JCM Accepted Manuscript Posted Online 25 November 2020
J Clin Microbiol doi:10.1128/JCM.02342-20
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Accepted Manuscript Posted Online
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Revised JCM02342-20

Evaluating ten commercially-available SARS-CoV-2 rapid serological tests using the STARD (Standards for Reporting of Diagnostic Accuracy Studies) method.

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Running title : Evaluation of SARS-CoV-2 rapid serological tests

Keywords: RTD; IgG; IgM; antibodies; COVID-19; analytical performances

Abstract : 228 words
Text : 3554 words
Figures : 4
Tables : 2
Supplemental figures : 4
Supplemental tables : 3
References : 31

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ABSTRACT

Numerous SARS-CoV-2 rapid serological tests have been developed, but their accuracy has usually been assessed using very few samples, and rigorous comparisons between these tests are scarce. In this study, we evaluated and compared 10 commercially-available SARS-CoV-2 rapid serological tests using the STARD methodology (Standards for Reporting of Diagnostic Accuracy Studies). 250 sera from 159 PCR-confirmed SARS-CoV-2 patients (collected from 0 to 32 days after onset of symptoms) were tested with rapid serological tests. Control sera (N=254) were retrieved from pre-COVID periods from patients with other coronavirus infections (N=11), positive rheumatoid factors (N=3), IgG/IgM hyperglobulinemia (N=9), malaria (n=5), or no documented viral infection (N=226). All samples were tested using rapid lateral flow immunoassays (LFIA) from ten manufacturers. Only four tests achieved ≥98% specificity, with other tests ranging from 75.7%-99.2%. Sensitivities varied by the day of sample collection, from 31.7%-55.4% (Days 0-9), 65.9%-92.9% (Days 10-14), and 81.0%-95.2% (>14 days) after the onset of symptoms, respectively. Only three tests evaluated met French Health Authorities' thresholds for SARS-CoV-2 serological tests (≥90% sensitivity + ≥98% specificity). Overall, the performances between tests varied greatly, with only a third meeting acceptable specificity and sensitivity thresholds. Knowing the analytical performance of these tests will allow clinicians and most importantly laboratorians to use them with more confidence, could help determine the general population's immunological status, and may help to diagnose some patients with false-negative RT-PCR results.

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INTRODUCTION

Asymptomatic carriage of SARS-CoV-2 has been estimated in some studies to be as high as 86 % (1). Others posit that it may be responsible for up to two-thirds of viral transmission (1-4). As the world increasingly acknowledges the challenges this poses to disease containment, reliable testing has become central to monitoring the COVID-19 pandemic, informing health policy, rapidly responding to events as they evolve, and mitigating disease transmission (5, 6).

Yet, RT-PCR tests (Real-time reverse transcription-polymerase chain reaction), the gold standard for SARS-CoV-2 detection, have substantial limitations. PCR requires specialized, expensive laboratory equipment, is often only located in laboratories with biosafety level ≥2, and may be affected by sample transport and testing delays of 2-3 days, in which time COVID-19 suspects may further expose other patients and health workers (7-9). For SARS-CoV-2, RT-PCR testing also uses naso-pharyngeal swab samples that can be complex to obtain, pose considerable risk to health care workers with insufficient personal protective equipment (PPE), and produce false-negative results in up to 30 of confirmed COVID-19 patients (10-12). Chest radiography (CXR) and computed tomography (CT) scans are currently used to overcome PCR tests' lack of sensitivity but also require expensive equipment (11, 13). These challenges limit current molecular and imaging approaches' ability to be scaled up in epidemic settings where rapid, reliable, and easy population screening is needed.

Thus, serological confirmation of COVID-19 antibodies could provide an important complementary tool to PCR testing by identifying previously exposed individuals (8, 12). SARS-CoV-2 seroconversion occurs 7-14 days after the onset of symptoms (8, 14-16). Classic ELISA tests (enzyme-linked immunosorbent assays) are currently available, but considerable effort has been made by manufacturers to offer faster answers with rapid diagnostic tests (RDTs) (17). According to the Foundation for Innovative New Diagnostics (FIND), 177 SARS-

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CoV-2 antibody RDTs were commercially-available on June 15th, 2020 (18). Most information were directly submitted by test suppliers or obtained from publicly available sources and were not independently verified. Neither their analytical performance nor their usefulness in a clinical setting has yet been rigorously evaluated with a sufficient panel of samples (19, 20). In addition, validation criteria seem to be different from one country to another (21-23).

We carried out a retrospective clinical evaluation of ten commercially available RDTs, comparing their performance, according to the time between the onset of symptoms and sampling, severity of the disease and usability of the tests. Our study was designed using the 2015 Standards for Reporting of Diagnostic Accuracy Studies (STARD) (24). We aim to provide accurate clinical performance data to assess the RDTs' utility and their ability to be integrated into adapted diagnostic algorithms across health systems and epidemiological contexts, especially in areas with limited resources (24).

METHODS

Study design

We conducted a retrospective study on 250 serum samples collected between March 11 till April 3rd from 159 patients, with documented RT-PCR positive results for SARS-CoV-2 using nasopharyngeal swabs (eSwabs™-Virocult, Copan, Italy). Real-time RT-PCR targeting RNA-dependent RNA polymerase and E genes were used to detect the presence of SARS-CoV-2 as described by Corman *et al.* (7). All patients were from 2 University hospitals located in the south of Paris (Bicêtre and Paul Brousse Hospitals) and provided between one and four serum samples. Sera from COVID-19 patients were randomly selected and grouped according to the time between onset of symptoms and patient's blood sampling (0-9 days, 10-14 days, and > 14 days) (Fig. 1A).

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To assess specificity, an additional 254 sera collected prior to December 2019 were selected, and which had previously been tested positive for a separate agent or pathology that could potentially interfere with SARS-CoV-2 testing results, either other coronavirus (n=11), other viral and parasitic infections (including EBV, CMV, Rubeola, toxoplasma; n=129), a rheumatoid factor (n=3), hyperglobulinemia IgG (n=6) and IgM (n=3), malaria (n=5), or a *Treponema pallidum* hemagglutination assay (TPHA) (n=97) (Fig. 1B).

Each RDT was evaluated on the same collection of sera. The minimum sample size was calculated assuming an expected sensitivity of 90 (with 5% accuracy) and a specificity of 98 (with 2% accuracy), amounting to 250 true positive samples and 254 true negative samples (power 0.80, alpha 0.05).

Sample preparation

Selected sera were randomly placed in working boxes so as not to bias technicians' interpretation of results. Two sets of these boxes were prepared and stored at 4°C prior to being used.

Selected Tests

Diagnostic tests were selected based on supply, expected performance (based on published literature), and on commercial brochures. Ten RDTs that could detect either all antibodies or specifically identified IgG or IgM (in blood, serum, or plasma) were evaluated: (RDT 1) NG-Test IgG-IgM COVID-19 (NG-Biotech, Guipry, France), (RDT 2) Anti SARS-CoV-2 rapid test (Autobio Diagnostic CO, Zhengzhou, China), (RDT 3) Novel Coronavirus -2019-nCOV-Antibody IgG/IgM (Avioq Bio-tech CO, Yantai, China), (RDT 4) NADAL® COVID-19 IgG/IgM Test (Nal Von Minden GmBH, Regensburg, Germany), (RDT 5) Biosynex®COVID-19 BSS (Biosynex, Illkirch-Graffenstaden, France), (RDT 6) 2019-nCoV Ab Test (Innovita

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Biological Technology CO, Qian'an, China), (RDT 7) 2019-nCoV IgG/IgM (Biolidics, Mapex, Singapore), (RDT 8) COVID-19-CHECK-1 (Veda.Lab, Alençon, France), (RDT 9) Finecare SARS-CoV2 Antibody test (Guangzhou Wondfo biotech, Guangzhou, China) and (RDT 10) Wondfo SARS-CoV2 Antibody test (Guangzhou Wondfo biotech, Guangzhou, China). Characteristics of these RDTs are summarized in Table S1. Tests were performed at room temperature by trained laboratory technicians. All tests followed the manufacturers' instructions, strict biosecurity measures, and good microbiological practices and procedures (8).

The intensity of the reaction line was recorded in 3 gradations: No signal (0), very weak but definitively positive (1), and medium to high intensity (2). Values were not recorded when a control line did not appear and tests were subsequently repeated (Fig. S1A and B).

Visual test interpretation was conducted independently by two separate readers and recorded on data collection sheets. Readings were determined based on two of three readers' interpretations. In cases where all three interpretations were different; results were registered as unknown.

Data analysis

Each RDT's sensitivity and specificity was calculated with its respective confidence interval 95 (CI95) using VassarStats (http://vassarstats.net/).

The cumulative positivity at different points of illness (from symptom appearance until day 31 post-appearance) was determined as follows (i) a positive result on Day N was followed by subsequent positive results on Days N+1, N+2, N-n, etc and (ii) a negative result on Day N was preceded by negative results on Days N-1, N-2, N-n, etc. Details of the calculation are presented in Figure S2.

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Cumulative curves were fitted to an asymmetrical (five-parameter) logistic equation using Graph Prism v6 (25). For comparative purposes, the point at which 50 cumulative positivity was reached was calculated for all RDTs and expressed as the number of days post-symptom onset (Fig. S3, Table 1).

