al.[81]	China	347		UR NP/OP + Serum + Other	N, S	Structural	RT-PCR /ELISA + CLIA	IgM + IgG	Yes
Eur Respir J	Lou B, Li TD, Zheng SF, et al. [82]	China	80		UR + LR + Serum + Other	N, ORF1Ab	Both	RT-PCR /ELISA + LFIA + CLIA	IgM + IgG	Yes
J Med Virol	Du Z, Zhu F, Guo F, Yang B, Wang T. [83]	China	60		Serum			RT-PCR /LFIA	IgM + IgG	No
J Infect	Pan Y, Li X, Yang G, et al. [84]	China	105		Serum			RT-PCR /LFIA	IgM + IgG	No
J Med Virol	Li Z, Yi Y, Luo X, et al.[85]	China	525					RT-PCR /LFIA	IgM + IgG	Yes
medRxiv	Liu Y, Liu Y, Diao B, et al.[86]	China	179		UR + Serum			RT-PCR /LFIA	IgM + IgG	Yes

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Emerg Microbes Infect	Yongchen Z, Shen H, Wang X, et al. [87]	China	21		UR + Serum + Other	ORF1Ab, single	Non-structural	RT-PCR /LFIA	IgM + IgG	No
Anal Chem	Chen Z, Zhang Z, Zhai X, et al.[88]	China	19		UR + Serum	N, single	Structural	RT-PCR /LFIA	IgG	Yes
medRxiv	Zhang P, Gao Q, Wang T, et al.[89]	China	163		UR + Serum	N, single	Structural	RT-PCR /LFIA	IgM + IgG	Yes
JAMA	Grasselli G, Zangrillo A, Zanella A, et al. [90]	Europe	1591		UR			RT-PCR		No
Radiology	Caruso D, Zerunian M, Polici M, et al. [91]	Europe	158	0.39	UR	N, E, RdRp	Both	RT-PCR		No
Travel Med Infect Dis	Lagier JC, Colson P, Tissot Dupont H, et al. [92]	Europe	337		UR +LR+ Other	N, E, S, RdRp	Both	RT-PCR		No
J Clin Virol	van Kasteren PB, van der Veer B, van den Brink S, et al. [93]	Europe	13		UR	S, N, E, RdRp, ORF1ab	Both	RT-PCR		No
Int J Mol Sci	Toptan T, Hoehl S, Westhaus S, et al. [94]	Europe	48		UR	M, E	Both	RT-PCR		No
Trop Med Infect Dis	Amrane S, Tissot-Dupont H, Doudier, et al. [95]	Europe	280		UR + LR + Other	E, single	Structural	RT-PCR		No
J Clin Microbiol	Lambert-Niclot S, Cuffel A, Le Pape S, et al. [96]	Europe	138		UR	E, single	Structural	RT-PCR		No
J Med Virol	Infantino M, Grossi V, Lari B, et al. [97]	Europe	61		Serum	S, N	Structural	RT-PCR /CLIA	IgM + IgG	Yes
Euro Surveill	Jääskeläinen AJ, Kekäläinen E, Kallio-Kokko H, et al. [98]	Europe	37		Serum	N, E, RdRp	Both	RT-PCR /ELISA	IgA + IgG	Yes
J Infect	Tré-Hardy M, Blairon L, Wilmet A, et al. [99]	Europe	182		Serum			RT-PCR /ELISA + CLIA	IgA + IgG	No
Orvo Hetil	Vásárhelyi B, Kristóf K, Ostorházi E, Szabó D, Prohászka Z, Merkely B. [100]	Europe	2310	0.06	UR + Serum			RT-PCR /LFIA	IgM + IgG	Yes
Infect Ecol Epidemiol	Hoffman T, Nissen K, Krambrich J, et al.[101]	Europe	153		Serum			RT-PCR /LFIA	IgM + IgG	Yes
J Med Virol	Cassaniti I, Novazzi F, Giardina F, et al. [102]	Europe	110		UR + Serum	E, RdRp	Both	RT-PCR /LFIA	IgM + IgG	No
medRxiv	Garcia FP, Perez Tanoira R, Romanyk Cabrera JP, et al. [103]	Europe	100		Serum	N, ORF1Ab	Both	RT-PCR /LFIA	IgM + IgG	Yes

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medRxiv	Paradiso AV, De Summa S, Loconsole D, et al.[104]	Europe	190		UR + Serum	N, RNAse P	Both	RT-PCR /LFIA	IgM + IgG	Yes
Public Health	Döhla M, Boesecke C, Schulte B, et al. [105]	Europe	49		Serum	E, single	Structural	RT-PCR /LFIA	IgM + IgG	Yes
J Emerg Infect Dis	Okba NMA, Muller MA, Li W, et al. [106]	Global	207		Serum			RT-PCR /ELISA	IgM + IgG	No
J Clin Virol	Smithgall MC, Scherberkova I, Whittier S, Green DA. [107]	North America	113		UR	E, ORF1Ab	Both	RT-PCR		No
J Med Virol	Pujadas E, Ibeh N, Hernandez MM, et al. [108]	North America	1006		UR	E, ORF1Ab	Both	RT-PCR		No
J Infect Dis	Burbelo PD, Riedo FX, Morishima C, et al. [109]	North America	100		Serum	N, single	Structural	RT-PCR		No
Am J Obstet Gynecol MFM	Penfield CA, Brubaker SG, Limaye MA, et al. [110]	North America	32		UR + Other	N, single	Structural	RT-PCR		No
medRxiv	Wyllie AL, Fournier J, et al. [111]	North America	44		UR + Other	N, single	Structural	RT-PCR		No
J Appl Lab Med	Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. [112]	North America	235		Serum	N, single	Structural	RT-PCR /CLIA	IgM + IgG	No
Clin Chem	Tang MS, Hock KG, Logsdon NM, et al. [113]	North America	201		UR + LR + Serum	N, E	Both	RT-PCR /CLIA + ELISA	IgG	Yes
medRxiv	Randad PR, Pisanic N, Kruczynski K, et al. [114]	North America	493		UR + Serum + Other	N, single	Structural	RT-PCR /ELISA	IgM + IgA + IgG	No
JMIR Public Health Surveill	Sullivan PS, Sailey C, Guest JL, et al. [115]	North America	159		UR + Serum	S, N	Structural	RT-PCR /ELISA	IgM + IgA + IgG	No
bioRxiv	Freeman B, Lester S, Mills L, et al.[116]	North America	618		UR NP/OP + Serum	N, single	Structural	RT-PCR /ELISA	IgM + IgG	Yes

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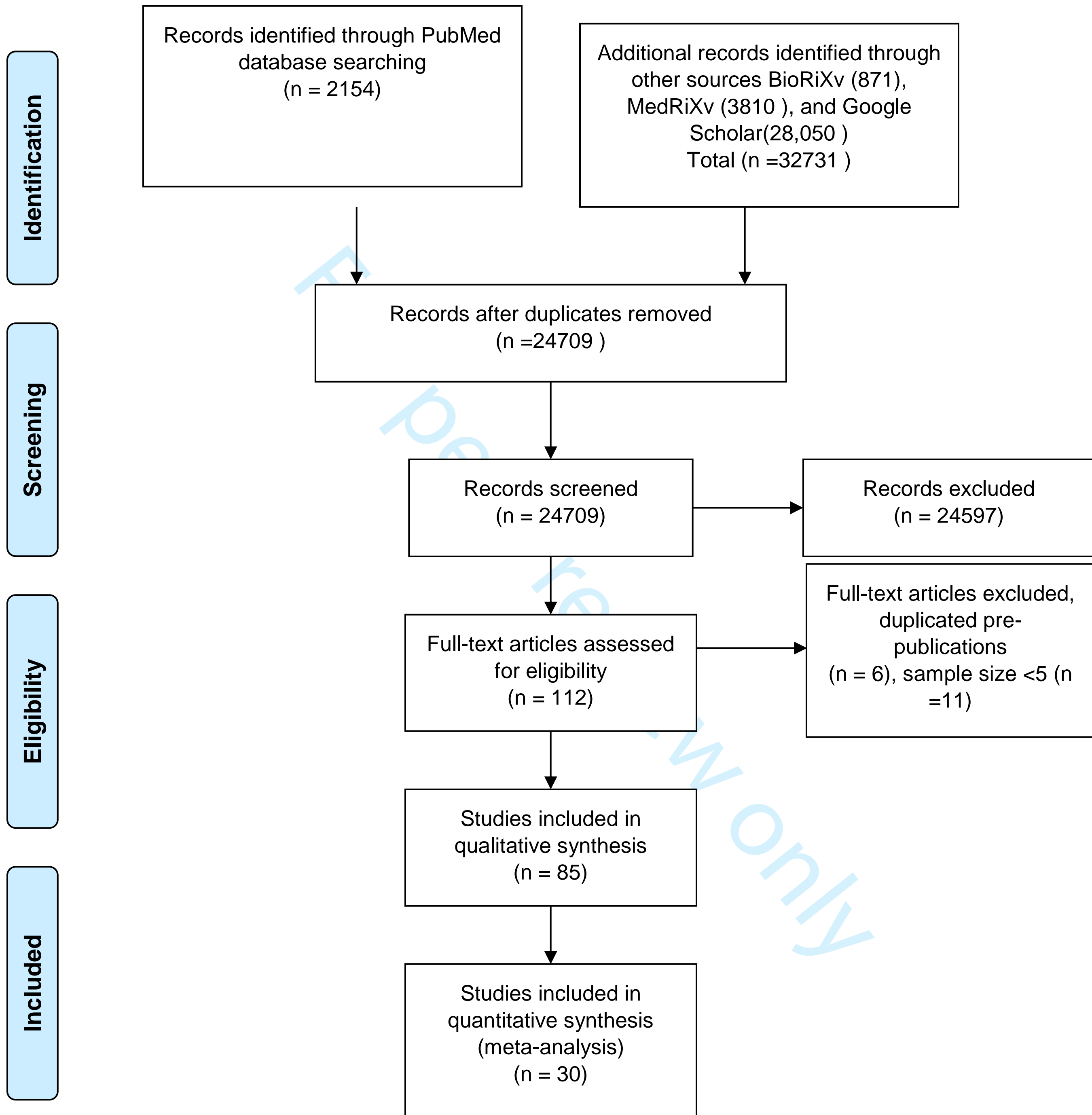
Fig S1. PRISMA Flowchart for Meta-Analysis and Qualitative Synthesis