The positive predictive value (PPV) and negative predictive value (NPV) were calculated as follows: PPV = (sensitivity x prevalence) / [(sensitivity x prevalence) + ((1 – specificity) x (1 – prevalence))], and NPV = (specificity x (1 – prevalence)) / [(specificity x (1 – prevalence)) + ((1 – sensitivity) x prevalence)].

Usability Evaluation

A self-administered user experience questionnaire using the Osgood scale was used for all tests and focused on the clarity of the instructions for the test user, the test's technical complexity, the ease of test result interpretation, and access to legal information (26).

Ethics

All samples were from a Bio-bank (BIOCOVID-19) after having received ethical clearance from the Patient Protection Committee (PPC) of the Ile-de-France VII (No. 2009-965). Blood samples from patients infected with the SARS-CoV-2 virus, who were subjected to routine testing as part of clinical management but whose serum samples had not been entirely used for clinical purposes, were approved for use in this study. The biobank is stored in CRB Paris South (BRIF: BB-0033-00089). The planning, conduct, and reporting of studies was in line with the Declaration of Helsinki.

RESULTS

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Clinical characteristics of COVID-19 patients

Overall, 250 sera collected from 159 COVID-19 patients were selected from the BIOCOVID-19 Bio-bank. The distribution of the tested sera was as follows: 1 serum for 93 patients; 2 sera for 42 patients; 3 sera for 23 patients, and 4 sera for one patient. The median age was 62.9 years (range 12.8 - 97.6) and the male/female ratio was 1.69 (100/59). Among these individuals, 4.4 % (7/159) were discharged after their initial visit to the emergency room (ER) and 95.6 % (152/159) were hospitalized. Over the study period, 44.1 % (67/152) of patients required ICU care while hospitalized. The overall death rate among hospitalized patients was 19.1 % (29/152); 10.5 % (9/85) among non-ICU patients and 29.9 % (20/67) among ICU patients. Most sera were sampled on Days 0-15 (85.5 %, 219/256) after symptoms appeared, though sera from later dates (up to Day 31) were also available (Fig. 1A).

Test Performance

Cumulative positivity rate rose with time, reaching 100 % at 20-days post-symptom onset for all RDTs (Fig. 2). More than 50 % of SARS-CoV-2 infected patients had detectable antibodies 7 to 10 days after symptoms appeared (Fig. 2). The time needed to reach >95 % sensitivity varied between 14 days (for half of the RDTs tested) and 18 days (for RDT 6) (Fig. 2). Asymmetrical (five-parameter) logistic analysis demonstrated that 50 % cumulative positivity (or the median time for seroconversion) varied from 7.0 to 9.6 days (Table 1). Failures in migration, as observed by the absence of control line was observed once RDT 2, and RDT 6, and three times for RDT8. For RDT 1 a weak control line was observed once. After retesting all gave correct control lines (Figure S1B).

As expected, overall test sensitivity was highest 15 days after the appearance of symptoms (Table 2), with all RDTs reaching >90 % sensitivity at that point, except for RDT 6 and RDT 8 (81.0 % and 88.5 %, respectively). For the 8 RDTs able to differentiate between IgM and IgG,

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combined detection significantly increased overall test sensitivity with the exception of RDT 1, RDT 4 and RDT 5 (for which IgM detection seemed to be nearly as sensitive as IgM + IgG detection) (Table 2).

Specificities, calculated with sera recovered from patients between 2017 and early 2019, ranged from 75.7 % to 99.2 %. Only four tests (RDT 1, RDT 4, RDT 5 and RDT 9), reached the >98 % threshold recommended by the French Health Authorities for serological diagnostic tests (Table 2) (23). The presence of a rheumatoid factor did not induce false positive results except in the case of the RDT 3, which systematically gave a positive IgM (3/3) and/or IgG (1/3) signal. Among the 11 sera with a non-SARS-CoV-2 agent (other coronaviruses) four tests produced one false positive result and one test produced two false positives. Notably, the false positives occurring in non-SARS-CoV-2 agent samples corresponded to one serum recovered from the same patient. No other patterns were detected for other false positive results (Table S2). The concordance between all tests varied from 77.0 % to 96.4 % except in the case of the RDT 8 test that had a lower concordance with other RDTs (<80). Other RDTs gave concordant results (usually ~90 % to 95 %) (Fig. 3).

The positive and negative predictive values (PPV and NPV respectively) describe the performance of a diagnostic test. A high result can be interpreted as indicating the accuracy of such a test. The PPV and NPV are not intrinsic to the test (as true positive rate and true negative rate are) but they depend also on the prevalence. As the prevalence increases, the PPV also increases but the NPV decreases. Similarly, as the prevalence decreases the PPV decreases while the NPV increases. As a consequence, having both VPN and PPV above a certain value can be quite challenging. Among the 10 RDTs evaluated only three presented PPV and NPVs above 95 % over a large window of population prevalence (RDT1, RDT 4, and RDT9) (Fig. S4). In France, depending on the region, the sero-prevalence was estimated around 5% in June

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2020, and local estimates report now values ranging from 5 % to 15 %, depending on regions more or less impacted by the virus (27). Thus, considering a 5%-15% prevalence range, the PPV (5 -15%) for RDT1, RDT 4 and RDT 9, would be 86-95.4 %, 85.8- 95.3 %, 75.8-91.3 %, respectively and NPV (5-15 %) 99.7- 98.9 %, 99.5-98.6 %, 99.7-99.2%, respectively. Overall, the 3 RDTs perform equally well, with a slight advantage for RDT1.

Band intensity

To compare the ease of reading the RDTs' banded results, the intensity of the reaction line was recorded according to 3 gradations: No signal (0), very weak but definitively positive (1), and medium to high intensity (2). As shown in Figure 4, the overall ease of reading was highest for sera recovered >14-days after the appearance of symptoms. Band intensity was most prominent in tests with combined antibody detection (i.e. both IgM and IgG detection; RDT 9 and RDT 10 tests) (Fig. 4A). Among the eight RDTs that differentiated between antibody types, IgM band intensity was most pronounced with RDT 1 test (Fig. 4B), with RDT 3, RDT 4 and RDT 5 tests closely following. Conversely, IgM bands obtained with the RDT 6, RDT 7, RDT 8 and, to a lesser extent, the RDT 2 tests were significantly less pronounced (Fig. 4B). For IgG tests, the bands produced by the RDT 1, RDT 2, RDT 3, RDT 7 and RDT 8 tests were more prominent than the RDT 4, RDT 5 and RDT 6 tests (Fig. 4C).

Ease-of-Use

All the tests were in cassette form and nearly all devices used standard colloidal gold antigen conjugated particles (Table S1). One test (RDT 9) used fluorescent antigen conjugated particles for visualisation using a specific reader. Ease-of-use could vary from one test to another, and all contained 'Instructions For User' (IFU) manuals that were in all cases considered easy to understand (Table S3). Only RDT 9's IFU did not provide figures explaining their methods or

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results interpretation. Most (6/10) IFUs contained figures explaining their methods and results interpretation, and 3/10 IFUs contained figures explaining results interpretation (Table S3). No users reported difficulty using the RDTs, though the RDT 2 test provided a dropper with no clear instruction as to how many drops should be used. Buffer for RDT 9 was included with every test tube. Less than half of the RDTs (RDT 1, RDT 3, RDT 4, and RDT 5) included single-use plastic pipettes or similar devices for transferring samples into the test wells. No users reported difficulties identifying sample and buffer wells. All tests' results interpretation, with the exception of RDT 6, were considered easy. The recommended time-to-read results ranged from 10-20 minutes (Table S1). From a packaging and legal point of view, all manufacturers except RDT 6 respected the CE-IVD regulation to describe needed storage conditions in the IFU, on test packaging, and in product references. RDT 6's reference test was not found on the box nor within the IFU. All tests were in a single, sealed package and included a desiccant pouch.

DISCUSSION

With no curative medications currently available for COVID-19 and vaccines in early stages of development, physical distancing and widespread testing have become the primary tools available to control an unprecedented global health crisis. Serological assays and RDTs are being increasingly used across the world to address other tests' limitations, but most commercially available RDTs have had their accuracy verified on only a small number of sera without including negative samples to evaluate cross-reactivity. Moreover, their usefulness for patient management in active hospital settings and among the general public has almost never been rigorously evaluated (28,29). By demonstrating the feasibility and accuracy of rapid serological immunoassays with a substantially more robust sample size than has previously been described, we add depth to the evolving conversation surrounding SARS-CoV-2 testing

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strategies. We hope that knowing the analytical performance of nearly a dozen commercially available tests, and by providing comparative detail, we will allow clinicians to select and use these tests with more confidence and certainty.

This study is, to our knowledge, the first to compare diagnostic performance and time-to-seropositivity in nearly a dozen SAR-CoV-2 RDTs using a large sample size (250 selected samples each for specificity and sensitivity, more than double other peer-reviewed, published RDT evaluations). Other studies evaluating antibody tests have also not included samples from patients with non-SARS-CoV-2 infections to evaluate specificity.