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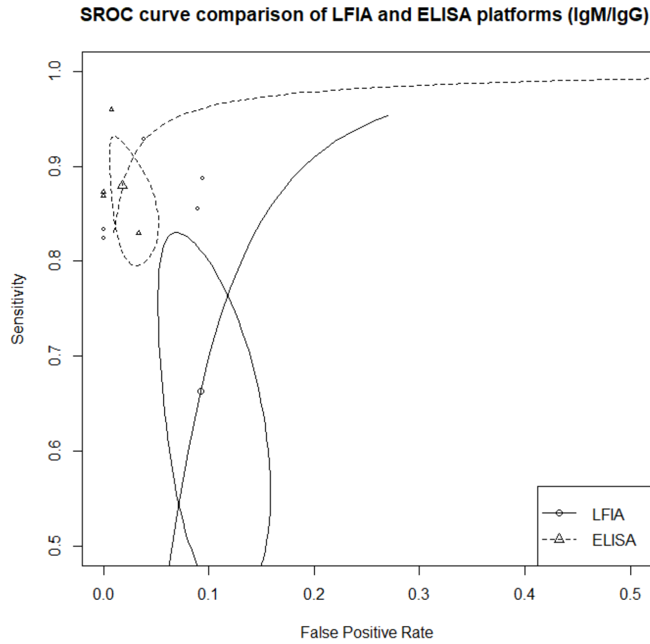

Fig S2.1 SROC comparison of LFIA and ELISA performance with 95% confidence contours

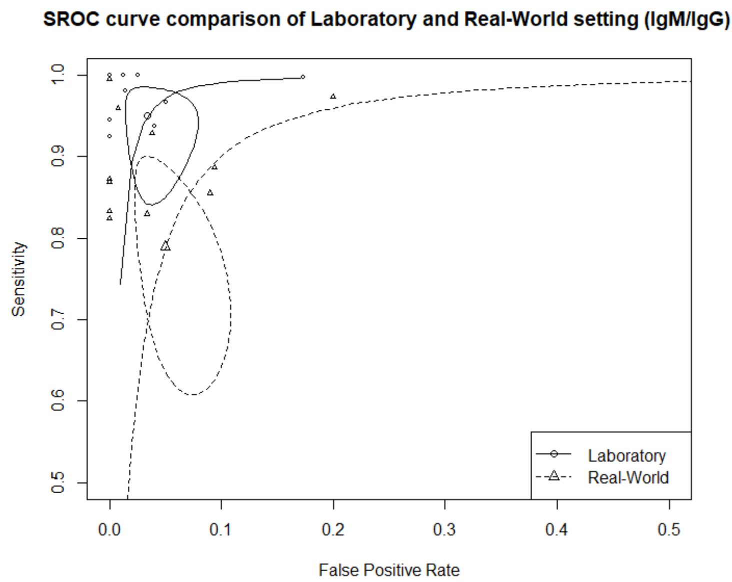

Fig S2.2 SROC comparison of Laboratory and Real-World performance with 95% confidence contours

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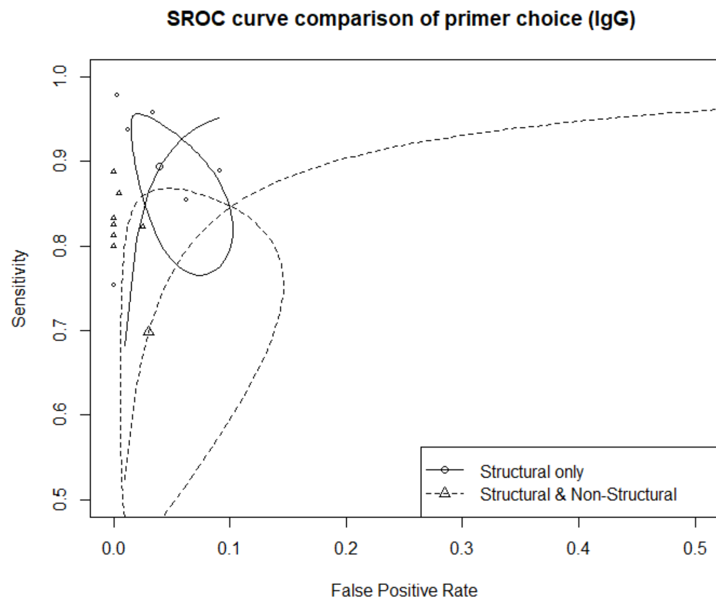

Fig S2.3 SROC comparison of primer configuration performance with 95% confidence contours

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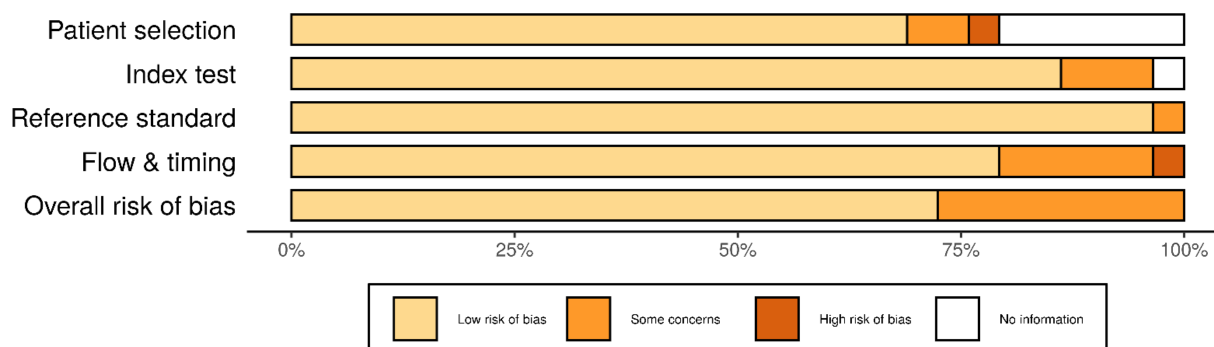
Fig S3. Summary plot of risk of bias for each study included in meta-analysis according to QUADAS-2 domain.



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PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	5
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	5
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6-7
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	6-7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	7
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	7
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	6
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	6-7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	6-7
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	7
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	7
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	7

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Section and Topic	Item #	Checklist item	Reported on page #
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Supp
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Supp
Study characteristics	17	Cite each included study and present its characteristics.	Supp Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Supp Fig 2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Figures
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Supp Fig 2
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Figures
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Figures
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Supp Fig 2
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Figures
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	10-14
	23b	Discuss any limitations of the evidence included in the review.	13
	23c	Discuss any limitations of the review processes used.	13
	23d	Discuss implications of the results for practice, policy, and future research.	14
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	15
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	15
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	14
Competing interests	26	Declare any competing interests of review authors.	15
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	14

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

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Meta-Analysis of the Robustness of COVID-19 Diagnostic Kit Performance During the Early Pandemic

Chandrakumar Shanmugam, M.D., M.B.A.,[1#] Michael Behring, Ph.D.,[2#] Vishwas Luthra, M.B.B.S.,[3] Sixto M Leal Jr., M.D., Ph.D.,[2] Sooryanarayana Varambally,[2, 4] PhD, George J Netto, M.D.,[2, 4] and Upender Manne, M.S., Ph.D.[2, 4,*]

Department of Pathology[1], MNR Medical College & Hospital, MNR Nagar, Fasalwadi, Sangareddy, Telangana, India 502279

Department of Pathology[2], University of Alabama at Birmingham, Birmingham, Alabama, USA 35274

Department of General Medicine[3], Sri Guru Ram Das Institute of Medical Sciences and Research, Mehta Road, Vallah, Amritsar, Punjab, India 143501

O'Neal Comprehensive Cancer Center[4], University of Alabama at Birmingham, Birmingham, Alabama, USA 35274

# Authors contributed equally

Running title: Robustness of Diagnostic Kits During Early Phase COVID 19: Lessons Learned

[*]Author for Correspondence:

Dr. Upender Manne
Professor of Pathology, Epidemiology and Surgery
University of Alabama at Birmingham
Birmingham, Alabama, USA 35274
Tel: 001 2052493895
Email: upendermanne@uabmc.edu

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ABSTRACT:

Background

Accurate detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is necessary to mitigate the coronavirus disease-19 (COVID-19) pandemic. However, the test reagents and assay platforms are varied and may not be sufficiently robust to diagnose COVID-19.

Methods

We reviewed 85 studies (21,530 patients), published from five regions of the world, to highlight issues involved in the diagnosis of COVID-19 in the early phase of the pandemic. All relevant articles, published up to May 31, 2020, in PubMed, BioRiXv, MedRiXv, and Google Scholar, were included. We evaluated the qualitative (9749 patients) and quantitative (10,355 patients) performance of RT-PCR and serologic diagnostic tests for real-world samples, and assessed the concordance (5,538 patients) between test performance in meta-analyses. Synthesis of results was done using random effects modelling and bias was evaluated according to QUADAS-2 guidelines.

Results

The RT-PCR tests exhibited heterogeneity in the primers and reagents used. Of 1,957 positive RT-PCR COVID-19 participants, 1,585 had positive serum antibody (IgM +/- IgG) tests (sensitivity 0.81, 95%CI 0.66-.90). While 3,509 of 3581 participants RT-PCR negative for COVID-19 were found negative by serology testing (specificity 0.98, 95%CI 0.94-0.99). The chemiluminescent immunoassay exhibited the highest sensitivity, followed by ELISA and lateral flow immunoassays. Serology tests had higher sensitivity and specificity for laboratory-approval than for real-world reporting data.

Discussion

The robustness of the assays/platforms is influenced by variability in sampling and reagents. Serological testing complements and may minimize false negative RT-PCR results. Lack of

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standardized assay protocols in the early phase of pandemic might have contributed to the spread of COVID-19.

Strengths and limitations of this study:

- This study offers the first evaluation of COVID-19 test performance with consideration of the heterogeneity of RT-PCR primers.
- We compare the performance of manufacturer-based, laboratory/approval data to the performance of the same test kits in a real-world setting in the early phase of the pandemic.
- We perform a qualitative analysis of RT-PCR assays using 85 studies (21,530 patients), and a quantitative meta-analysis of RT-PCR vs. serum antibody assays in a sub-set of 30 publications (10,355 patients).
- Much of the information in the early pandemic was reported from China, and often from non-peer reviewed, preprint sources.
- Data measuring duration of the infection was not available in majority of included studies.

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INTRODUCTION

In December 2019, there was a cluster of unexplained pneumonia cases in Wuhan, China, and a novel coronavirus was identified as the causative agent.[1] The virus was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease as coronavirus disease-19 (COVID-19).[2] The clinical spectrum ranges from asymptomatic forms to acute respiratory failure and multi-organ dysfunction syndrome, coagulopathy, and death.[3,4] On March 11th 2020, the World Health Organization described the spread of these infections as a pandemic, which persists as a global crisis. Robust diagnostic tests are required to mitigate the spread of this virus and thereby to minimize the impact of COVID-19 on the health, economy, and social well-being of mankind.

The standard diagnosis of COVID-19 is based on clinical and radiologic evidence and viral genome detection by RT-PCR in respiratory samples.[5] Gene-specific primers are used in the RT-PCR assays; structural genes include *envelope* (*E*), *nucleocapsid* (*N*), and/or *spike (S)*-genes; non-structural genes include *RNA-dependent RNA polymerase* (*RdRp*) or *open reading frame1ab* (ORF1ab) [6,7] Some studies used only a single-gene specific primer, and others used multiple-gene primers. Since studies published in the early phase of the pandemic reported a 3%-41% range of false-negativity by RT-PCR, a repeat RT-PCR testing was suggested.[8,9] Furthermore, false negativity was attributed to either mutations in the regions to which the primers bind or to sampling and laboratory practices, including collection, transportation, and handling.[10] Timing of sample collection with respect to the course of infection and the sample type also influence test results.[11] Alternatively, the diagnosis can be made by detection of antigens (E, N, or S) and/or antibodies (IgM or IgG or both) in blood samples.[12] However, these tests have the potential for false positives owing to cross-reactivity with other human coronaviruses.[13,14] Due to the unprecedented public health emergency, the FDA authorized, on June 1, 2020, EUA requests for more than 15 diagnostic and serologic tests. Though serology testing can detect the false positives of RT-PCR tests in clinically suspected patients, its value in COVID-19 diagnosis as a complementary assay in the

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mitigation of the pandemic is not well defined. However, given the complexities in COVID-19 testing, there is a need for a review of performance for tests commonly used.

In this systematic review and meta-analysis, we examine testing for the diagnosis of COVID-19 in the early pandemic and evaluate the sensitivity and specificity of serological tests relative to RT-PCR tests. Our objectives were to assess the uniformity of primer usage in RT-PCR assays and evaluate whether primers used in gold-standard RT-PCR tests affect the validity of serological tests. Furthermore, we compared the performance of serological tests/platforms in approval contrived/laboratory *vs.* real-world data.

METHODS

Literature Search

This research was accomplished according to standards outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[15] To find relevant studies, international databases, including PubMed, Embase, MedRiXv, BioRiXv, and Google Scholar, were searched for articles published until May 31, 2020. The following search terms were used (selected using English MeSH keywords and Emtree terms): [SARS-CoV-2 AND diagnosis] OR [2019-nCoV AND diagnosis]" OR ["COVID-19 AND diagnosis] and [SARS-CoV-2 AND RT-PCR] OR, [2019-nCoV AND RT-PCR]" OR ["COVID-19 AND RT-PCR] and [SARS-CoV-2 AND serology] OR [2019-nCoV AND serology]" OR ["COVID-19 AND serology]. Additional searches were performed for references listed in the included studies.

Eligibility Criteria

Relevant articles that reported diagnostic information for infected patients were included in the analysis. Pre-print articles with non-peer review were considered for inclusion. Articles were excluded if appropriate information was not reported or if they were in the Chinese language. Population sample sizes of <5 participants were not included; reviews and editorials were not considered. For meta-analysis and approval *vs*. real-world performance, studies that reported percent sensitivity/specificity without including patient numbers were also excluded.

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Data Extraction and Report Quality Evaluation

Two authors (CS and VL) screened and evaluated the literature independently. Discrepancies were resolved by consensus after evaluation by a third author (MB). The following were extracted for review and meta-analysis: journal name, authors, period of publication (end of May, 2020), location of study, total number of patients, tissue of origin for samples tested, whether samples were from upper or lower respiratory tract (or both), primers for RT-PCR, platforms for serology tests, and antibodies tested for serology. Counts of true positives, false negatives, true negatives, and false positives were used in the meta-analysis.

An author (MB) extracted and analyzed the approved testing kit performance data from the following sources: FDA EUA Authorized Serology Test Performance,[16] the Foundation for Innovative New Diagnostics (FIND) SARS-CoV-2 diagnostic pipeline,[17] and package inserts provided on company websites for each product. Real-world sample testing data from kits in meta-analyses were compared against the performance of the same kits, or platforms, reported in approval documentation. Variables abstracted were study authors/test developer, name of test, test platform, and true positives, false negatives, true negatives, and false positives for each antibody or antibody combination measured (IgM, IgG, IgA, combined, and Pan-Ig). Risk of bias within individual studies of meta-analysis was assessed using the QUADAS 2 tool for assessment of diagnostic studies.[18] QUADAS 2 has been developed specifically for evaluating bias in the meta-analyses of diagnostic test accuracy.

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Patient and Public Involvement

Since we performed a meta-analysis and systematic review, it was not appropriate or possible to involve patients or the public in the design, conduct, reporting, or dissemination plans of our research.

Statistical Analysis

Statistical analyses were performed with R version 6.3.2 (2019-12-12).[19] The package "meta" was used for meta-analyses.[20] Random effects models were used to measure sensitivity and specificity of outcomes across studies. Subgroup analysis was performed to evaluate the effect of assay, RT-PCR primer type, and setting (laboratory *vs*. real-world) upon serum test performance. Heterogeneity across studies and subgroups was evaluated using Cochrane's *Q* statistic, and residual heterogeneity was quantified as a percentage with the I^2 statistic. An I^2 measure of 0% shows no observed heterogeneity, with increasing values from 0%-100% indicating higher levels of heterogeneity. [21] An assumption of homogeneity was rejected for p-values < 0.1. The evaluation of publication bias was not possible using FDA and EU reported approval data.

RESULTS

Search Results and Population Characteristics

Our search generated 112 publications with potential relevance to the performance of COVID-19 diagnostic tests. After excluding duplicate publications, manuscripts that did not report numbers of patients used for sensitivity/specificity calculations and studies with a sample size of <5 patients, 85 studies were selected for qualitative synthesis of RT-PCR primer usage. From this set, a sub-set of 30 publications were selected for the quantitative meta-analysis of serologic *vs.* RT-PCR diagnostic testing for COVID-19 (**Table S1**). Ancillary analysis compared the performance of these 30 real-world studies to that reported in laboratory approval data from 47 diagnostic serum-based tests. In all, our qualitative synthesis of RT-PCR studies included 85 studies and 21,530 patients. From this synthesis, a group of 30 studies with 10,355 patients from 5 regions of the world were selected for meta-analysis and comparison to performance from laboratory approval data (**Fig S1**).

Uniformity of Primer Usage in RT-PCR Diagnostic Tests

We reviewed use of single primer of structural genes as compared to use of both structural and non-structural gene primers in 56 population-based studies with 9,872 participants.

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Overall, high proportions of studies employed both structural and non-structural gene primers in RT-PCR testing [55% (31 in 56) in studies and 56% (5484 in 9872) of total participants]. Additionally, 29 studies (11,658 patients) did not report RT-PCR primer data. Single markers were most frequently tested in China and North American studies (**Table 1**). In general, the most tested samples were from the upper respiratory tract, regardless of primer status. Sample source and location in the respiratory tract were not reported for 8-20% of patients, and this was more common for studies using single gene primer.

Meta-Analysis: RT-PCR *vs.* Serum Antibody Testing

In general, patient sera were tested for IgM and IgG antibodies. China was the region with the highest frequency of antibody testing, and lateral flow immunoassay (LFIA) and chemiluminescent immunoassay (CLIA) testing platforms were most often utilized. Of the 45 studies included in the qualitative synthesis, 30 manuscripts reported both serum antibody testing and RT-PCR testing for the same patients. Key characteristics of this population include: China as the regional location for research; lack of reporting of RT-PCR primer information for ~33% (10/30) of all studies; most studies used IgM and IgG serum-based antibody tests; and LFIA, CLIA, and enzyme-linked immunosorbent assay (ELISA) platforms were common across studies (**Table 2**).