Overall, after the appearance of symptoms, seroconversion occurred on Days 7-9 for 50 of COVID positive patients (Table 1), with >95 % seroconverting after 14 days using RDT 1, RDT3, RDT 4, RDT 9 and RDT 10) and 18 days for RDT 6) (Fig. 2). The specificities ranged from 94.5-99.2 %, except for RDT 8 test (75.7 %). Notably, the RDT 3 test produced systematic false positive results with sera of patients who had a high level of rhumatoid factor (Table S2).

Thresholds for sensitivity and specificity for RDTs have been set by many National Health Authorities (21-23). For diagnosis in symptomatic patients, high sensitivity is required (generally ≥90 %), while specificity is less critical as some false-positives may be tolerated as other potential diagnoses are considered in parallel (RT-PCR and/or CT scans). However, if LFIAs were deployed as an individual-level approach to inform release from quarantine or immune-protection, then high specificity (>98) is essential, as false-positive results return non-immune individuals to risk of exposure (23). Using the French health authority (21) acceptable limits for SARS-CoV-2 serological tests (≥90 % sensitivity; ≥98 % specificity) our evaluation validated only three RDTs for clinical use, namely NG-Test IgG-IgM COVID-19 (RDT 1, NG-Biotech), NADAL® COVID-19 IgG/IgM Test (RDT4, Nal Von Minden GmBH) and Finecare SARS-CoV2 Antibody test (RDT 9, Guangzhou Wondfo biotech).

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Appraisal of test performance should also consider the influence of population prevalence, as it may change over time, geography and within different population groups. The potential risk of a test providing false positive results is crucial for release from lock-down of non-immune individuals. Among the 10 RDTs evaluated only three presented PPV and NPVs above 95 % over a large window of population prevalence (RDT1, RDT 4, and RDT9).

These serological tests were able to independently diagnose COVID-19, especially in those with ≥2 weeks of symptoms, and could be used to triangulate unclear or false negative results from PCR and CT testing. They could also be used to monitor the status of medical and non-medical frontline workers and, over the longer term, to establish population level immunity as countries' social restrictions ease. In the US (Santa Clara County, California) rapid antibody tests were used to evaluate the population prevalence of antibodies (ranging from 2.49-4.16) and helped authorities to understand that infection was far more widespread (55-fold) than indicated by the number of confirmed cases. These data are crucial to calibrate epidemic and mortality projections (30).

Among the three RDTs fulfilling the French health authorities' criteria, only NG-Test IgG-IgM COVID-19 (NG-Biotech) might be considered a self-test since it includes all materials needed for self-puncture and capillary blood recovery. Nevertheless, we only authenticated this using serum, since its use has been previously established in capillary whole blood and our results in serum confirm those of the initial study (31). Namely, that this bedside fingerprick test confirmed infection in <15 minutes and could be performed by a medical practitioner without specialized training or a pathology laboratory (31).

Our study is limited in the following ways: (1) RT-PCR detection was based on upper respiratory tract specimens from patients with severe symptoms. None were asymptomatic patients (who did not access care). (2) Most study participants' diagnoses were based on

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positive findings from an RT-PCR test using respiratory samples. Patients with negative RT-PCR results but with chest imaging compatible with COVID-19 were not included. (3) Because the epidemic situation in France was very recent at the time of study, samples were collected during the acute phase of illness. Accordingly, we do not yet have sera from later stages to evaluate antibody persistence. (4) Only 10 out of more than 170 available RDTs have been evaluated.

The COVID-19 pandemic has revealed gaps in our diagnostic arsenal and is highlighting the essential role of sero-diagnostics in public health response (32). With the use of carefully verified assays, appropriately designed serologic studies will help characterize transmission dynamics, refine disease burden estimates, diagnose suspected cases, and confirm clinically diagnosed patients without access to RT-PCR testing.

Though this assessment demonstrates varied analytical performance across a sample of current SARS-CoV-2 RDTs, they nevertheless hold real utility as tool for establishing population level exposure: many people have been exposed more than 3 weeks prior to antibody testing and would benefit from the nearly 100 % sensitivity (in all tests evaluated) after 3 weeks' time. However, highly sensitive (as early as 7 days) and specific tests are needed, both to achieve sufficiently high positive predictive values since population prevalence is often estimated to be low (≤5 %), and to be clinically useful as an initial diagnostic assay and a complement to direct RNA testing. Only three of the evaluated assays met the thresholds needed (sensitivity of >90 % at 14 days after symptom appearance and >98 % specificity).

Serological assays are simple, cheap, rapid, easy to interpret, and practical (can be stored at room temperature). They detect IgM, IgG, or both and can be performed directly at a patient's bedside, at a general physician's office, or when triaging in an emergency department, as most have been validated using whole blood.

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DECLARATION OF INTERESTS

The authors declare no conflict of interest

FUNDING

This research was supported by Assistance Publique – Hôpitaux de Paris (APHP), Médecins Sans Frontières (MSF), and by a Grant from the French Defence Innovation Agency (AID).

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488 **Table 1.** Median times for SARS-CoV-2 seroconversion using 10 commercially available

489 RDTs, Paris, France, June 2020.

490

RDT	Median time to seroconversion	
	Days after symptom onset	CI95
1	8.3	(8.2 - 8.4)
2	7.4	(7.3 - 7.6)
3	7.0	(6.8 - 7.1)
4	7.2	(7.0 - 7.3)
5	7.8	(7.6 – 7.9)
6	9.6	(9.5 - 9.7)
7	8.2	(8.1 – 8.4)
8	7.5	(7.4 - 7.7)
9	7.0	(6.8 - 7.1)
10	7.0	(6.8 - 7.1)

491 CI95, 95 confidence interval

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Table 2. Performance of 10 rapid serological tests for SARS-CoV-2 antibodies, Paris, France, June 2020.

Tests	N	Tests Not Interpretable	Sensitivity by time elapsed after symptom onset, % (CI 95 %)				Specificity		
			N[a]	Ig type	0-9 days	10-14 days	>14 days	N[b]	Ig type	(CI95)
				IgM or IgG	**42.0 (32.3 - 52.3)**	**75,0 (64.1 - 83.5)**	**93.7 (83.7 - 97.9)**		**IgM or IgG**	**99.2 (96.9-99.9)**
RDT 1 (IgM/IgG)	499	0	247	IgM	42.0 (32.3 - 52.3)	75,0 (64.1 - 83.5)	93.7 (83.7 - 97.9)	252	IgM	99.6 (97.5 - 100.0)
				IgG	33.0 (24.1 - 43.2)	70.2 (59.1 - 79.5)	85.7 (74.1 - 92.9)		IgG	99.2 (96.9-99.9)
				IgM or IgG	**52.0 (41.8 - 62.0)**	**87.1 (77.6 - 93.1)**	**90.3 (79.5 - 96.0)**		**IgM or IgG**	**94.5 (90.7-96.8)**
RDT 2 (IgM/IgG)	500	0	247	IgM	46.0 (36.1 - 56.2)	81.2 (70.9 - 88.5)	82.3 (70.0 - 90.4)	253	IgM	96.0 (92.6 - 98.0)
				IgG	44.0 (34.2 - 54.3)	83.5 (73.6 - 90.4)	83.9 (71.9 - 91.6)		IgG	97.6 (94.7 - 99.0)
				IgM or IgG	**46.5 (36.6 - 56.7))**	**76.5 (65.6 - 84.9)**	**91.8 (81.2 - 96.9)**		**IgM or IgG**	**94.1 (90.1-96.6)**
RDT 3 (IgM/IgG)	482	1	243	IgM	42.6 (32.9 - 52.8)	75.3 (64.3 - 83.9)	86.9 (75.2 - 93.8)	238	IgM	95.4 (91.7 - 97.6)
				IgG	45.5 (35.7 - 55.7)	75.3 (64.3 - 83.9)	91.8 (81.2 - 96.9)		IgG	95.8 (92.2 - 97.9)
				IgM or IgG	**55.4 (45.2 - 65.2)**	**90.6 (81.8 - 95.6)**	**92.1 (81.7 - 97.0)**		**IgM or IgG**	**99.2 (96.9-99.9)**
RDT4 (IgM/IgG)	503	0	249	IgM	54.5 (44.3 - 64.3)	88.2 (79.0 - 93.9)	90.5 (79.8 - 96.1)	254	IgM	100.0 (98.1 - 100)
				IgG	18.8 (12.0 - 28.1)	54.1 (43.0 - 64.9)	90.5 (79.8 - 96.1)		IgG	99.2 (96.9-99.9)
				IgM or IgG	**48.0 (38.0 - 58.2)**	**84.3 (74.3 - 91.1)**	**90.5 (79.8 - 96.1)**		**IgM or IgG**	**92.4 (83.6 - 96.9)**
RDT5 (IgM/IgG)	495	0	246	IgM	48.0 (38.0 - 58.2)	80.7 (70.3 - 88.3)	90.5 (79.8 - 96.1)	249	IgM	97.5 (90.3 - 99.6)
				IgG	22.0 (14.6 - 31.6)	69.9 (58.7 - 79.2)	77.8 (65.2 - 86.9)		IgG	94.9 (86.9 - 987.4)
				IgM or IgG	**31.7 (23.0 - 41.8)**	**65.9 (54.7 - 75.6)**	**81.0 (68.7 - 89.4)**		**IgM or IgG**	**98.4 (95.7-99.5)**
RDT 6 (IgM/IgG)	502	0	249	IgM	22.8 (15.3 - 32.4)	54.1 (43.0 - 64.9)	61.9 (48.8 - 73.6)	253	IgM	99.2 (96.9 - 99.9)
				IgG	21.8 (14.4 - 31.3)	60.0 (48.8 - 70.3)	71.4 (58.5 - 81.8)		IgG	98.8 (96.3 - 99.7)