We used the IgM+/-IgG serum antibody test since it was most commonly utilized across studies. Of 1,957 participants (pooled sensitivity 0.81, 95%CI 0.66-0.90) with a positive RT-PCR COVID-19 result, 1,585 were also detected as positive with serum antibody tests. Of 3,581 true negatives in RT-PCR, 3,509 negatives were also found by serum antibody testing (pooled specificity 0.98, 95%CI 0.94-0.99). For both models, heterogeneity between studies was significant (p<0.01 for both, I^2=97% and I^2 =98% for sensitivity and specificity, respectively).

Sub-analyses of differences based on the testing platform found that sensitivity between groups differed (p <0.0001), with CLIA tests performing best (0.99, 95%CI 0.97-0.99); ELISA as next-best (0.89, 95%CI 0.82-0.93); and LFIA as having the poorest sensitivity (0.67, 95%CI

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0.50-0.81). LFIA test sensitivity also showed heterogeneity between studies ($p<0.01$, I^2 95%). For IgM/IgG tests, specificity did not differ significantly by platform (p= 0.06). However, a performance trend followed sensitivity, with LFIA underperforming (**Figure 1 and Figure S2.1**).

Serum Antibody Testing Performance: Approval Data vs. Real-World Data

For manufacturer-based, laboratory approval data, IgM+/-IgG testing detected COVID-19 positivity for 1,045 of 1,068 RT-PCR-determined “true” positive patients (sensitivity 0.98, 95%CI 0.92-1.0). In the same group, serum testing correctly identified 1,928 of 1,967 (specificity 0.98, 95%CI 0.95-.099) true negatives by RT-PCR. For both models (sensitivity and specificity), there was evidence of heterogeneity (p <0.01 for both and I^2=93% and I^2=94% for sensitivity and specificity, respectively).

We evaluated IgM+/-IgG serum test performance in subgroup analyses comparing laboratory approval performance data to real-world performance in study data. In manufacturer data presented for approval, serum antibody testing detected 1,047 of 1,068 “true positive” cases of COVID-19 (sensitivity 0.98, 95%CI 0.92-1.0). Real-world use of serum IgM+/-IgG testing was evident for 2,450 of 3,025 participants diagnosed with COVID-19 by RT-PCR (sensitivity 0.81, 95% CI 0.66-0.90). For both groups, there was heterogeneity between studies (p <0.01 for both, I^2=93% and I^2 =97% for approval and real-world specificity, respectively) (**Figure 2 and Figure S2.2**). In addition, the overall sensitivity between approval and real-world testing groups differed significantly (Q=8.37, p=0.004). An analysis of specificity by the same subgroups found no significant difference between laboratory approval and real-world data. Laboratory data identified 1,928 of 1,967 participants with true COVID-19 negative status (specificity 0.98, 95% CI 0.95-0.99). Real-world data found 5,437 of 5,548 true negatives (specificity 0.98, 95% CI, 0.96-0.99) (analysis not shown).

Since, in IgM+/-IgG tests, there were differences in sensitivity between platforms, we evaluated the effect of approval-based data *vs.* real-world data by the type of platform. In an analysis stratified for ELISA, CLIA, and LFIA, there was no significant difference in specificity

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between approval and real-world data (data not shown). However, for ELISA tests, real-world capacity to detect true positives was lower than in laboratory-based analyses. In real-world studies, the sensitivity of ELISA was 0.89 (95% CI, 0.82-0.93), different from laboratory sensitivity for the same platform (0.94, CI95% 0.91-0.96, Q =4.74, p=0.03). The LFIA platform also showed a trend of lower real-world sensitivity (0.67, 95% CI, 0.50-0.81) compared to laboratory approval sensitivity (0.99, CI95% 0.90-0.99, Q =8.56, p 0.003). Laboratory/real-world groups for CLIA platforms were too small to be tested reliably (1 and 2 groups, respectively).

Serum Antibody Testing Performance: Effect of Primer Choice on Test Validity

To evaluate the reliability of RT-PCR as a gold standard for serum-based test performance, we tested the consequences of using structural and non-structural primers in RT-PCR reference testing of serum. Analyses were divided into three subgroups based on antibody targets: IgM, IgG, and IgG+/-IgM combined. In IgM and combined IgG+/-IgM testing, the primer choice had no significant influence on sensitivity or specificity. However, for IgG antibody tests, use of both a structural and a non-structural gene-specific primers in RT-PCR resulted in reduced sensitivity for serum testing (**Figure 3 and Figure S2.3**, Q=6.17, p=0.013). Furthermore, although not statistically significant, the sensitivity of both IgM and IgG+/-IgM combined data sets was lower when using a referent RT-PCR test with both primer types.

DISCUSSION

Because of the highly infectious nature of COVID-19, a prompt, accurate, and early diagnosis is necessary to deal with the ongoing pandemic, for such diagnoses can help reduce the spread of infection and its associated risk for mortality. Currently, the COVID-19 diagnosis is generally based on RT-PCR assays.[8] Alternative methods such as antigen- and antibody-based serology tests, although available, have uncertain value. The current systematic review and meta-analysis addresses the challenges encountered in the diagnosis of COVID-19 by various methods. It also analyzes differences between the FDA-approved EUA data and real-

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world data. There is worldwide non-uniformity in the performance of RT-PCR, including the number and types of primers and reagents used for COVID19 diagnosis, which raise questions about its generalized applicability. Similarly, the studies based on serological tests showed diagnostic inaccuracies owing to individual differences in mounting an immune response as well as dependency on the time duration after the onset of symptoms. Overall, the sensitivity between RT-PCR and serology tests was 0.81 (95% CI, 0.66-0.90), and specificity was 0.98 (95% CI, 0.94-0.99). Among the various platforms for serodiagnosis, the highest sensitivity was exhibited by ELISA, followed by CLIA and LFIA. Furthermore, use of primers (structural, non-structural, or both) had a variable effect on sensitivity based on antibody targets. Sensitivity was significantly higher for IgG serology tests using structural-primer-only RT-PCR tests as a referent. Serology tests had higher sensitivity for approval-based data than for real-world reporting. This difference was significant for ELISA-based platforms, and a non-significant trend towards inflated approval-based sensitivity was evident for both CLIA and LFIA platforms. These observations highlight the inconsistencies/challenges in the COVID-19 diagnosis by RT-PCR, which is the current gold standard, as well as in serologic testing.

For RT-PCR assays, the targets in SARS-CoV-2 include structural genes like *E, N* and *S*, and nonstructural genes, including that for *RdRp* or *ORF1ab.*[22] In the early phase of the pandemic, some studies used a two-step diagnosis that included an initial screening phase using structural genes followed by a confirmatory phase using nonstructural genes. [6,7,23] The test is considered positive when both structural and non-structural markers are positive.[24,25] However, currently both types of primers are used simultaneously to diagnose COVID-19. The viral load or copy number of the viral genome is expressed as a Ct-value, which when <37 is indicative of a positive test, and a value of ≥ 40 is considered negative. A Ct value between >37 and < 40 requires repetition of RT-PCR analysis to confirm the diagnosis.[24] However, the Ct value range varies widely according to assays and laboratory practices. A COVID-19-RdRp/Hel assay has a higher sensitivity than a conventional RdRp-P2 assay irrespective of the type of sample.[26] Overall, higher proportions of studies (58%) employed both structural

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and non-structural gene primers in RT-PCR testing. Single markers were used in some Chinese and North American studies. These findings are indicative of non-uniformity in the RT-PCR methodology. We note that half of the positive, symptomatic patients became negative by the second week, when they became asymptomatic. In contrast, the asymptomatic, positive patients became negative two days after hospital admission, indicating the importance of a temporal factor in COVID-19 diagnosis by RT-PCR.[27,28]

Published in the early phase of the pandemic, 11 of 85 studies had clinically suspected COVID-19 patients. In these studies, the average test positivity by RT-PCR, regardless of the sample source, was 44% (Supplementary Table 1), and test sensitivity was influenced by sample source (upper *vs*. lower respiratory *vs.* other samples), issues related to testing performance, and delay after onset of symptoms.[29] In the early phase of the COVID-19 pandemic, for studies evaluating suspected COVID-19 cases, the total positive RT-PCR for throat swabs was in the range of 30–60% at initial presentation.[8,30] One study reported a yield of 72-93% positive cases for lower respiratory samples (bronchioalveolar lavage and sputum) as compared to 32-63% positivity for upper respiratory samples (oral and nasopharyngeal swabs) and 29% for stool samples.[29] Hence, a negative COVID-19 test based only on an upper respiratory sample at a single time point is questionable. For most studies, the testing sample was from the upper respiratory tract, regardless of primer type used. However, the sample source was not reported for 8-20% of patients, which was more common for studies using only structural gene primers. For stool samples testing positive for COVID-19, 66.7% also tested positive on pharyngeal swabs. Of the stool samples, 64.3% remained positive after pharyngeal clearance of the virus.[31] In contrast, none of the patients showed a positive test on upper respiratory samples after the anal swabs tested negative.[31] These findings raise concerns about whether patients with negative respiratory swabs are truly virus-free, and sampling of additional body sites is needed. As determined by various studies, the performance of the RT-PCR depends on usage of comparable protocols, including primers

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and reagents.[32] Additionally, it is unknown whether the currently used RT-PCR primers detect all SARS-Cov-2 strains.

The specific immune response to SARS-CoV-2 can be measured by serological testing. Several rapid serological tests, including point-of-care tests, are being developed. Even though some of these tests have been approved by the FDA through EUA, their accuracy needs to be validated.[33] A minimum of 1–2 weeks after the onset of infection is needed for seroconversion. Hence, antibody testing is of no value in the early phase of infection. Additionally, its value is limited by its cross-reactivity with other coronaviruses.[34,35] The initial RT-PCR positivity during the early stages (<15 days) of SARS-CoV-2 infection declines to 66.7% in the later phase (15-39 days), during this period, the antibody test can supplement RT-PCR in the diagnosis of COVID-19.[34,35] Additionally, serology testing becomes valuable for clinically suspected and RT-PCR negative (false-negative) individuals.

This research has limitations. Due to the dynamic reporting of COVID-19 testing research and inconsistencies in reporting of predictive variables across studies, bias in sampling may have some effect on our results. Patient flow analysis suggests that lack of consistent RT-PCR reference standard given to patients in the same study, as well as the unclear reporting of patient selection methods could contribute to bias in these results (**Fig. S3**). In addition, the observed heterogeneity between studies in the meta-analysis suggests that we must consider the possibility that the differences in results may be due to chance. Lastly, it is questionable to compare two separate testing methods of RT-PCR and seroprevalence in sensitivity/specificity analysis. In particular, given the relationship between time since diagnosis and accuracy of serology testing, a contributor to the observed differences in performance is time. Furthermore, because of each diagnostic kit having differing cut points for positive/negative, threshold effect as a source of heterogeneity in sensitivity and specificity cannot be ruled out.

The effective containment of COVID-19 involves accurate diagnoses and isolation of SARS-CoV-2-infected persons. Robustness of the assays/platforms is determined by

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variability of the samples, primers, and reagents used. Serological tests alone are of value only during the latter times of infection; however, they complement RT-PCR when used in conjunction and minimize false negative RT-PCR results. Additionally, some of the approved serological assays/platforms, particularly those developed using contrived/laboratory data, perform poorly when applied to real-world samples. We are currently in a new phase of the pandemic, and there is a need for a reliable/robust diagnostic test to mitigate the spread of COVID-19.

Our analyses of studies published in the early-phase of the pandemic have highlighted issues related to COVID-19 diagnosis that need to be addressed as follows: 1) The high mutational rate exhibited by the SARS-CoV-2 virus may lead to the generation of new strains. Therefore, like for influenza virus, the existing diagnostic kits need to be modified constantly to optimize the detection of new strains; 2) Though RT-PCR diagnosis of COVID-19 is the gold standard, its combination with a serologic test may increase the accuracy of SARS-CoV-2 detection; 3) Approval agencies must account for the type of data (contrived versus real world) presented by diagnostic kit developer; 4) Although agencies employed EUA processes for the approval of diagnostic kits, there is a need to monitor their performance and assess their robustness in real-world samples, to permit continued use of these kits; and 5) Standardized assay protocols need to be developed and continually updated to mitigate the COVID-19 pandemic.

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DATA SHARING

Data is available upon request

ETHICAL APPROVAL STATEMENT

Not applicable

FUNDING STATEMENT

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AUTHOR CONTRIBUTIONS

CS, MB, and UM made substantial contributions to the conception and design of the work. CS, MB and VL contributed to the acquisition of the study data. Data were analyzed by CS, MB and UM. MB was responsible for the statistical analysis. CS, MB, SML, SV, GJN, and UM contributed to the interpretation of the data, and to drafting and revising of the manuscript. CS, MB and VL have accessed and verified the data. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work. All authors have read and approved the manuscript. All authors declare no competing interests.

REGISTRATION AND PROTOCOL

The work in this review was not registered and has no available review protocol.

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Table 1. **Characteristics of the studies included in qualitative analysis**

	Total Studies	Total pop.	Structural gene primers		Structural and non-Structural gene primers		Non-Structural gene primers		Not reported	
			N studies	N pop.	N studies	N pop.	N studies	N pop.	N studies	N pop.
Total	**85**	**21530**	**22**	**4265**	**31**	**5484**	**3**	**123**	**29**	**11658**
Location										
Asia (excl. China)	6	378	2	53	4	325				
China	28	12187	8	1802	17	3047	3	123	24	7215
Europe	12	5757	4	528	8	993			4	4236
North America	10	3001	8	1882	2	1119				
Global		207							1	207
Primers										
N -single	11	2016	11	2016						
E -single	4	759	4	759						
S -single	1	412	1	412						
N, E	2	226	2	226						
S, N	4	852	4	852						
ORF1Ab, single	2	59					2	59		
RdRp, single	1	64					1	64		
E+ORF1Ab	2	1119			2	1119				
E + RdRp	2	259			2	259				
M, E	1	48			1	48				
N+ORF1Ab	14	2703			14	2703				
N + E + RdRp	4	333			4	333				
S, N, E, RdRp, ORF1ab	1	13			1	13				
N, E, ORF1ab	1	33			1	33				
N, RNAse P	1	190			1	190				
S, N, RdRp, ORF1ab, E, M	1	52			1	52				
N, S, RdRp	1	273			1	273				
N, E, S, RdRp	2	349			2	349				
S, ORF1Ab	1	112			1	112				
Sample Source										
Upper Respiratory	23	6748	3	575	9	2633	1	64	10	3476
Upper & Lower Respiratory	1	52			1	52				
Upper Respiratory + Other*	9	751	3	368	2	44	1	38	3	301
Lower Respiratory + Other*	1	273			1	273				
Upper Respiratory + Serum	20	6407	7	1473	9	1432			4	3502
Upper Respiratory + Serum + Other*	4	941	2	840	1	80	1	21		
Upper & Lower Respiratory + Other*	4	678	1	280	3	398				
Upper & Lower Respiratory + Serum + Other*	2	518			1	132			1	386
Serum	18	2376	6	729	4	440			8	1207
Other*	1	199							1	199
Not reported	2	2587							2	2587

* Other = bronchioalveolar lavage, feces, urine, neonatal, amniotic fluid, and breast milk. N pop. = patient population

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Table 2. Characteristics of studies included in quantitative meta-analysis

	N studies	N pop.
Total	30	10355
Location		
Asia (excl. China)	2	261
China	19	6375
Europe	7	2900
North America	2	819
PCR primers		
Structural		
N, single	5	1084
E, single	1	49
N, E	1	201
N, S	2	408
Structural and Non-structural		
with ORF1Ab	8	1115
with RdRp	2	186
N, RNAse P	1	190
not reported	10	7122
Ab tested		
IgG	2	220
IgM + IgG	25	7828
IgA + IgG +IgM	1	208
IgA + IgG	1	37
not reported	1	2062
Serum Ab		
CLIA	8	3705
ELISA	8	1908
LFIA	10	3800
CLIA + ELISA	2	548
LFIA + ELISA	1	80
not reported	1	314

CLIA = chemiluminescent immunoassay, LFIA = lateral flow immunoassay, ELISA = enzyme-linked immunosorbent assay

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Figure Legends

Figure 1. **Comparison of Performance (Sensitivity and Specificity) of Serology Tests (IgM/IgG) Based on Assay Platforms**

Figure 2. **Comparison of Sensitivity of Laboratory setting versus Real World setting of RT-PCR and Serology (IgM/IgG) kits**

Figure 3. **The Effect of Primer Choice in RT-PCR Referent on Sensitivity of Tests based on Serum IgG**

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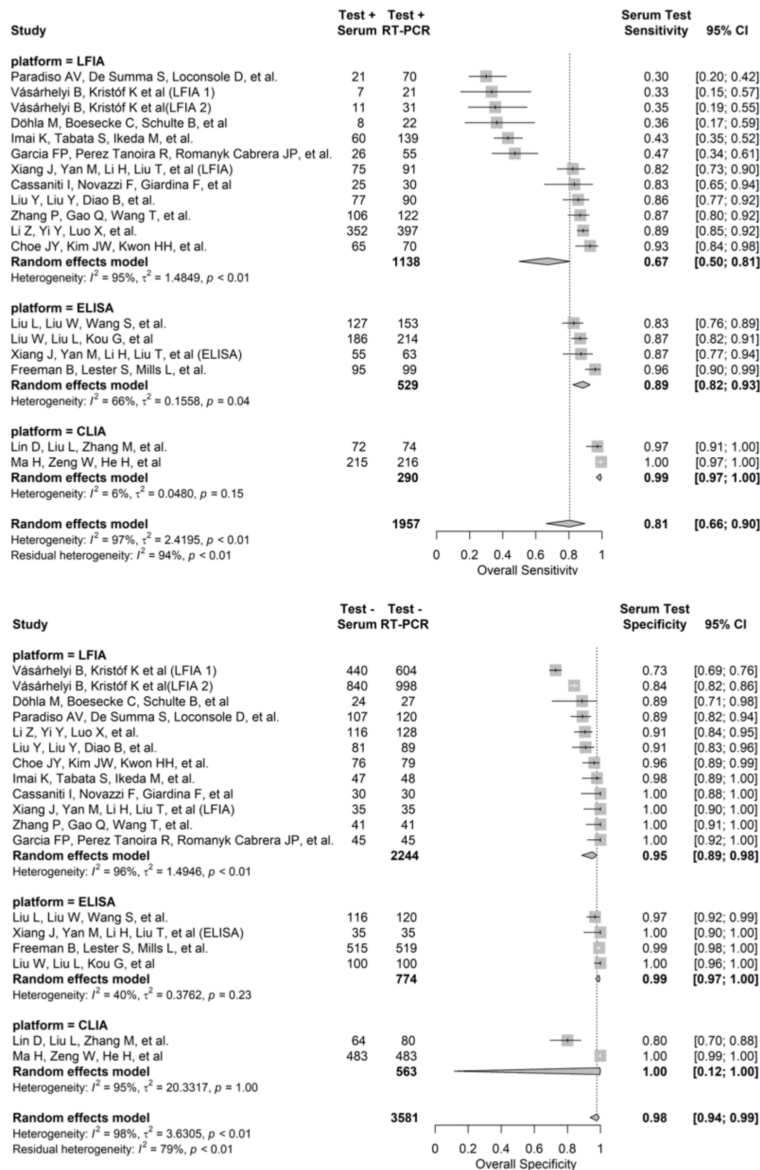

Figure 1. Comparison of Performance (Sensitivity and Specificity) of Serology Tests (IgM/IgG) Based on Assay Platforms