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				IgM or IgG	**35.7 (24.9 - 48.1)**	**78.8 (64.9 - 88.5)**	**93.3 (80.7 - 98.3)**		**IgM or IgG**	**92.4 (83.6 - 96.7)**
RDT 7 (IgM/IgG)[c]	246	0	167	IgM	20.0 (11.7 - 31.6)	32.7 (20.7 - 47.3)	53.3 (38.0 - 68.1)	79	IgM	97.5 (90.3 - 99.6)
				IgG	32.9 (22.4 - 45.2)	76.9 (62.8 - 87.0)	93.3 (80.7 - 98.3)		IgG	94.9 (86.9 - 98.4)
				IgM or IgG	**55.7 (45.2 - 65.6)**	**81.3 (70.6 - 88.8)**	**88.5 (77.2 - 94.9)**		**IgM or IgG**	**75.7 (69.8-80.8)**
RDT 8 (IgM/IgG)	488	3	238	IgM	42.3 (32.4 - 52.7)	70.0 (58.6 - 79.5)	65.6 (52.2 - 77.0)	247	IgM	79.8 (74.1 - 84.5)
				IgG	46.4 (36.3 - 56.8)	71.3 (59.9 - 80.5)	85.2 (73.3 - 92.6)		IgG	87.9 (83.0 - 91.5)
RDT 9 (Total Ig)	500	0	249	**Total Ig**	**55.4 (45.2 - 65.2)**	**92.9 (84.7 - 97.1)**	**95.2 (85.8 - 98.8)**	251	**Total Ig**	**98.4 (95.7 - 99.4)**
RDT10 (Total Ig)	503	0	249	**Total Ig**	**55.4 (45.2 - 65.2)**	**92.9 (84.7 - 97.1)**	**92.1 (81.7 - 97.0)**	254	**Total Ig**	**96.5 (93.2 - 98.3)**

[a] N corresponds to the number of tested sera from COVID+ patient

[b] N corresponds to the number of tested sera from COVID negative patient

[c] The RDT 7 test was evaluated only on half of the total sera collection (only 250 tests received)

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FIGURE LEGENDS

Figure 1. Sera collection used for the evaluation. A. Distribution of 250 sera from COVID positive patients according to the number of days after onset of symptoms. B. Distribution of the 254 control sera.

Figure 2. Cumulative positivity rate obtained with 10 RDTs in sera from COVID-19 patients stratified by the number of days after appearance of symptoms. The day after symptom appearance with >95 % positivity is indicated by a coloured bar (red for RDT 1, black for the other tests). The abscisses correspond to days post symptoms.

Figure 3. Results agreement between RDTs. Percent agreement is indicated across all RDT combinations. RDTs were considered positive if any of IgG and/or IgM was detected.

Figure 4. Results (visible band) intensity for IgM + IgG (panel A), IgM only (panel B), and IgG only (panel C) tests.

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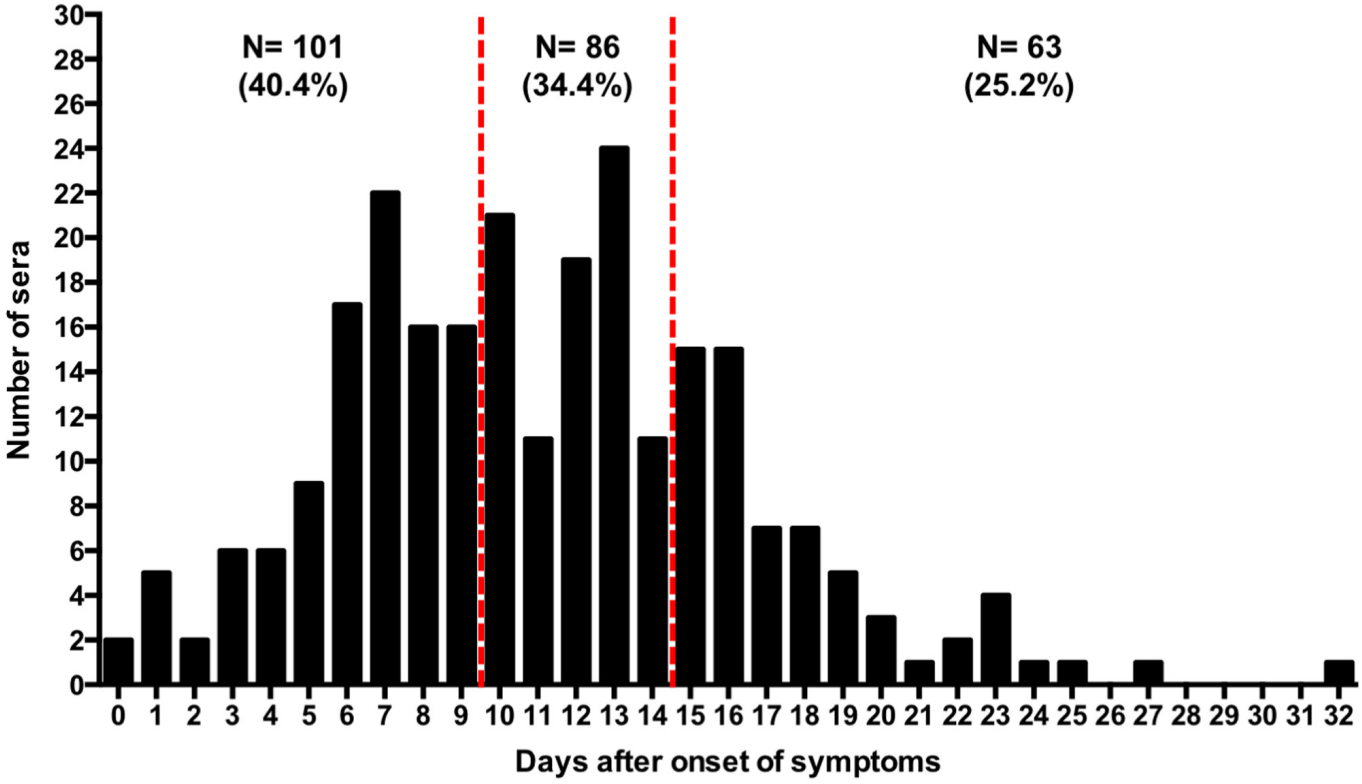
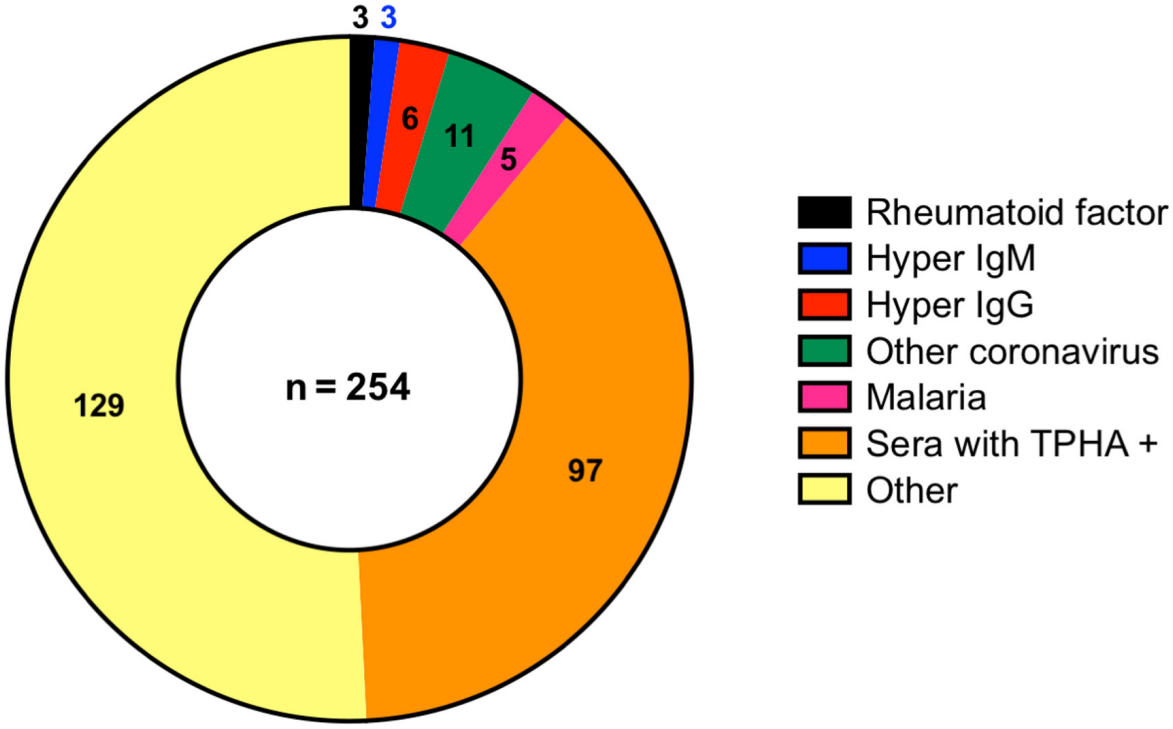
A



B



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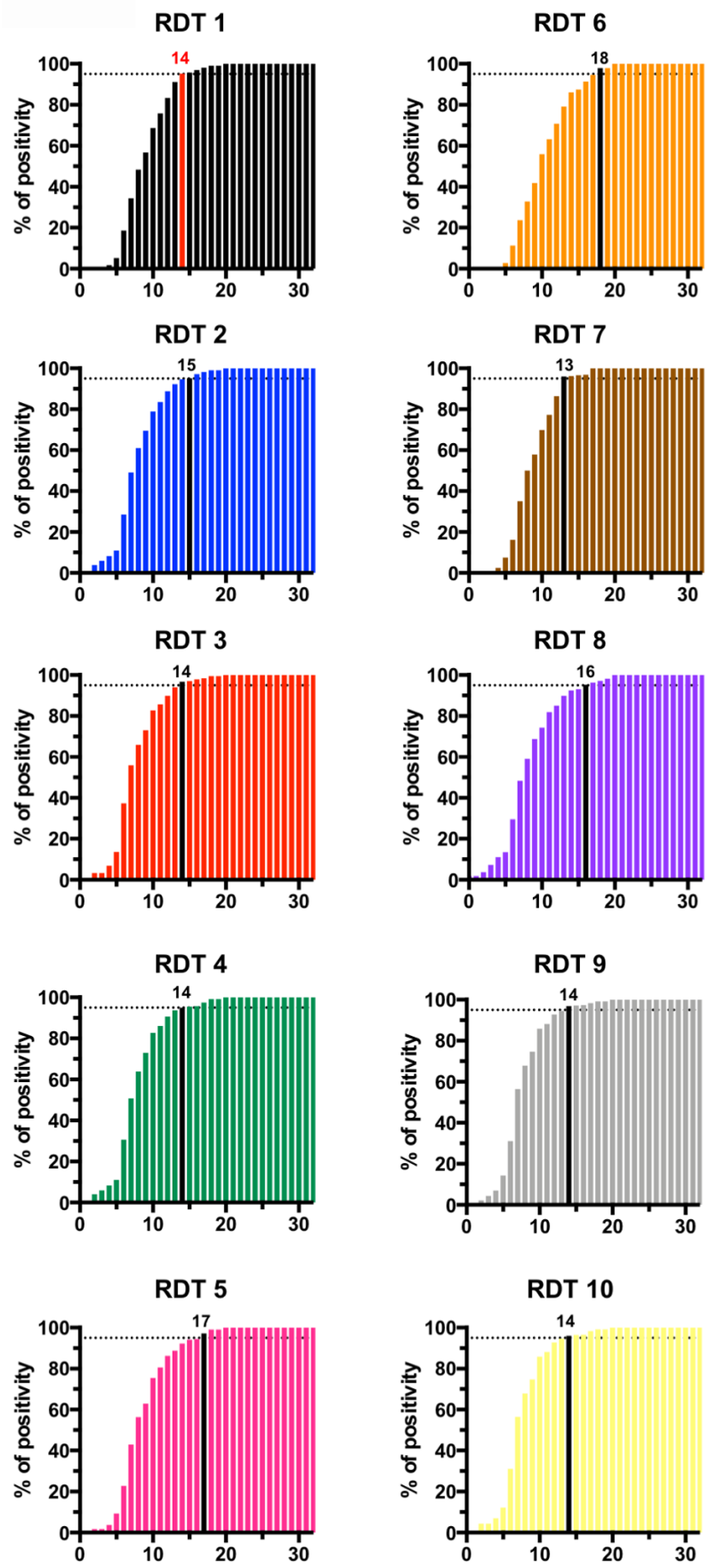

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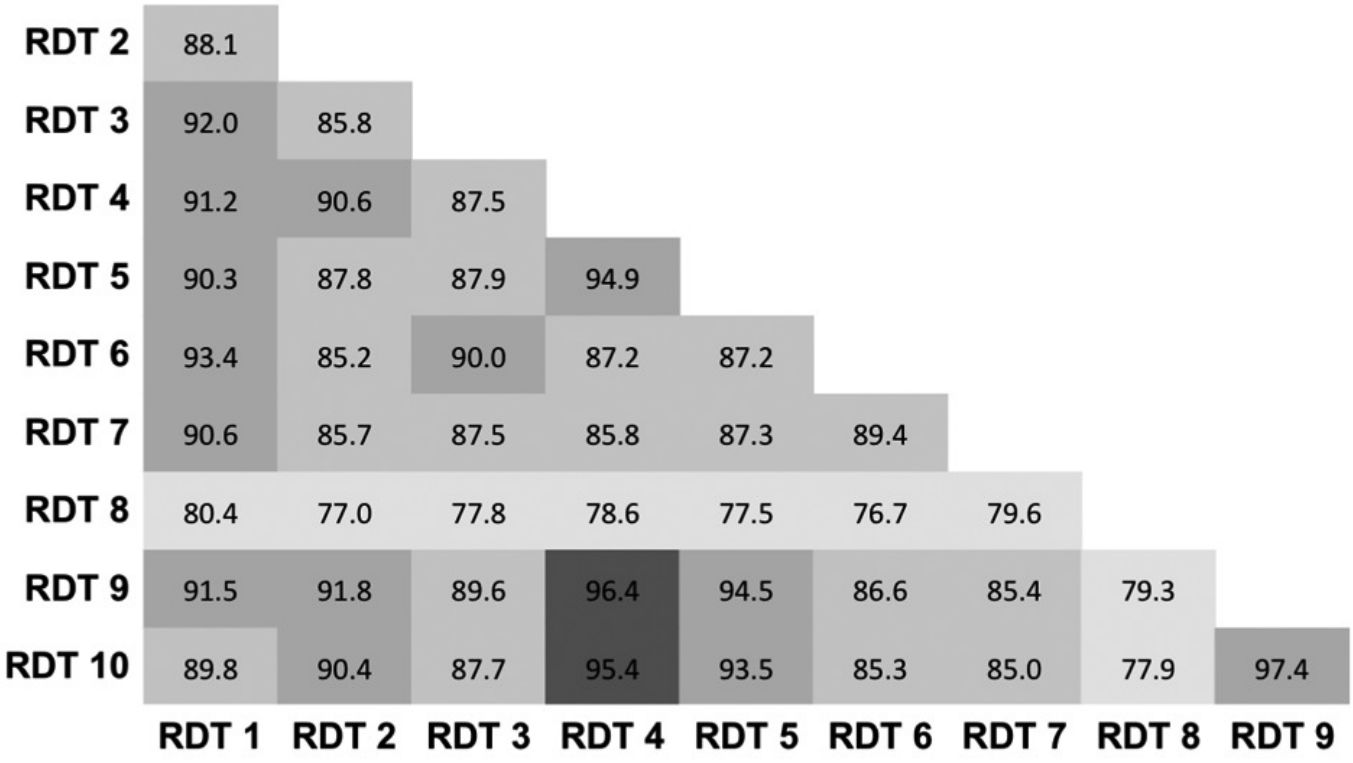

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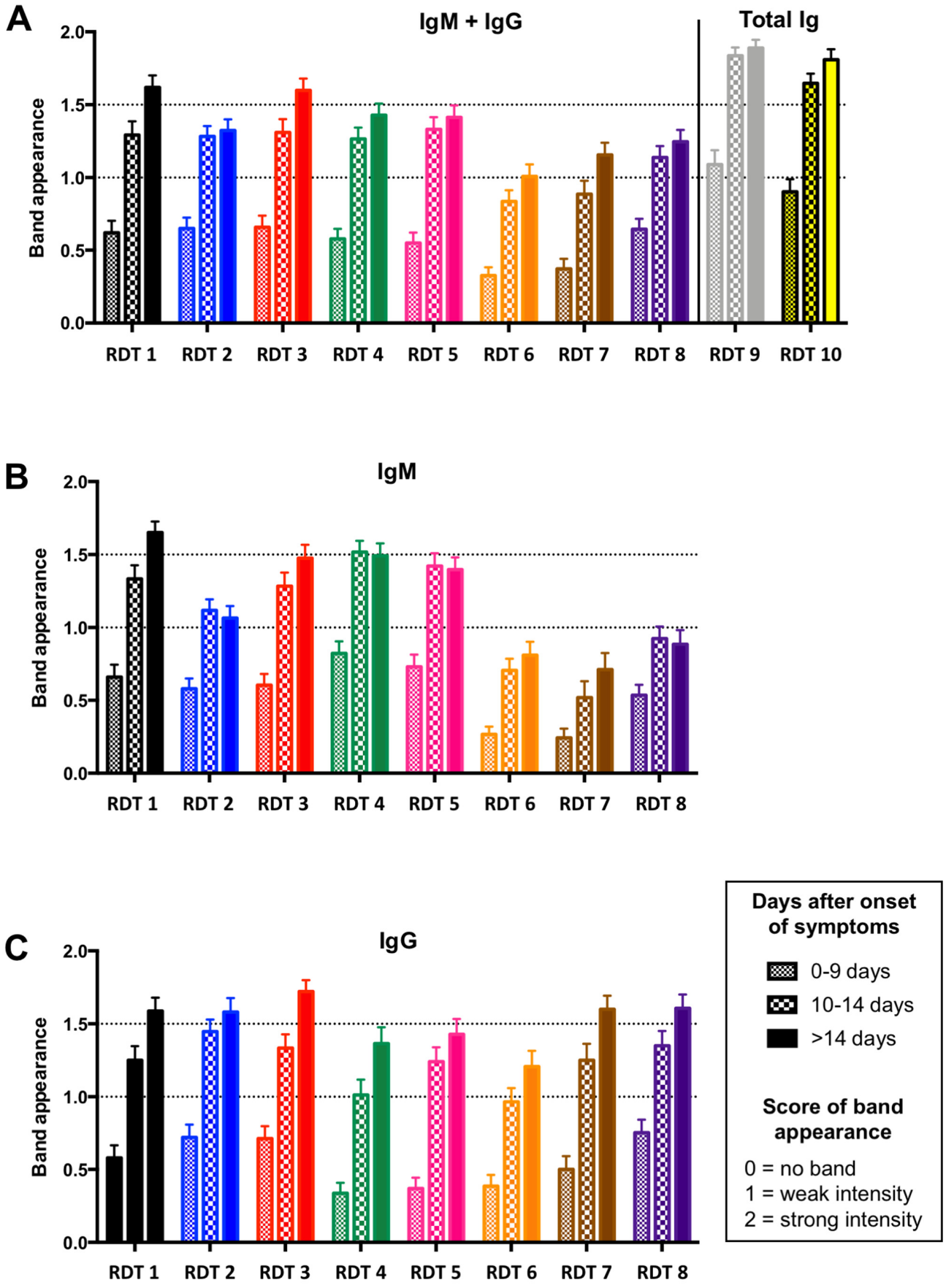