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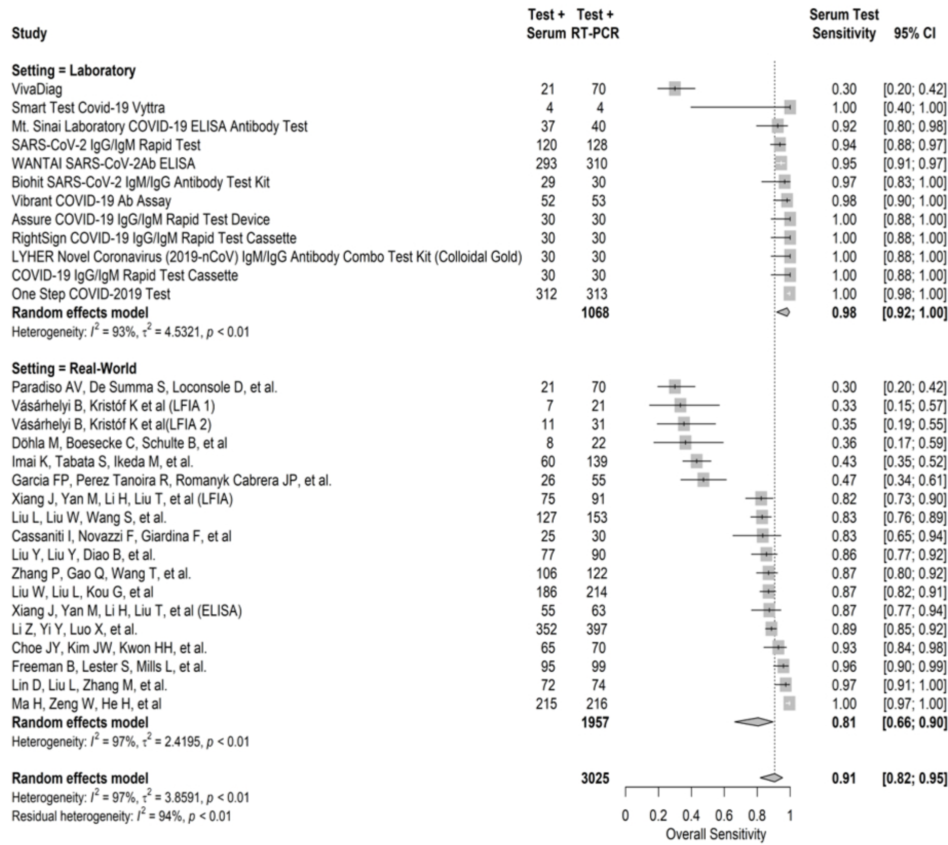

Figure 2. Comparison of Sensitivity of Laboratory setting versus Real World setting of RT-PCR and Serology (IgM/IgG) kits

164x141mm (150 x 150 DPI)

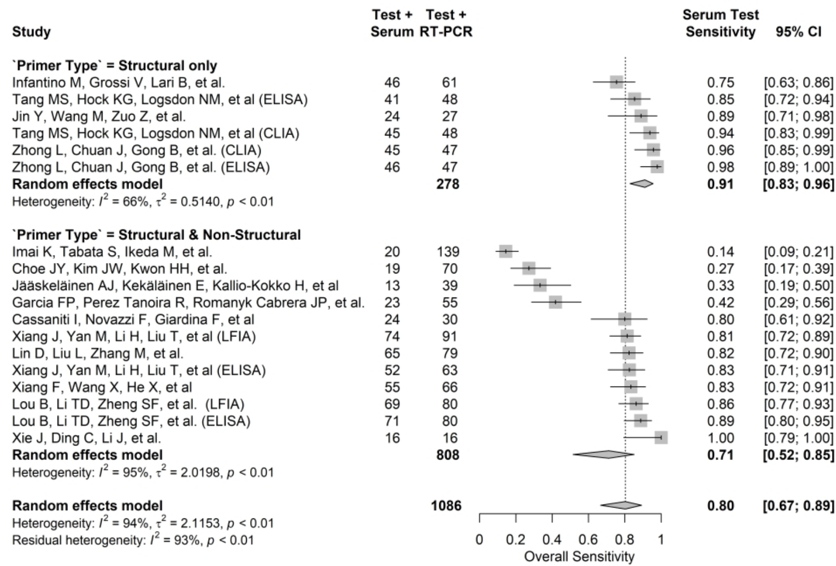

Figure 3. The Effect of Primer Choice in RT-PCR Referent on Sensitivity of Tests based on Serum IgG

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Meta-Analysis of Robustness of COVID-19 Diagnostic Kits During Early Pandemic. Supplemental information

Table S1. Description of 55 Studies Included for Qualitative Synthesis (gray) and 30 Studies Included in the Meta-Analysis (tan)

Journal	Authors	Region	N (patients)	Percent COVID-19 positivity	Sample origin	RT-PCR primers	RT-PCR primer type	RT-PCR/Serology platform	Serology targeted antibody	Study included in meta-analysis
J Clin Virol	Rahman H, Carter I, Basile K, et al. [36]	Asia	52		UR+LR	S, N, E, RdRp, ORF1ab, M	Both	RT-PCR		No
Exp Neurobiol	Won J, Lee S, Park M, et al. [37]	Asia	12		UR	N, E, S, RdRp	Both	RT-PCR		No
Jpn J Infect Dis.	Okamaoto K, Shirato K, Nao N, et al. [38]	Asia	25		UR	N, E	Structural	RT-PCR		No
J Med Virol	Choe JY, Kim JW, Kwon HH, et al. [39]	Asia	149	0.47	Serum	E, RdRp	Both	RT-PCR /CLIA	IgM + IgG	Yes
Lancet Infect Dis	Yong SEF, Anderson DE, Wei WE, et al. [40]	Asia	28		UR + Serum	N, single	Structural	RT-PCR /ELISA	IgG	No
J Clin Virol	Imai K, Tabata S, Ikeda M, et al. [41]	Asia	112		UR + Serum	S, ORF1Ab	Both	RT-PCR /LFIA	IgM + IgG	Yes
Emerg Microbes Infect	Xu Y, Xiao M, Liu X, et al. [42]	China	6		Serum			ELISA + LFIA	IgM	No
Radiology	Ai T, Yang Z, Hou H, Zhan C, et al. [8]	China	1014	0.59	UR			RT-PCR		No
NEJM	Cao B, et al.[43]	China	199		Other			RT-PCR		No
Radiology	Bai HX, Hsieh B, Xiong Z, et al.[44]	China	256		UR			RT-PCR		No
Lancet	Chen H, Guo J, Wang C, et al. [45]	China	9		UR + Other			RT-PCR		No
AJR Am J Roentgenol	Liu D, Li L, Wu X, et al. [46]	China	15		UR			RT-PCR		No
Eur J Radiol	Long C, Xu H, Shen Q, et al. [47]	China	87		UR			RT-PCR		No
Pediatr Pulmonol	Xia W, Shao J, Guo Y, Peng X, Li Z, Hu D. [48]	China	20		UR			RT-PCR		No
Am J Obstetr Gynecol	Yan J, Guo J, Fan C, et al. [49]	China	116	0.56	Other			RT-PCR		No
J Hosp Infect	Ye G, Li Y, Lu M, et al. [50]	China	91	0.52	UR			RT-PCR		No
J Med Virol	Zhang J, Wang S, Xue Y. [51]	China	14		UR + Other			RT-PCR		No
Respir Res	Zhang G, Zhang J, Wang B, Zhu X, Wang Q, Qiu S. [52]	China	95		UR			RT-PCR		No

Lancet	Zhou F, Yu T, Du R, et al. [53]	China	191		UR			RT-PCR		No
J Clin Microbiol	Liu W, Liu L, Kou G, et al.[54]	China	314		UR + Serum			RT-PCR	IgM + IgG	Yes
J Med Virol	Li, Y et al. [55]	China	610	0.40	UR	N, ORF1Ab	Both	RT-PCR		No
medRxiv	Diao B, Wen K, Chen J, et al.[56]	China	239		UR + Serum	N, ORF1Ab	Both	RT-PCR		No
J Clin Microbiol	Chan JF, Yip CC, To KK, et al. [26]	China	273		UR + Other	N, S, RdRp	Both	RT-PCR		No
Nature Microbiol	Kong WH, Li Y, Peng MW, et al. [57]	China	640		UR	N, ORF1Ab	Both	RT-PCR		No
Front Med	Liu W, Wang J, Li W, Zhou Z, Liu S, Rong Z. [58]	China	38	0.53	UR + Other	N, ORF1Ab	Both	RT-PCR		No
Int J Biol Sci	Lo IL, Lio CF, Cheong HH, et al. [59]	China	10		UR + LR + Other	N, ORF1Ab	Both	RT-PCR		No
Travel Med Infect Dis	Wu J, Liu J, Li S, Peng Z, et al. [60]	China	132		UR + LR + Serum + Other	N, E, RdRp	Both	RT-PCR		No
Int J Infect Dis	Xu T, Chen C, Zhu Z, et al. [61]	China	51		UR + LR + Other	N, ORF1Ab	Both	RT-PCR		No
J Med Virol	Yuan Y, Wang N, et al. [62]	China	6		UR + Other	N, E, RdRp	Both	RT-PCR		No
AJR Am J Roentgenol	Cheng Z, Lu Y, Cao Q, et al. [63]	China	33	0.33	UR	N, E, ORF1ab	Both	RT-PCR		No
Arch Pathol Lab Med	Schwartz, DA [64]	China	38		UR + Other	ORF1Ab, single	Non-structural	RT-PCR		No
Radiology	Wong HYF, Lam HYS, Fong AH, et al. [65]	China	64		UR	RdRp, single	Non-structural	RT-PCR		No
Chin Med J	Ling Y, Xu SB, Lin YX, et al. [66]	China	292		UR + Other	E, single	Structural	RT-PCR		No
Clin Infect Dis	Zhao R, Li M, Song H, et al. [67]	China	412		UR	S, single	Structural	RT-PCR		No
medRxiv	Ma H, Zeng W, He H, et al.[68]	China	699		UR + Serum			RT-PCR /CLIA	IgM + IgG	Yes
medRxiv	Cai X, Chen J, Hu J, et al.[69]	China	443		Serum			RT-PCR /CLIA	IgM + IgG	Yes
medRxiv	Qian C, Zhou M, Cheng F, et al. [70]	China	2062					RT-PCR /CLIA	IgM + IgG	Yes
J Infect Dis	Zhang G, Nie S, Zhang Z, Zhang Z. [71]	China	112		UR + Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	No

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medRxiv	Lin D, Liu L, Zhang M, et al.[72]	China	159		UR + Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	Yes
J Med Virol	Xie J, Ding C, Li J, et al. [73]	China	56		UR +Serum	N, ORF1Ab	Both	RT-PCR /CLIA	IgM + IgG	Yes
Nature Med	Long QX, Liu BZ, Deng HJ, et al. [74]	China	285		UR+ Serum	S, N	Structural	RT-PCR /CLIA	IgM + IgG	No
Int J Infect Dis	Jin Y, Wang M, Zuo Z, et al. [75]	China	76	0.57	Serum	N, single	Structural	RT-PCR /CLIA	IgM + IgG	Yes
Emerg Microbes Infect	Zhang W, Du RH, Li B, et al. [76]	China	278		UR + Other			RT-PCR /ELISA	IgM + IgG	No
Clin Infect Dis	Zhao J, Yuan Q, Wang H, et al.[34]	China	386		UR + LR + Serum			RT-PCR /ELISA	IgM + IgG	Yes
Euro Surveill	Perera RA, Mok CK, Tsang OT, et al. [77]	China	51		Serum			RT-PCR /ELISA	IgM + IgG	Yes
Clin Infect Dis	Xiang F, Wang X, He X, et al. [78]	China	216		UR + Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
medRxiv	Xiang J, Yan M, Li H, Liu T, et al. [79]	China	154		Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
medRxiv	Liu L, Liu W, Wang S, et al.[80]	China	238		UR + Serum	N, ORF1Ab	Both	RT-PCR /ELISA	IgM + IgG	Yes
Clin Infect Dis	Guo L, Ren L, Yang S, et al. [12]	China	208	0.39	Serum	N, single	Structural	RT-PCR /ELISA	IgM + IgA + IgG	Yes
Sci China Life Sci	Zhong L, Chuan J, Gong B, et al.[81]	China	347		UR NP/OP + Serum + Other	N, S	Structural	RT-PCR /ELISA + CLIA	IgM + IgG	Yes
Eur Respir J	Lou B, Li TD, Zheng SF, et al. [82]	China	80		UR + LR + Serum + Other	N, ORF1Ab	Both	RT-PCR /ELISA + LFIA + CLIA	IgM + IgG	Yes
J Med Virol	Du Z, Zhu F, Guo F, Yang B, Wang T. [83]	China	60		Serum			RT-PCR /LFIA	IgM + IgG	No
J Infect	Pan Y, Li X, Yang G, et al. [84]	China	105		Serum			RT-PCR /LFIA	IgM + IgG	No
J Med Virol	Li Z, Yi Y, Luo X, et al.[85]	China	525					RT-PCR /LFIA	IgM + IgG	Yes
medRxiv	Liu Y, Liu Y, Diao B, et al.[86]	China	179		UR + Serum			RT-PCR /LFIA	IgM + IgG	Yes

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Emerg Microbes Infect	Yongchen Z, Shen H, Wang X, et al. [87]	China	21		UR + Serum + Other	ORF1Ab, single	Non-structural	RT-PCR /LFIA	IgM + IgG	No
Anal Chem	Chen Z, Zhang Z, Zhai X, et al.[88]	China	19		UR + Serum	N, single	Structural	RT-PCR /LFIA	IgG	Yes
medRxiv	Zhang P, Gao Q, Wang T, et al.[89]	China	163		UR + Serum	N, single	Structural	RT-PCR /LFIA	IgM + IgG	Yes
JAMA	Grasselli G, Zangrillo A, Zanella A, et al. [90]	Europe	1591		UR			RT-PCR		No
Radiology	Caruso D, Zerunian M, Polici M, et al. [91]	Europe	158	0.39	UR	N, E, RdRp	Both	RT-PCR		No
Travel Med Infect Dis	Lagier JC, Colson P, Tissot Dupont H, et al. [92]	Europe	337		UR +LR+ Other	N, E, S, RdRp	Both	RT-PCR		No
J Clin Virol	van Kasteren PB, van der Veer B, van den Brink S, et al. [93]	Europe	13		UR	S, N, E, RdRp, ORF1ab	Both	RT-PCR		No
Int J Mol Sci	Toptan T, Hoehl S, Westhaus S, et al. [94]	Europe	48		UR	M, E	Both	RT-PCR		No
Trop Med Infect Dis	Amrane S, Tissot-Dupont H, Doudier, et al. [95]	Europe	280		UR + LR + Other	E, single	Structural	RT-PCR		No
J Clin Microbiol	Lambert-Niclot S, Cuffel A, Le Pape S, et al. [96]	Europe	138		UR	E, single	Structural	RT-PCR		No
J Med Virol	Infantino M, Grossi V, Lari B, et al. [97]	Europe	61		Serum	S, N	Structural	RT-PCR /CLIA	IgM + IgG	Yes
Euro Surveill	Jääskeläinen AJ, Kekäläinen E, Kallio-Kokko H, et al. [98]	Europe	37		Serum	N, E, RdRp	Both	RT-PCR /ELISA	IgA + IgG	Yes
J Infect	Tré-Hardy M, Blairon L, Wilmet A, et al. [99]	Europe	182		Serum			RT-PCR /ELISA + CLIA	IgA + IgG	No
Orvo Hetil	Vásárhelyi B, Kristóf K, Ostorházi E, Szabó D, Prohászka Z, Merkely B. [100]	Europe	2310	0.06	UR + Serum			RT-PCR /LFIA	IgM + IgG	Yes
Infect Ecol Epidemiol	Hoffman T, Nissen K, Krambrich J, et al.[101]	Europe	153		Serum			RT-PCR /LFIA	IgM + IgG	Yes
J Med Virol	Cassaniti I, Novazzi F, Giardina F, et al. [102]	Europe	110		UR + Serum	E, RdRp	Both	RT-PCR /LFIA	IgM + IgG	No
medRxiv	Garcia FP, Perez Tanoira R, Romanyk Cabrera JP, et al. [103]	Europe	100		Serum	N, ORF1Ab	Both	RT-PCR /LFIA	IgM + IgG	Yes

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medRxiv	Paradiso AV, De Summa S, Loconsole D, et al.[104]	Europe	190		UR + Serum	N, RNAse P	Both	RT-PCR /LFIA	IgM + IgG	Yes
Public Health	Döhla M, Boesecke C, Schulte B, et al. [105]	Europe	49		Serum	E, single	Structural	RT-PCR /LFIA	IgM + IgG	Yes
J Emerg Infect Dis	Okba NMA, Muller MA, Li W, et al. [106]	Global	207		Serum			RT-PCR /ELISA	IgM + IgG	No
J Clin Virol	Smithgall MC, Scherberkova I, Whittier S, Green DA. [107]	North America	113		UR	E, ORF1Ab	Both	RT-PCR		No
J Med Virol	Pujadas E, Ibeh N, Hernandez MM, et al. [108]	North America	1006		UR	E, ORF1Ab	Both	RT-PCR		No
J Infect Dis	Burbelo PD, Riedo FX, Morishima C, et al. [109]	North America	100		Serum	N, single	Structural	RT-PCR		No
Am J Obstet Gynecol MFM	Penfield CA, Brubaker SG, Limaye MA, et al. [110]	North America	32		UR + Other	N, single	Structural	RT-PCR		No
medRxiv	Wyllie AL, Fournier J, et al. [111]	North America	44		UR + Other	N, single	Structural	RT-PCR		No
J Appl Lab Med	Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. [112]	North America	235		Serum	N, single	Structural	RT-PCR /CLIA	IgM + IgG	No
Clin Chem	Tang MS, Hock KG, Logsdon NM, et al. [113]	North America	201		UR + LR + Serum	N, E	Both	RT-PCR /CLIA + ELISA	IgG	Yes
medRxiv	Randad PR, Pisanic N, Kruczynski K, et al. [114]	North America	493		UR + Serum + Other	N, single	Structural	RT-PCR /ELISA	IgM + IgA + IgG	No
JMIR Public Health Surveill	Sullivan PS, Sailey C, Guest JL, et al. [115]	North America	159		UR + Serum	S, N	Structural	RT-PCR /ELISA	IgM + IgA + IgG	No
bioRxiv	Freeman B, Lester S, Mills L, et al.[116]	North America	618		UR NP/OP + Serum	N, single	Structural	RT-PCR /ELISA	IgM + IgG	Yes

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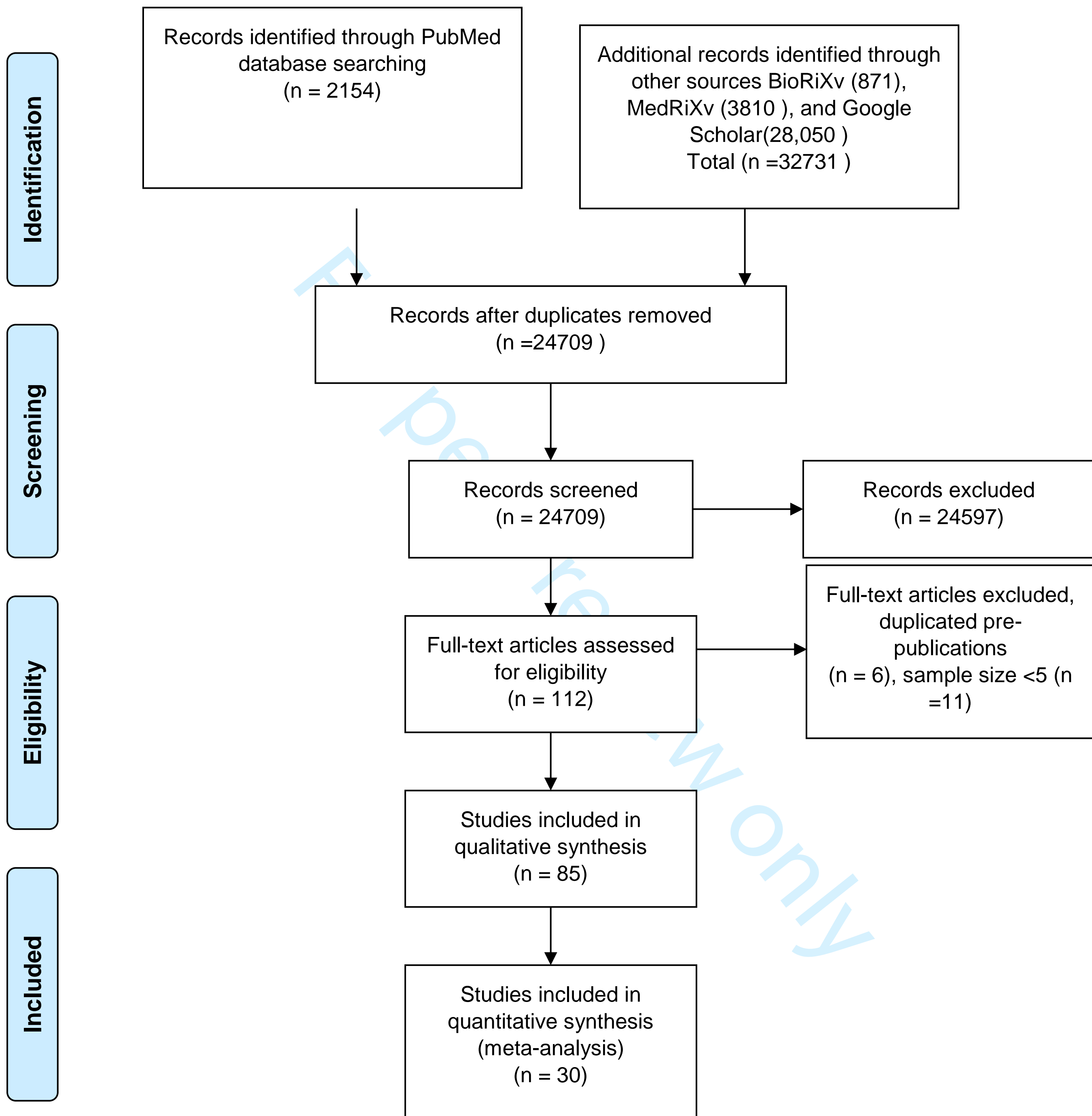
Fig S1. **PRISMA Flowchart for Meta-Analysis and Qualitative Synthesis**



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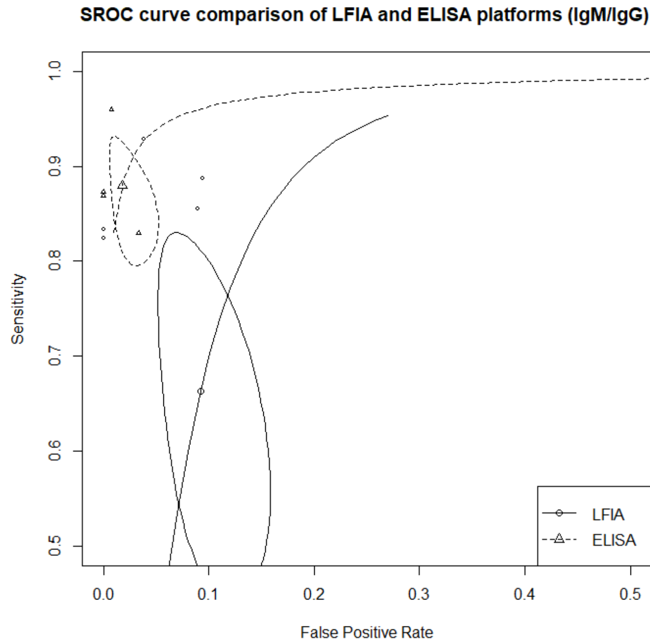

Fig S2.1 SROC comparison of LFIA and ELISA performance with 95% confidence contours

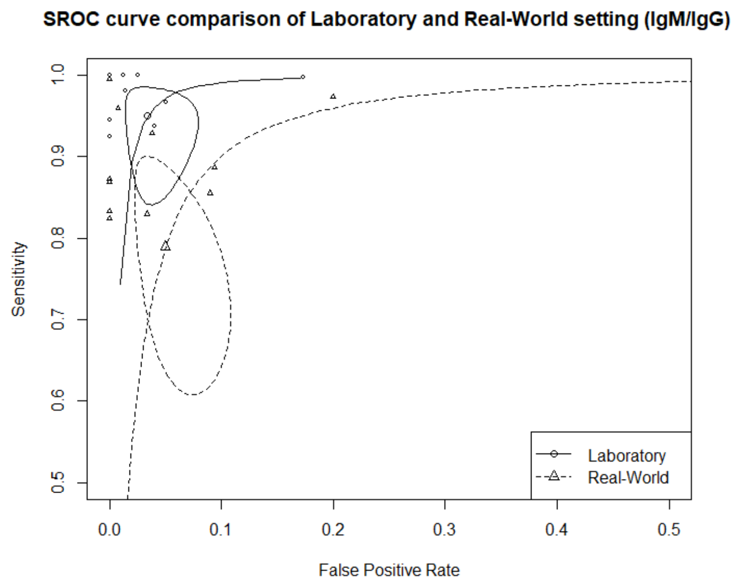

Fig S2.2 SROC comparison of Laboratory and Real-World performance with 95% confidence contours

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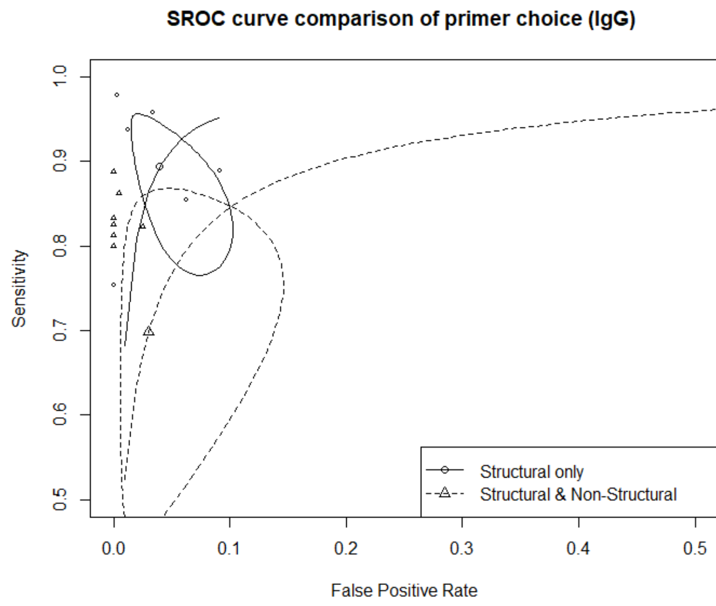

Fig S2.3 SROC comparison of primer configuration performance with 95% confidence contours

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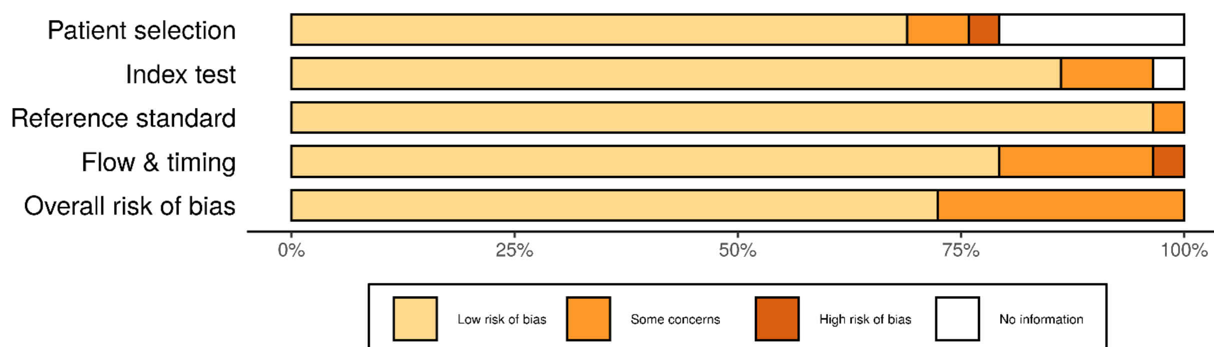
Fig S3. Summary plot of risk of bias for each study included in meta-analysis according to QUADAS-2 domain.



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PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	5
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	5
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6-7
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	6-7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	7
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	7
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	6
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	6-7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	6-7
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	7
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	7
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	7

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Reported on page #
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Supp
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Supp
Study characteristics	17	Cite each included study and present its characteristics.	Supp Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Supp Fig 2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Figures
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Supp Fig 2
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Figures
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Figures
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Supp Fig 2
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Figures
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	10-14
	23b	Discuss any limitations of the evidence included in the review.	13
	23c	Discuss any limitations of the review processes used.	13
	23d	Discuss implications of the results for practice, policy, and future research.	14
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	15
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	15
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	14
Competing interests	26	Declare any competing interests of review authors.	15
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	14

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