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Supplementary data

Revised JCM

Evaluating ten commercially-available SARS-CoV-2 rapid serological tests using the STARD (Standards for Reporting of Diagnostic Accuracy Studies) method.

Supplemental figures: 4
Supplemental tables: 3

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Supplemental Figures

Figure S1. Index (panel A) and results of negative, weak positive, medium/high positive, and undetermined tests.

A

Rating index	Reading intensity scale
0	Not reactive
1	Very weak, but definitely reactive
2	Medium to high reactivity
U	Undetermined

B

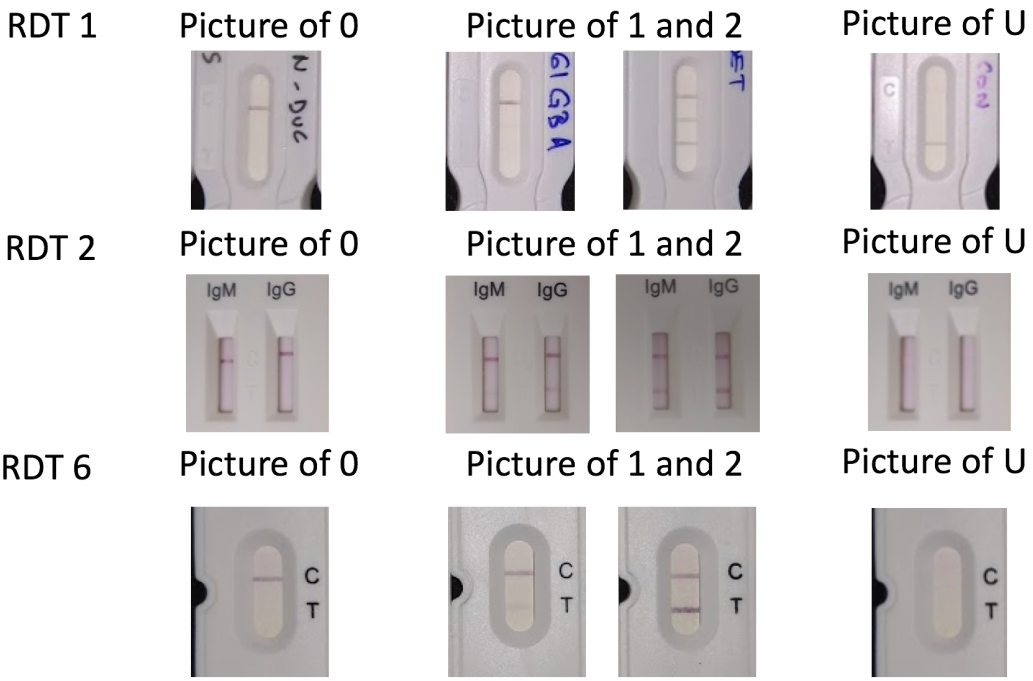

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Figure S2. Assessment of cumulative positivity stratified by the number days after symptom appearance.

Day after onset of symptoms	1	2	3	4	5	6	...	N
Patient 1	n	n	n	n	**N**			
Patient 2	**N**							
Patient 3					**P**	p	p	p
Patient 4	n	n	**N**				**P**	p
Patient 5	**N**	n	n	**N**		**P**	p	p
Patient 6	**P**	p	p	p	p	p	p	p

Cumulative number of negative results	4	3	3	2	1	0	0	0
Cumulative number of positive results	1	1	1	1	2	3	4	4
Cumulative % of positivity	**20**	**25**	**25**	**33,33**	**66,67**	**100**	**100**	**100**

P Sample tested positive

N Sample tested negative

p Sample interpreted as positive

n Sample interpreted as negative

Only one serum was available (and tested) for patients 1, 2, 3 and 6
Two sera were available (and tested) for patients 4
Three sera were available (and tested) for patients 5

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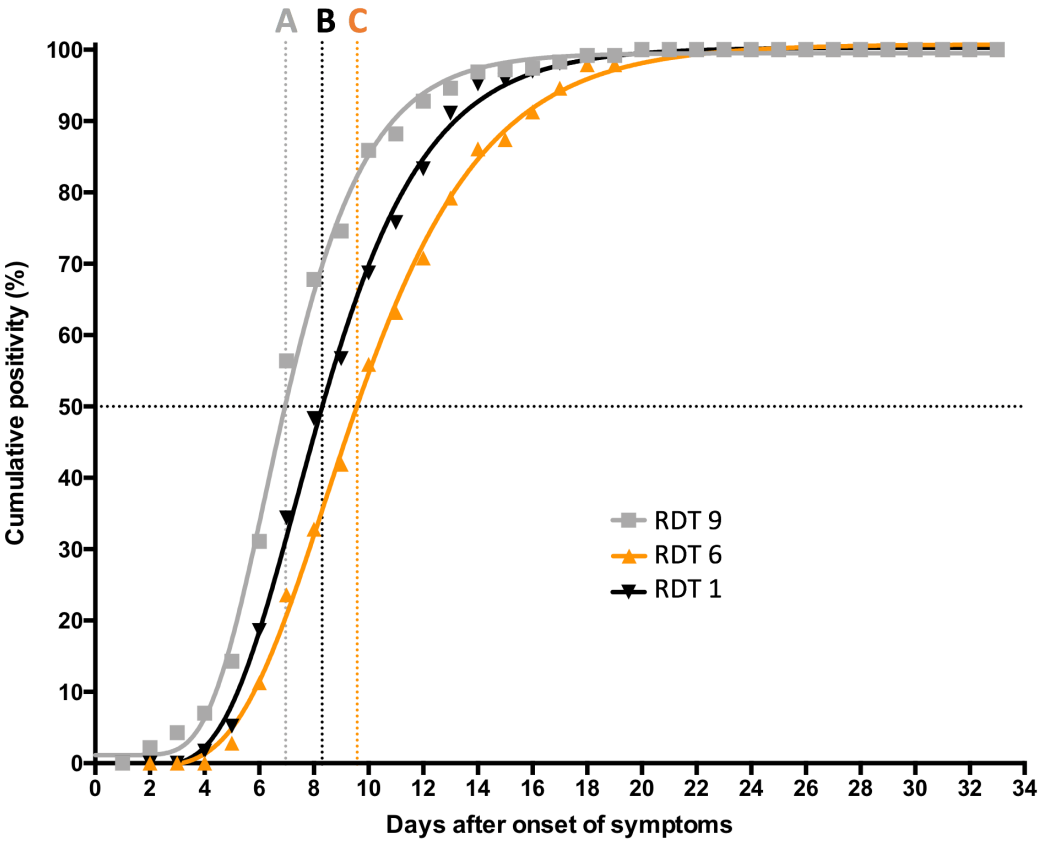
Figure S3. Best fit asymmetric curve for RDT 1, RDT 6 and RDT 9 test cumulative positivity.



A = 6.962 (CI95%: 6.837 – 7.087)

B = 8.297 (CI95%: 8.185 – 8.409)

C = 9.579 (CI95%: 9.437 – 9.722)

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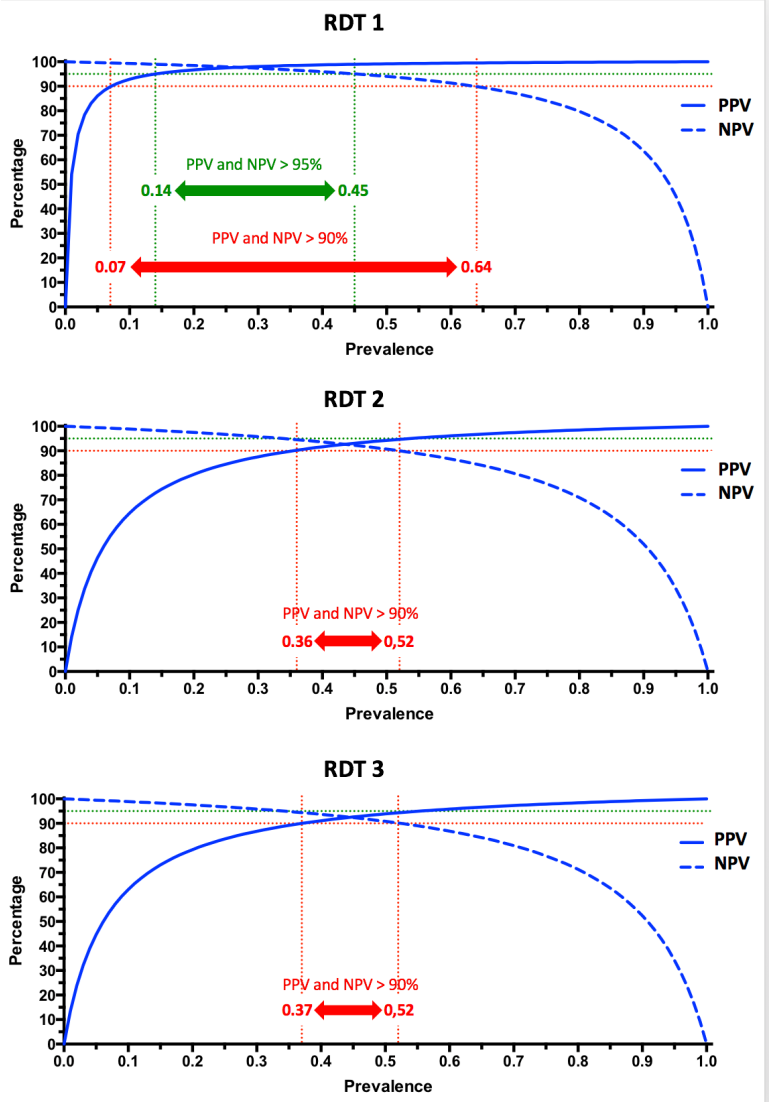
Figure S4. Influence of population prevalence of seropositivity on assay performance. Scenarios with increasing population prevalence (x-axis) are shown for each RDT. PPV (Positive Predictive value) and NPV (Negative predictive value) expressed in percentage (y axis) have been calculated using VassarStats (http://vassarstats.net/). Zones for which both PPV and NPV are above 90% (red zone) or above 95% (green zone) are indicated.



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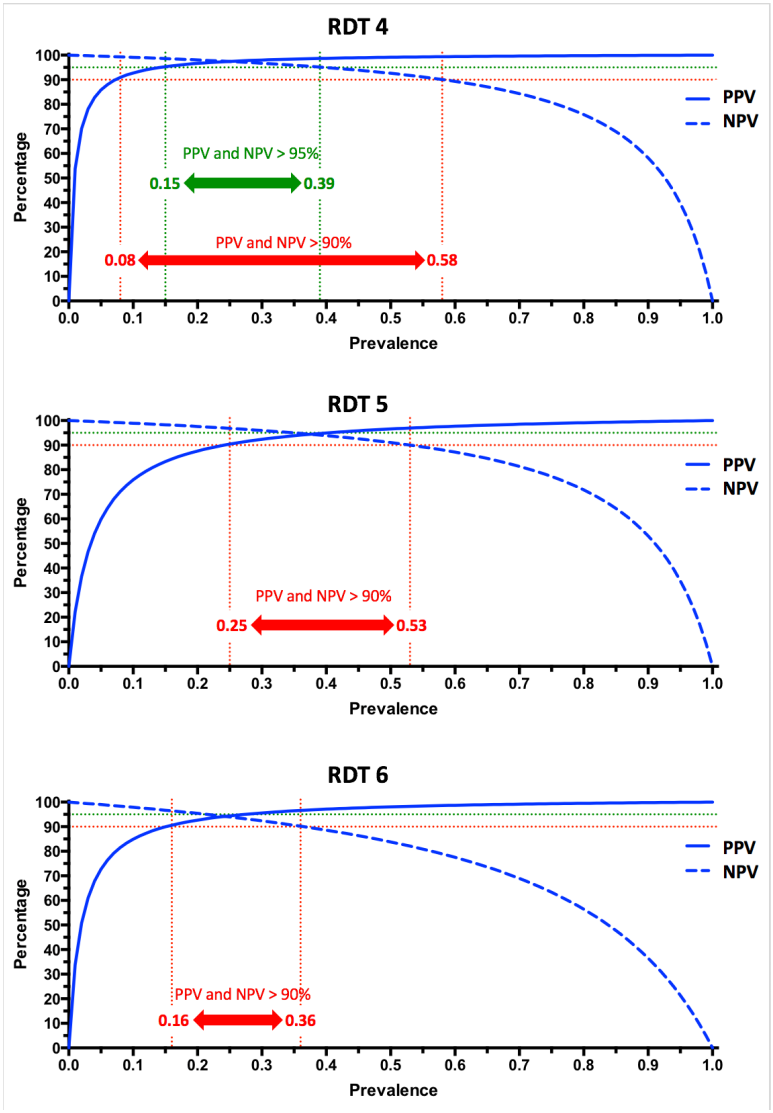

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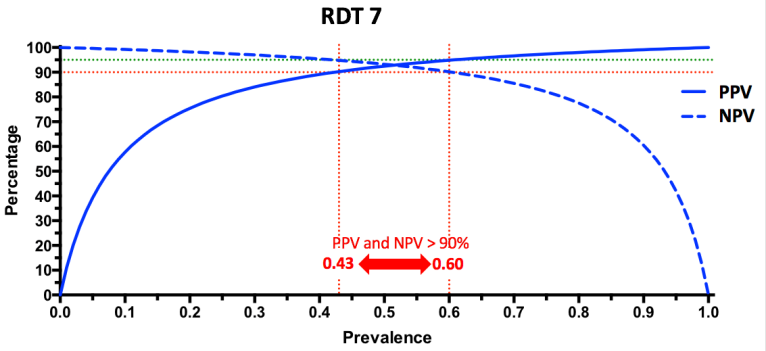

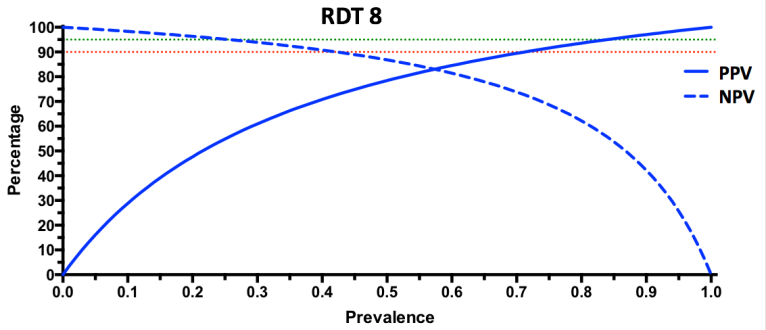

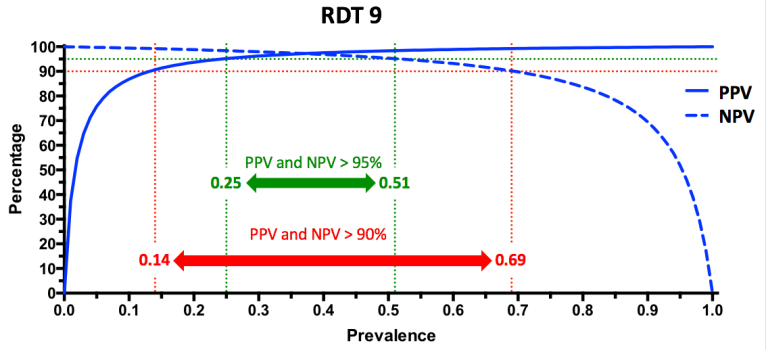

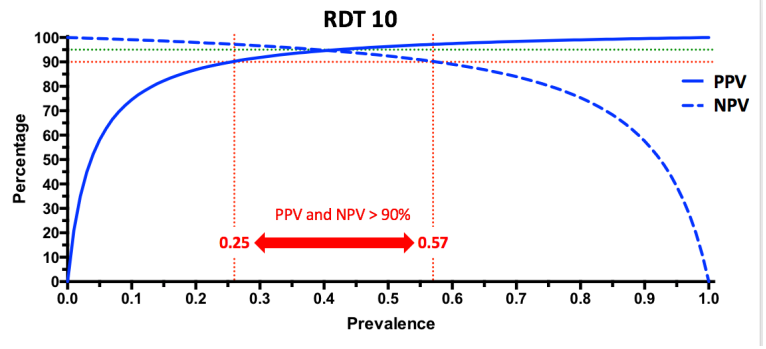

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Table S1: Immunoassay kit and manufacturer information

	RDT 1	RDT 2	RDT 3	RDT 4	RDT 5	RDT 6	RDT 7	RDT 8	RDT 9	RDT 10
Name	**NG-Test IgG-IgM COVID-19**	**Anti-SARS-CoV-2 Rapid test**	**Novel Coronavirus (2019-nCoV) Antibody IgG/IgM Assay Kit**	**NADAL COVID-19 IgG/IgM Test**	**Biosynex COVID-19 BSS**	**2019-nCoV Ab Test**	**2019-nCoV IgG/IgM**	**COVID-19-CHECK-1**	**Finecare SARS-CoV-2 Antibody Test**	**Wondfo SARS-CoV-2 Antibody Test**
Manufacturer	**NG Biotech SA, Guipry, France**	**Autobio Diagnostics Co, Ltd, Zhengshou, China**	**Aviog Bio-tech Co, Ltd, Shandong, China**	**Nal Von Minden Co, Ltd, Moers, Germany**	**Biosynex SWISS SA, Fribourg, Switzerland**	**Innovita (Tangshan) Biological Technology Co, Ltd, Hebei, China**	**Biolidics Co, Ltd, Mapex, Singapore**	**Vedal Lab SA, Alençon, France**	**Wondfo Biotech Co, Ltd, Guangzhou, China**	**Wondfo Biotech Co, Ltd, Guangzhou, China**
Catalogue No./manufacturer Ref	NGB-COV-W23-002	RTAQ202	------	COV20030034	SW40005	------	C88-F015016-B1	200081-4-2-3L	W276	W195
Lot number tested	200414-01	21C22-J01	20200201	243001	COV20040003	20200402	V5020032352	23040-46	F27614309AD	
Product description										
Antibody detection	IgG-IgM	IgG-IgM	IgG-IgM	IgG-IgM	IgG-IgM	IgG-IgM	IgG-IgM	IgG-IgM	Total Ab	IgG/IgM 1 test,1 line
Antigens *	NP, SP	------	------	------	------	NP, SP	------	------	------	------
Detection conjugate	Colloidal gold	Colloidal gold	Colloidal gold	Colloidal gold	Colloidal gold	Colloidal gold	Colloidal gold	Colloidal gold	Fluorescent conjugate	Colloidal gold
Type of reading	Visual	Visual	Visual	Visual	Visual	Visual	Visual	Visual	UV automatic reader	Visual
Format	cassette with single lane and different band for IgG and IgM	cassette with separate lane for IgG and IgM	cassette with single lane and different band for IgG and IgM	cassette with single lane and different band for IgG and IgM	cassette with single lane and different band for IgG and IgM	cassette with separate lane and different band for IgG and IgM	cassette with single lane and different band for IgG and IgM	cassette with single lane and different band for IgG and IgM	cassette with single lane and single band for both IgG and IgM	cassette with single lane and single band for both IgG and IgM
Specifications										
Sample type	WB/Serum/Plasma	WB/Serum/Plasma	WB/Serum/Plasma	WB/Serum/Plasma	WB/Serum/Plasma	WB/Serum/Plasma	WB/Serum/Plasma	WB/Serum/Plasma	WB/Serum/Plasma	WB/Serum/Plasma
Sample volume	10µL	5µL	10µL	5µL (S, P), 10µL (WB)	10µL	10µL	20µL	10 µL	10µL	10 µL
Pipette for sample volume provided	Not provided but system integrated to device for direct transfer for Capillary WB	Not provided	transfert system, lancet	Not provided	Plastic disposable pipettes	Not provided	Not provided	Not provided	pipette tips and tubes of detection buffer	Not provided
Diluent volume	2 drops	60µL	2 drops (30-70µL)	2 drops	2 drops (80 µL)	2 drops (80 µL)	3 drops	3 drops (100 µL)	------	2-3 drops
Diluant bottle format	1.5 mL	4,5 mL	4.5 mL	3 mL	------	5 mL	5 mL	3 mL	25 tubes of detection buffer	
Time to result	15 min	15-20 min	15 min	15 min	20 min	15 min	10 min	10-15 min	10 min	15 min
Limit Of Detection	------	------	------	3,4 ng/mL (IgG), 210 ng/mL (IgM)	------	------	------	------	------	------
Interference reported	None reported	None reported	None reported	None reported	SARS-CoV Ab, Rheumatoid Factors, MERS-CoV Ab	None reported	------	None reported	None reported	None reported
Cross -reactivity reported on IFU	None reported	None reported	None reported	None reported	None	None	------	None reported	None reported	None reported
Shelf-life (months)	24 m	12 m	18 m	24 m	24 m	18 m	24 m	12 m	12 m	12 m
Storage temperature (°C)	2-30°C	2-30°C	4-30°C	2-30°C	2-30°C	4-30°C	4-30°C	2-30°C	4-30°C	4-30°C
Package size	5 test/ box	20 test/box	20 test/box	10 test/bags	25 test/box	40 test/box	50 test/box	20 test/box	25 test/box	20 test/box
Controls	Internal control line	Internal control line	Internal control line	Internal control line	Internal control line	Internal control line	Internal control line	Internal control line	Internal control line	Internal control line
Performance notes	------	Some band smearing	------	------	------	Some band smearing	------	------	------	------
Regulatory approval										
IVD Certification	CE-IVD	CE IVD, Chinese FDA-EUA,	CE IVD, Chinese FDA-EUA,	CE-IVD	CE-IVD	CE IVD, Chinese FDA-EUA,	CE-IVD	CE-IVD	CE, Chinese FDA-EUA, Taiwan FDA	CE, Chinese FDA-EUA, Taiwan FDA
Pictures of the Kit content										
Kit Acquisition for study	Provided by supplier Free of charge	Purchased from supplier	Provided by supplier Free of charge	Purchased from supplier	Purchased from supplier	Purchased from supplier	Purchased from supplier	Purchased from supplier	Provided by supplier Free of charge	Provided by supplier Free of charge

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Table S2. Detail results obtained with the 254 sera of COVID negative patients

Tests	Rheumatoid factor		Hyper IgG		Hyper IgM		Sera with TPHA +		Other coronavirus		Other			Malaria		Total		
	TN[a]	FP	TN	FP	TN	FP	TN	FP	TN	FP	TN	FP	NI	TN	FP	n	TN	FP
RDT 1	3	0	6	0	3	0	94	1G[b]	11	0	128	1MG	0	5	0	252	250	2
RDT 2	3	0	6	0	3	0	89	5M + 2G + 1MG	11	0	122	3M + 2G +1MG	1	5	0	254	239	14
RDT 3	0	2M +1MG	5	0	2	1MG	86	2M + 1G + 1MG	10	1MG	121	2G + 2MG	0	ND	ND	238	224	14
RDT 4	3	0	6	0	3	0	97	0	11	0	127	2G	0	5	0	254	252	2
RDT 5	3	0	5	0	3	0	92	1M + 1G	10	0	124	2M + 3G	0	4	1M	249	241	8
RDT 6	3	0	6	0	2	1G	95	1M	10	1G	129	0	0	4	1MG	253	249	4
RDT 7*	3	0	5	0	2	1G	12	2M	10	1G	41	2G	0	ND	ND	79	73	6
RDT 8	3	0	4	1G	1	2G	72	10M + 4G + 6MG	8	2G	95	24M + 6G + 4MG	0	4	1M	247	187	60
RDT 9	3	0	6	0	2	1T	96	1T	11	0	127	2	0	2	0	251	247	4
RDT 10	3	0	5	1T	2	1T	94	3T	10	1T	126	3	0	5	0	254	245	9

[a] TN, True negative ; FP, False positive; NI, Not interpretable; ND, Not determined
[b] M = IgM, G = IgG, MG = IgM + IgG, T= Total Ig
*Only part (79/254) of the collection was tested due to a limited number of tests received

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Table S3. Usability of the ten RDTs

RDTs	1	2	3	4	5	6	7	8	9	10
Clarity of instruction for user										
Manufacturer instructions	Very clear	Very clear	Clear	Very clear	Very clear	Very Clear	Clear	Very Clear	Clear	Clear
Presence of pictures, schemas	methods and results	methods and results	methods and results	methods and result	methods and results	methods and results	results only	results only	none	methods only
Technical complexity										
Technical complexity	Very easy	Easy	Very easy	Very easy	Very easy	Very easy	Very easy	Very easy	Easy	Very easy
Number of steps	3	3	3	3	3	3	3	3	3	3
Exact measurements or volumes for specimens	No (Drop)	Yes (µl)	No (Drop)	Yes (µl)	Yes (µl)	Yes (µl)	No (Drop)	Yes (µl)	Yes (µl)	Yes (µl)
All equipment present in the kit to use test	Yes	No	Yes	Yes	Yes	No	No	No	Yes	No
Easy to identify the well to deposit the sample	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Easy to identify the well to deposit buffer	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Results interpretation										
Easiness of results interpretation	Very easy	Very easy	Very easy	Very easy	Very easy	Difficult	Very easy	Very easy	Very easy	Very easy
Reading type Time to results (min)	Visual <15	Visual < 15	Visual <15	Visual <15	Visual <15	Visual <15	Visual <15	Visual <15	Visual <15	Visual 15-20
Packaging, legal information										
T° storage conditions available	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Product reference available	Yes	Yes	No	Yes	Ye	No	Yes	Yes	Yes	Yes
Single sealed package	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pouch dessicant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes