Report
Status of the validation of point-of-care serology tests for SARS-CoV-2 diagnostics: considerations for use

Status as at 15 July 2020

Data collection and reporting by the Serology Taskforce, which is part of the Dutch National Testing Capacity Coordination Structure (Landelijke Coördinatiestructuur Testcapaciteit, LCT)

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Colophon

This is a living document. New versions of it will be issued regularly, in which the data will be updated, depending on the validation data provided by the laboratories.

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Version control:

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- [a terminology change in the Dutch version]
- Status of POCT validation in the Netherlands, updated after 20 April (Section 2)
- Evaluation supplemented with data from Franciscus Vlietland Groep Foundation; Twente Achterhoek Microbiology Laboratory; Maastricht University Medical Centre (Sections 2 and 3)

Version dated 27 April 2020: third version
- Status of POCT validation in the Netherlands, updated after 27 April (Section 2)
- Added the numbers of samples that the sensitivity and specificity are based on (Section 3)
- Specificity calculated over the total number of samples from various laboratories (Section 3)
- Evaluation supplemented with data from NoordwestZiekenhuisgroep (Sections 2 and 3)

Version dated 5 May 2020: fourth version
- Short introduction added (Section 1)
- Status of POCT validation in the Netherlands, updated after 5 May (Section 2)
- Evaluation supplemented with data from Saltro and COMICRO (Sections 2 and 3)

Version dated 19 May 2020: fifth version
- Status of POCT validation in the Netherlands, updated after 19 May (Section 2)
- Sensitivity calculated over the total number of samples from various laboratories (Section 3.2)
- Evaluation supplemented with data from Leiden University Medical Centre; Jeroen Bosch Hospital; PAMM Laboratories; NoordwestZiekenhuisgroep and COMICRO (Section 3). This involved adding additional data for the following tests (Section 3.2): Zhejiang Orient Gene COVID-19 IgM/IgM Rapid Test Cassette; Wantai SARS-CoV-2 Ab rapid test; BIOSYNEX COVID-19 BSS; BIOZEK Coronavirus covid rapid test; BOSON 2019-nCoV IgM/IgM combo test; Wantai SARS-CoV-2 Ab rapid test.

Version dated 26 May 2020: sixth version
- Copyright updated (Acknowledgements)
- Status of the validation updated after 26 May (Section 2)

Version dated 16 June 2020: seventh version
- Status of POCT validation in the Netherlands, updated after 16 June (Section 2)
- Adjusted cut-off in days after onset of illness from 10 to 14 days for sensitivity. Additionally, all data of which the duration of material collection after onset of illness is unknown was removed. All sensitivity is recalculated for a, b and c (Section 3.2).
- Evaluation supplemented with data from Star-SHL, Maastricht UMC+, Erasmus MC, Franciscus and Diakonessenhuis (Section 3).

Version dated 15 July 2020: eighth version
- Report is translated in English
- Status of POCT validation in the Netherlands, updated after 15 July (Section 2)
- Specificity for IgM is added for all tests. This involved adding additional data for all tests (Section 3.2)
- Evaluation supplemented with data from Franciscus, LUMC, Jeroen Bosch hospital, NoordwestZiekenhuisgroep, Amsterdam UMC, PAMM, Saltro, Star-SHL, RIVM-IDS (Section 3).

Table of contents

1 Introduction and considerations

The report you are reading is "Status of the validation of point-of-care serology tests for SARS-CoV-2 diagnostics: considerations for use", in the version dated 15 July 2020. The data collection and reporting has been done by the Serology Taskforce, which is part of the Dutch National Testing Capacity Coordination Structure (Landelijke Coördinatiestructuur Testcapaciteit, LCT). The data described comes from seventeen different ISO 15189 accredited laboratories in the Netherlands with a flexible scope in the fields Medical Microbiology or Medical Immunology with relevant elements. Chapter 1 explains the backgrounds and the considerations for using antibody tests, aimed at a wider audience. Experts in the subject matter are advised to start with Chapter 2, which is where the descriptions of the more technical aspects of this report start.

Starting 17 July 2020, the LCT will be terminated because of the transition of the Dutch response structure to COVID-19 from crisis management to management embedded in the regular pre-crisis structure. Therefore, this is the last version of this report published by the Serology Taskforce. Identification of knowledge gaps, knowledge generation, the national sharing and support regarding the laboratory preparedness and response to COVID-19 and policymaking concerning serology in the COVID-19 response will continue as part of this normalized structure. This includes the continuation of these reports in which validation data is shared; the specifics and frequency are to be determined during the summer of 2020.

1.1 Background: the possibilities of antibody testing

The human body makes antibodies in response to foreign materials that penetrate it: antibodies are part of the immune system. Antibodies and immune cells work together to combat an invading pathogen and can play a role in protecting against subsequent infections by the same pathogen. It can take quite a while (several weeks) before the antibody production gets going. Antibodies are custom-made for each pathogen, which means that they are quite specific. Antibodies against an influenza virus do not bind to a coronavirus and vice versa, although there can be a certain amount of cross-reactivity within groups of related viruses.

When antibodies protect against new infections, they are referred to as protective antibodies. Their presence indicates immunity. Antibodies are capable of doing this against a wide range of pathogens. However, the pathogens' escape mechanisms are sometimes so good that even large numbers of antibodies do not offer protection. Whether antibodies provide immunity or not varies from one pathogen to the next.

Possible applications of antibody tests are:
- Evaluations of whether someone has had an infection (recently or in the past)
- Evaluations of whether someone is immune

In the current SARS-CoV-2 pandemic, there has been some highly vocal backing for antibody tests to be used for determining what proportion of the population has already had the viral infection, in order to see who may be immune. That could present numerous options, such as policy differentiation between people who may be immune and those who do not have immunity yet. If the size of the fraction of the population with immunity is known, it is possible to model what the effects of measures being taken or relaxed will be. An incorrect assumption is often made here that the presence of antibodies correlates with complete immunity against reinfection.

In response to the growing need for test capacity worldwide during the COVID-19 pandemic, point-of-care antibody tests (POCT) are being offered by various manufacturers for use in or outside a laboratory setting to determine whether someone has COVID-19 by quickly determining the presence of antibodies against SARS-CoV-2. Blood is used most frequently, often from a finger prick (in theory, collected saliva could also be used). This type of test is called POCT because they can be used right next to the patient – at the point of care – and generate a result quickly, generally within 10 to 30 minutes. The tests are almost all 'lateral flow tests', meaning that the sample is drawn in over a strip, usually of nitrocellulose. The European Centre for Disease prevention and Control (ECDC) has warned that, despite most available POCTs for antibody detection meeting European regulations, there are also POCTs with CE markings on the market that have false documentation, incomplete technical validation and unsubstantiated performance claims (https://www.ecdc.europa.eu/en/publications-data/overview-rapid-test-situation-covid-19-diagnosis-eueea). The World Health Organization (WHO) advised on 8 April 2020 that these POC antibody tests should only be used for research purposes. They also encourage more research into the use of POCTs for infectious disease surveillance and epidemiological research, but not for individual patient diagnostics (https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19). In addition to antibody tests, virus neutralisation tests (VNT and PRNT) can be used. These use the fact that specific antibodies in the serum can inhibit viral multiplication in cell cultures. This is generally seen as an indication of the presence of potentially protective antibodies. There are however only a few laboratories in the Netherlands as yet that routinely culture viruses. On top of that, culturing SARS-CoV-2 has to be done under stringent safety conditions (BSL3 conditions). In the Netherlands, virus neutralisation tests for human diagnostics are currently available (as far as we are aware) at RIVM-IDS and the Erasmus MC.

This report describes considerations relating to antibody tests in general and POC tests in particular. It also carries out an initial comparative study of POCTs for detecting antibodies that were evaluated in Dutch laboratories and shares the provisional results and conclusions. This report will be updated regularly as more validation data comes in at the Serology Taskforce.

1.2   The limitations of antibody tests

The limitations of antibody tests break down into two large categories, namely (i) the human biology and (ii) the characteristics of the antibody tests.

Limitations deriving from the biology of antibodies:
1) It takes some time before antibodies are produced. The first reports about SARS-CoV-2 show that it takes a month after the first day of illness for the proportion of infected people who have produced antibodies to exceed 90%. That is a limitation during a rapidly spreading epidemic as it means that a large proportion of people will generate a negative antibody test during the first weeks after infection. The results of antibody tests lag at least two to four weeks behind the actual number of infections. For the above reasons, a second blood sample is often needed to determine whether someone has recently had an infection with a virus. This second blood sample is used to assess the kinetics of antibodies, such as the switchover from negative to positive, increased positive response or change of antibody class (e.g. transition from IgM to IgG).
2) There are various types of antibodies against different parts of the virus and the tests available at the moment differ in what they measure. For reliable interpretation of the result, it is important that the exact specifics of the tests are known. That information is not always available (company confidential). Because of the diversity of antigens and human immune

responses, the tests also need to be evaluated in a large group of patients before definitive conclusions can be drawn.

3) A proportion of people who were infected by SARS-CoV-2 and remained asymptomatic or only had mild symptoms appear to produce little or nothing in terms of antibodies. That has been seen not only in current preliminary research but also in asymptomatic infections with H5N1. That means that population screening programmes or studies of people in critical professions will underestimate the actual number of infections. It is unclear by how much this will be the case because insufficient research has yet been done into asymptomatic and mild infections with SARS-CoV-2 to allow that question to be answered. It is also unclear whether people with low antibody counts do perhaps have partial protection.

4) Antibodies are 'sticky proteins' that are mostly not as specific as we would like for answering our questions. For the question of whether people have already had the infection, a lack of sufficient specificity is problematic because SARS-COV-2 is related to other coronaviruses that are commonplace. There can also be disruptive factors that have nothing to do with infections, such as rheumatoid factors. The antibodies that you detect using a SARS-CoV-2 test can in reality also be antibodies against some other coronavirus. A lack of specificity therefore results in <u>false-positive test results</u>.

5) Antibodies often disappear after some time. The rate at which antibodies disappear is subject to individual variation and depends on both the pathogen and the severity of the infection suffered. The disappearance of antibodies gives negative test results that can lead to the erroneous conclusion that someone has not had the infection. The outcome in a population screening programme is then an underestimate of the number of people who have had the infection.

6) For SARS-CoV-2, we do not yet know whether and to what extent the presence of antibodies is correlated to immunity. Although it is reasonable to think that there will be some degree of immunity, caution is needed given plentiful experience with other respiratory pathogens showing that such an association is not necessarily the case. In parallel with other respiratory pathogens, including the four "common cold coronaviruses", it is currently assumed that reinfections will be possible, in which people will probably be less sick – perhaps a great deal less sick – while possibly being contagious. This cannot be excluded and research will have to make this clear over the coming years. This is the reason why it is not guaranteed safe to let people with antibodies care for COVID-19 patients without protective measures.

<u>Limitations of antibody tests in general:</u>
Antibody tests are developed for specific applications. An antibody test that is intended for demonstrating acute infections in sick patients has to meet different requirements than a test used in a population screening programme or a test for demonstrating whether health sector employees have had an infection. If a test is used outside the scope of its applicability, unreliable results will be generated.
The specific problems with antibody tests are as follows:

1) The tests have not been validated for the purpose for which they are being used or sold. Many of the tests currently on offer have been validated by research into COVID-19 patients with severe complaints, comparing them against healthy subjects. Those are the two extremes of the spectrum and there is insufficient information to allow statements to be made about the level of cross-reactions (<u>false-positive test results</u>) or the sensitivity of tests in people who have had a mild infection or remained asymptomatic (<u>false-negative test results</u>).

2) Lack of sensitivity: the sensitivity is the test's ability to detect the intended antibodies. The antibodies are detected by making them adhere (bind) to components of the pathogen. If it is to work well, the correct parts of the pathogen must be used and the three-dimensional shape of those components must have been properly retained. The latter aspect turns out by no means always to be the case. Additionally, each body has to 'invent the wheel' itself when producing the appropriate antibodies. As a result, there are individual differences between the antibodies that are produced. The components that one person makes antibodies against may not be the same as those made by a different person. These factors mean that many antibody tests do not have a sensitivity of 100% or anywhere close to it. A shortfall in the sensitivity results in <u>false negatives</u>.

3) Lack of specificity: the specificity is the ability of a test to flag people as negative if they do not have the required antibodies (i.e. have not had the infection). Antibodies are sticky molecules. They sometimes adhere to test components that are not relevant. If pathogens are related to one another, antibodies against one such pathogen can bind to components of the other pathogen. A good antibody test uses components of the pathogen that are as unique as possible. If the specificity is less than 100%, it means that <u>false positives</u> can arise.

4) The antibody tests that measure the amounts of protective antibodies are labour-intensive and difficult to carry out on a large scale. The commercially available tests have mostly not been validated for their suitability for determining the amounts of protective antibodies.

5) Because this is about detecting antibodies against a novel virus, the method in this situation will be a new one and there is only very limited experience with it at this stage. Using these tests in large groups will reveal the potential problems such as e.g. false positives or false negatives when certain medicines are used, variability of sensitivity in different age groups or during pregnancy, the stability of the tests after storage and so forth.

<u>Specific limitations of POC antibody tests (rapid tests)</u>

1) Similar to serological validation: in general there is often only very limited information available about the patients whose blood was used for characterising the test's performance. Relevant information that is missing includes (1) the relationship between the moment of blood sampling and the first day of illness, (2) how severely ill the patients were, (3) whether whole blood was used (such as from a finger prick) or serum, (4) the patient characteristics for the negative samples and (5) whether cross-reactivity with antibodies against other human coronaviruses was examined. Points (1) to (3) are determinants of the test's <u>sensitivity</u>; points (4) and (5) are determinants of the <u>specificity</u>. Because the information about these points is missing, the POCTs need to be accurately assessed to make it possible to define what populations they can be used in and at what time after infection.

2) The tests were developed for use by the general population and the way the test is read is subjective. Can you see a line or not? For many of these tests, it is important that the test is read at the right moment (for example 15 minutes after applying the drop of blood). When assessing the test in home situations, people who have doubts about the intensity of the band often leave the test standing for longer, which causes many bands to colour more intensely, resulting in a lot of false positives.

   In general, experience in using and reading tests increases the reliability. The more often you do it, the better you know how to handle the test and what the meaning of a weak signal is, for example. Standardised material (serum) and a standardised method of usage both help produce reliable results. In short, the test characteristics determined in a laboratory do not automatically apply to a situation where people use and read their own tests themselves.

3) Another discrepancy that occurs when using a POCT at home is the use of blood from a finger prick. Finger prick blood is a form of whole blood. This also contains all of the cell types in the blood. Serum is the fluid that remains when blood plasma is left to clot and that clot is centrifuged off. The blood cells have then also been removed from the serum. The volume difference between serum and whole blood is about 60%. If the same volume of whole blood or serum is used for the test (for example one large drop), the serum has more antibodies than whole blood. This makes serum tests more effective than whole blood tests. Although the package leaflets usually do not mention it, the manufacturers will probably have used serum to validate their tests, because serum is mainly used for antibody tests worldwide. Blood samples are also generally stored in the form of serum.

1.3   What margin of error is acceptable?

The limitations mentioned above give an impression of the complexity of antibody tests. There are no antibody tests at all as yet that are infallible – not even among tests at an advanced stage of development such as those for HIV. The tests that have been developed against SARS-CoV-2 are still at an early stage of development and studies into the reliability of these tests are limited or virtually non-existent. It would therefore be sensible to determine the reliability of these tests first in the light of the context in which they are used before implementing their use as part of policy.

How often may a test give a false result if it is to remain usable? That depends on the consequences of the outcome. If someone only wants to know whether they have had a SARS-CoV-2 infection as a matter of interest, an incorrect result will probably have little effect. If someone with a false-positive result believes they are immune and therefore behaves in a high-risk way, the consequences could be severe. In the extreme situation where measures are scaled down nationwide based on the assumption that a large proportion of the population is immune (for which modelling shows an immunity level of 50-60% is needed in the population at large) and a significant proportion of the test results underpinning that decision are false positives, a renewed major outbreak could arise. If a significant proportion of the results are false negatives, measures could remain in place longer than necessary. This is apart from the fact that it is not yet sufficiently clear to what extent the presence of IgG correlates to actual protection.

The antibody tests that are currently being offered on a large scale for detecting antibodies against SARS-CoV-2 have been developed to show acute infections among people who have or have recently had significant symptoms. They come with impressive specifications stating high sensitivity and specificity. These tests have generally been validated using samples from hospital patients. That is a selective patient population with severe symptoms, in whom we have in the meantime learned do produce large quantities of antibodies. These evaluations have scarcely looked at samples (if at all) from people with mild symptoms or asymptomatic infections. Neither cross-reactivity with other coronaviruses nor all kinds of other conditions in people that can cause cross-reactivity have been examined. Finally, these tests were not developed to test the presence of immunity in people or to do population screening. Using them for those purposes would lead to high percentages of incorrect results.

An example calculation:
Suppose that 3% of the Dutch population has been infected with SARS-CoV-2. We then attempt to determine this using a serological test that has a sensitivity of 99% and a specificity of 97%. Those would be impressive test characteristics for a serological test. Many antibody tests at an advanced stage of development that are used in hospitals on a daily basis have figures that are not as good.

Testing the population with a test like that would however lead to about half of all the positive test results being incorrect! The positive predictive value is 50%. The test is then doing no better than flipping a coin. Is that acceptable?

How is this possible? Out of every 100 people, only 3 will have had an infection. The test's specificity is 97%, so 3 people will also get a false-positive test result. All 3 infected people will indeed probably be found, as the sensitivity is 99%. But only 3 of the 6 positive results are correct. The test's positive predictive value is low. The negative predictive value is much better, though, at 99.9%.

If 20% of the population has had SARS-CoV-2, the positive predictive value using the same test is around 93%, which is much better. In a hospital population, where the a priori chance of the given condition is high (the test will have been requested because something is specifically suspected), the problem of a lack of specificity is less than in untargeted screening, where the prevalence is much lower. This shows that the test has to be seen in the context of the population and the situation in which it is used. The results of antibody tests are therefore not easy to interpret, even though they may be easy to carry out in the GP practice or at home.

1.4   Recommendation that antibody tests should be used sensibly

The antibody tests for SARS-CoV-2 have only been developed very recently. They have been developed for determining infections in patients who have been admitted to hospitals: people who are suffering severe symptoms and with a high a priori likelihood of infection with SARS-CoV-2. The specifications may seem impressive, but the independent research carried out so far shows that those specifications cannot be replicated if a wider patient population is used. Application of a test outside the intended target group for that test can result in a large number of incorrect results. Despite the high degree of urgency, using tests is not desirable before they have had the requisite thorough evaluation.

The World Health Organization (WHO) made a statement on 8 April 2020 about the use of POCT tests and recommends that these tests should only be used for research purposes. They should not be used for any other purpose, such as clinical diagnostics or underlying evidence for policymaking, until more evidence has been provided and collected about the use for specific indications (https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19).

Chapters 2 and 3 below share the provisional results of evaluations in the Netherlands of the possible applications of POCT.

2. Status of the validation of POC antibody tests

Status as at 15 July 2020

Inventories of the validation status of serological tests were carried out via the Dutch Society for Medical Microbiology (NVMM). Fifty-three laboratories responded to these inventory requests, showing that there were 27 different POC antibody tests at various stages of validation in the Netherlands as at 15 July 2020. A total of 204 different POC tests were available on the market worldwide on 14 July 2020 (https://www.finddx.org/); since the National Consortium for Medical Devices was put in place, triage and selection were done centrally. Table 1 shows the 27 tests that are at some stage of validation in the Netherlands, including five tests for which validation data has not yet been obtained. This list was compiled based on information from the laboratories that responded to the request for information and it may not be complete.

Table 1. POC antibody tests at various stages of validation in the Netherlands as at 15 July 2020

POCT	Manufacturer	Regulatory	Stage of evaluation (n labs)		
			Finished	Started	Planned
2019 nCOV IgG/IgM Rapid test	Dynamiker Biotechnology (Tianjin) Co., Ltd	CE-IVD	2	0	0
Cellex qSARS-CoV-2 IgG/IgM cassette, Rapid test	Celllex Inc.	CE-IVD	3	0	0
InTec Rapid SARS-CoV-2 antibody (IgM/IgG) test	InTec PRODUCTS Inc.	CE-IVD	2	1	0
COVID-19 IgM/IgM Rapid Test Cassette	Zhejiang Orient Gene Biotech Co., Ltd./Healgen	CE-IVD	5	0	0
BIOSYNEX COVID-19 BSS	BIOSYNEX	CE-IVD	2	0	0
BIOZEK Corona virus COVID rapid test	Biozek medical	CE-IVD	7	1	0
Acro Biotech COVID-19 Rapid POC test	Acro Biotech	CE-IVD	3	0	0
Biomerica COVID-19 IgG/IgM Rapid test	Biomerica Inc.	CE-IVD	1	0	0
DiagnoSure COVID-19 IgG/IgM rapid test cassette	GritOverseas Pte. Ltd	unknown	1	0	0
Diagnostic kit for antibody IgM/IgG of Novel Coronavirus COVID-19	Shanghai LiangRun, Biomedicine Tech. Co., Ltd	CE-IVD	1	0	0
2019-nCoV IgM/IgM combo test	BOSON Biotech	CE-IVD	7	0	0
2019-nCoV IgG/IgM Test Cassette	Prometheus Bio Inc.	CE-IVD	1	0	0
VivaDiag COVID-19 IgM/IgG Rapid Test	VivaChek Biotech (Hangzhou) Co. Ltd.	CE-IVD	1	0	0
COVID-19 IgG/IgM Rapid Test Cassette	Vomed	unknown	1	0	0
Wantai SARS-CoV-2 Ab rapid test	Beijing Wantai Biological	RUO	2	0	0
The non-invasive MEGA test of SARS-CoV-2	Absea Biotechnology Ltd	In development	1	0	0
OnSite COVID-19 IgG/IgM Rapid Test	CTK Biotech, Inc.	CE-IVD	3	0	0
SureScreen COVID-19 Coronavirus Rapid Test Cassette	SureScreen Diagnostics	CE-IVD	1	0	0
PRIMA COVID-19 IgG/IgM Rapid Test	PRIMA Lab S.A.	CE-IVD	1	0	0
SARS-CoV-2 Antibody Test (LF method)	Guangzhou Wondfo Biotech Co Ltd	CE-IVD	1	0	0
COVID-19 rapid test	Medea Medical Co.	CE-IVD	1	0	0

AFIAS COVID-19 Ab, IgM/IgG	Boditech Med. Inc.	CE-IVD	1	0	0
COVID PRESTO Ag	AAZ-LMB	unknown	0	1	1
COVID PRESTO®	AAZ-LMB	CE-IVD	0	1	0
Alltest 2019-nCoV IgG/IgM Rapid Test	Hangzhou Alltest Biotech Co. Ltd	CE-IVD	0	1	0
Bioeasy COVID-19 Coronavirus IgG/IgM GICA Rapid Test Kit	Shenzhen Bioeasy Biotechnology	CE-IVD	0	0	1
STANDARD Q Covid-19 Ag	SD Biosensor	CE-IVD	0	0	1

The implementation of the plans for further research into these POC tests and subsequent validation of such tests depends on the availability and delivery of the tests. Various laboratories have stated that there were problems with the delivery of some of the POC tests listed above. This resulted in some validations being carried out less extensively than would normally be desired.

3 Results and conclusions of POC antibody test validation in Dutch laboratories

3.1 Scope and criteria

Status as at 15 July 2020

The available results from validations of POC tests as at 15 July 2020 are mainly the outcomes of validation processes that are limited because some kits are or were not available in large quantities. The data in this report can therefore be seen as an initial screening for the applicability of the POC antibody tests. Tests that perform well can potentially be selected for thorough validation if they are available in sufficient numbers.

Because SARS-CoV-2 has only recently appeared in the Netherlands, the sensitivity and specificity of the IgG antibodies (versus IgA and IgM) are important if they are to be used as a marker for having had the infection. The use of serology (and with it, serological POCT) is at the moment only advised, if sufficiently reliable, for acute patient care. The criteria that antibody tests must meet differ depending on where the test is to be applied. In this initial screening of POC antibody tests[1], the following criteria were used (expert opinion) to assess a test as promising:

- For individual patient diagnostics: IgG and IgM antibodies: both *separately*, with a specificity of >98% and sensitivity of >95% from 14 days[2] after either severe or mild symptoms appear.
- Once national and international research has given a better understanding of how the presence of antibodies can be an indication for protective immunity against reinfection (and possibly for reduced contagiousness), it may be useful to test whether people in specific populations or subpopulations (such as health care workers and family-based carers) have had a SARS-CoV-2 infection: Only IgG: specificity >98%, sensitivity >85% from 14 days[1] after symptoms appear.
- Epidemiological and serological prevalence studies: Only IgG: specificity >98%, sensitivity >95%

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[1] The POCTs in this report are on the market for IgG and IgM assays. (No IgA or total IgG assays)

[2] International consultations (in the WHO laboratory/technical working group and elsewhere) are increasingly showing that it is only possible to determine with the highest level of certainty using serology whether someone has had an infection from 4 weeks after symptoms start showing. This is a living document and amendments will be supplied as data about the kinetics of immunological responses in various populations becomes more robust.

These are not absolute criteria, but recommendations from the Serology Taskforce based on expert opinion. The applicability of these criteria will have to be assessed by the local expert in each situation.

3.2   Results and conclusions for each point-of-care antibody test

Status as at 15 July 2020

The results and conclusions for each POCT for detecting antibodies (all detect both IgG and IgM which can be read separately) are described below, stating four points consecutively each time:

   a. IgM and IgG sensitivity in patients (confirmed positive by RT-PCR) with severe symptoms and with serum samples taken > 14 days after onset of illness.
   b. IgM and IgG sensitivity in patients (confirmed positive by RT-PCR) with severe symptoms and with serum samples taken < 14 days of the first symptoms appearing. It should be noted here that the sensitivity of a test in this category cannot be assessed properly due to the sampling moment being so early in the course of the infection.
   c. IgM and IgG sensitivity in populations (confirmed positive by RT-PCR) with no symptoms or mild symptoms with serum samples taken > 14 days after onset of illness.
   d. IgM and IgG specificity.

Where multiple laboratories have evaluated the same test in patients from the same group, the results are bundled for calculating the overall sensitivity or specificity.

The results in this report are provisional; many laboratories are still carrying out follow-up tests with e.g. different patient groups.

Dynamiker Biotechnology 2019 nCOV IgG/IgM Rapid test (2 labs; total panel sensitivity n=44; specificity n=13)

   a. The sensitivity for IgG/IgM combined reported only (71.4%, n=7) does not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.
   b. The sensitivity for IgM/IgG combined reported only is 19.2% (n=26) in patients with severe infections where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.
   c. The IgM and IgG sensitivities (both 63.6%, n=11) do not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.
   d. The IgM and IgG specificities (both 92.3%, n=13) do not meet the predetermined criteria. Because these percentages are based on a limited number of samples, the specificity must be determined with higher confidence with a larger number of samples.

Cellex qSARS-CoV-2 IgG/IgM cassette Rapid test (3 labs; total panel sensitivity n=193; specificity n=112)

   a. The IgG sensitivity (100%, n=36) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. The IgM sensitivity (47.2%), or IgM/IgG combined reported only (0%, n=2) do not meet the predetermined criteria. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are both 57.1% (n=7), or 59.0% for IgM/IgG combined reported only (n=83) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM and IgG sensitivities (21.5% and 75.4%, n=65) do not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

d. The IgM and IgG specificities (99.1% and 98.2%, n=112) meet the predetermined criteria. Confirmation with a larger number of samples is needed.

InTec Rapid SARS-CoV-2 antibody (IgM/IgG) Test (2 labs; total panel sensitivity n=175; specificity n=112)

a. The IgG sensitivity (100%, n=36) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. The IgM sensitivity is lower, at 83.3%, and does not meet the predetermined criteria. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are both 75.0% (n=8), or 86.8% for IgM/IgG combined reported only (n=68) in patients where samples were collected ≤ 14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM and IgG sensitivities (28.6% and 69.3%, n=63) do not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected after >14 days after onset of illness. Confirmation with a larger number of samples is needed.

d. The IgM and IgG specificities (87.5 and 95.5% (n=112) do not meet the predetermined criteria. Confirmation with a larger number of samples is needed.

Zhejiang Orient Gene COVID-19 IgM/IgM Rapid Test Cassette (5 labs; total panel sensitivity n=349; specificity n=210)

a. The IgG sensitivity (100%, n=63) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. The IgM sensitivity (88.9%), or IgM/IgG combined reported only (90.5%, n=21) do not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are 52.9% and 47.1% (n=17) or 60.1% for IgM/IgG combined reported only (n=158) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The sensitivities for IgM and IgG (94.6% and 93.2%, n=74), or for IgM/IgG combined reported only (77.8%, n=9) do not meet all predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected after >14 days. Confirmation with a larger number of samples is needed.

d. The IgG specificity (98.6%, n=136) meets the predetermined criteria, while the IgM specificity (92.6%) and IgM/IgG combined reported only (97.3%, n=73) do not meet the predetermined criteria. Confirmation with a larger number of samples is needed.

BIOSYNEX COVID-19 BSS (2 labs; total panel sensitivity n=158; specificity n=53)

a. The IgG sensitivity (96.6%, n=58) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. The IgM sensitivity (93.1%) does not meet the predetermined criteria for diagnosis in patients with

       severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are 65.0% and 44.0% (n=100) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The characteristics in mild SARS-CoV-2 infections have not yet been evaluated and no statements can therefore be made about them.

d. The IgG specificity (100%, n=53) meets the predetermined criteria, while the IgM specificity (90.6%) does not meet the predetermined criteria. Because these percentages are based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

BIOZEK Coronavirus COVID rapid test (7 labs; total panel sensitivity n=379; specificity n=489)

a. The IgG sensitivity (95.4%, n=130) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. The IgM sensitivity (53.8%) does not meet the predetermined criteria in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are 39.0% and 58.3% (n=228) for patients where samples were collected ≤14 days after onset of illness.

c. The IgM and IgG sensitivities (28.6% and 85.7%, n=21) do not meet all predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected after >14 days. Confirmation with a larger number of samples is needed.

d. The IgG specificity (98.2%, n=489) meets the predetermined criteria, while the IgM specificity is lower (95.9%) and does not meet the predetermined criteria.

Acro Biotech COVID-19 Rapid POC test (3 labs; total panel sensitivity n=75; specificity n=50)

a. The IgM and IgG sensitivities (37.8% and 91.9%, n=37) do not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are 48.1% and 66.7% (n=27) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM and IgG sensitivity (27.3% and 90.9%, n=12) do not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

d. The IgG specificity (98.0%, n=50) meets the predetermined criteria, while the IgM specificity (96.0%) does not meet the predetermined criteria. Because these percentages are based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

Biomerica COVID-19 IgG/IgM Rapid test (1 lab; total panel sensitivity n=22; specificity n=25)

a. The IgG sensitivity (100%, n=5) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. The IgM sensitivity (40.0%) does not meet the predetermined criteria in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are 50.0% and 62.5% (n=8) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM and IgG sensitivities (0% and 77.8%, n=9) do not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

d. The IgG specificity (100%, n=25) meets the predetermined criteria, while the IgM specificity (96.0%) does not meet the predetermined criteria. Because these percentage are based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

DiagnoSure COVID-19 IgG/IgM rapid test cassette (1 lab; total panel sensitivity n=23; specificity n=25)

a. The IgM and IgG sensitivities (both 100%, n=5) meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are 50.0% and 37.5% (n=8) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM and IgG sensitivities (50% and 0%, n=10) do not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

d. The IgM and IgG specificities (both 100%, n=25) meet the predetermined criteria. Because these percentages are based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

Shanghai LiangRun Diagnostic kit for antibody IgM/IgG of Novel Coronavirus COVID-19 (1 lab; total panel sensitivity n=22; specificity n=25)

a. The IgG sensitivity (100%, n=5) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. The IgM sensitivity (0%) does not meet the predetermined criteria in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are 25.0% and 50.0% (n=8) in patients with severe infections where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM and IgG sensitivities (22.2% and 33.3%, n=9) do not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

d. The IgM and IgG specificities (both 100%, n=25) meet the predetermined criteria. Because these percentages are based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

BOSON 2019-nCoV IgM/IgG combo test (7 labs; total panel sensitivity n=228; specificity n=103)

a. The IgG sensitivity (96.8%, n=62) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. The IgM sensitivity (61.3%) does not meet the predetermined criteria for diagnosis in patients with

severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM and IgG sensitivities are 50.8% and 57.7% (n=130), or 70.8% for IgM/IgG combined reported only (n=48) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM and IgG sensitivities (48.1% and 50.0%, n=15), or 68.6% for IgM/IgG combined reported only (n=35) do not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Because this percentage is based on a limited set of samples, the sensitivity in this population must be determined with higher confidence with a larger number of samples.

d. The IgM and IgG specificities (83.5 and 94.2%, n=103) do not meet the predetermined criteria. Confirmation with a larger number of samples is needed.

Prometheus 2019-nCoV IgG/IgM Test Cassette (1 lab; total panel sensitivity n=20 ; specificity n=0)

a. The sensitivity for IgG/IgM combined reported only (0%), based on only 2 samples, does not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM/IgG combined reported only is 22.2% (n=18) for patients where samples were collected ≤14 days of onset of illness. Confirmation with a larger number of samples is needed.

c. The characteristics in mild SARS-CoV-2 infections have not yet been evaluated and no statements can therefore be made about them.

d. The IgM and IgG specificities in prepandemic control groups and/or potentially cross-reactive infections have not yet been evaluated and no statements can therefore be made about them.

VivaDiag COVID-19 IgM/IgG Rapid Test (1 lab; total panel sensitivity n=9; specificity n=10)

a. The IgM/IgG sensitivity combined reported only (100%, n=8) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The characteristics in severe SARS-CoV-2 infections where samples are collected ≤14 days after onset of illness have not yet been evaluated and no statements can therefore be made about them.

c. The IgM/IgG sensitivity combined reported only (100%, n=1) meets the predetermined criteria in populations with mild symptoms or asymptomatic infections where the sample was collected >14 days after onset of illness. Because this percentage is based on only one sample, the sensitivity in this population must be determined with a larger number of samples.

d. The IgM/IgG specificity combined reported only (100%, n=10) meets the predetermined criteria. Because this percentage is based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

Vomed COVID-19 IgG/IgM Rapid Test Cassette (1 lab; total panel sensitivity n=31; specificity n=23)

a. The IgM/IgG sensitivity combined reported only (100%, n=13) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM/IgG combined reported only is 50.0% (n=10) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM/IgG sensitivity combined reported only (75.0%, n=8) does not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Because this percentage is based on a limited set of samples, the sensitivity in this population must be determined with higher confidence with a larger number of samples.

d. The IgM/IgG specificity combined reported only (95.7%, n=23) does not meet the predetermined criteria. Because this percentage is based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

Wantai SARS-CoV-2 Ab rapid test (2 labs; total panel sensitivity n=52; specificity n=9)

a. The IgM/IgG sensitivity combined reported only (87.5%, n=16) does not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

b. The IgM/IgG sensitivity combined reported only is 65.7% (n=35) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM/IgG sensitivity combined reported only (100%, n=1) meets the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Because this percentage is based on only one sample, the sensitivity in this population must be determined with higher confidence with a larger number of samples.

d. The IgM/IgG specificity combined reported only (100%, n=9) meets the predetermined criteria. Because this percentage is based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

CTK OnSite COVID-19 IgM/IgG Rapid Test (3 labs; total panel sensitivity n=63; specificity n=81)

a. The IgM/IgG sensitivity combined reported only (0%, n=2) does not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed, because this is only based on two samples.

b. The IgM/IgG sensitivity combined reported only is 38.9% (n=18) for patients with severe infections where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The IgM/IgG sensitivity combined reported only (52.4%, n=42) does not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. The IgM/IgG sensitivity combined reported only or patients with mild infections is 38.9% (n=18). Because these percentages are based on very limited sets of samples, the sensitivity in this population must be determined with higher confidence with a larger number of samples.

d. The IgG specificity (100%, n=25) and the IgM/IgG combined reported only (98.2%, n=56) meet the predetermined criteria, while the separately reported IgM specificity (88.0%) does not meet the predetermined criteria. Because these percentages are based on limited sets of

samples, the specificity must be determined with higher confidence with a larger number of samples.

Surescreen COVID-19 Coronavirus Rapid Test Cassette (1 lab; total panel sensitivity n=37; specificity n=56)

a. The characteristics in severe SARS-CoV-2 infections where samples are collected >14 days after onset of illness have not yet been evaluated and no statements can therefore be made about them.

b. The characteristics in severe SARS-CoV-2 infections where samples are collected ≤14 days after onset of illness have not yet been evaluated and no statements can therefore be made about them.

c. The IgM/IgG sensitivity combined reported only (40.5%, n=37) does not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Because this percentage is based on a very limited set of samples, the sensitivity in this population must be determined with higher confidence with a larger number of samples.

d. The IgM/IgG specificity combined reported only (98.2%, n=56) meets the predetermined criteria. Because this percentage is based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

PRIMA COVID-19 IgM/IgG Rapid Test (1 lab; total panel sensitivity n=20; specificity n=0)

a. The IgM/IgG sensitivity combined reported only (0%, n=2) does not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed, because this is only based on two samples.

b. The IgM/IgG sensitivity combined reported only is 16.7% (n=18) for patients where samples were collected ≤<14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The characteristics in mild SARS-CoV-2 infections have not yet been evaluated and no statements can therefore be made about them.

d. The IgM and IgG specificities in prepandemic control groups and/or potentially cross-reactive infections have not yet been evaluated and no statements can therefore be made about them.

Wondfo SARS-CoV-2 antibody Test (1 lab; total panel sensitivity n=20; specificity n=0)

a. The IgM/IgG sensitivity combined reported only (0%, n=2) does not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed, because this is only based on two samples.

b. The IgM/IgG sensitivity combined reported only is 30.0% (n=20) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

c. The characteristics in mild SARS-CoV-2 infections have not yet been evaluated and no statements can therefore be made about them.

d. The IgM and IgG specificities in prepandemic control groups and/or potentially cross-reactive infections have not yet been evaluated and no statements can therefore be made about them.

Medea Medical COVID-19 Rapid Test (2 labs; total panel sensitivity n= 34; specificity n=22)

    a. The characteristics in severe SARS-CoV-2 infections where samples are collected >14 days after onset of illness have not yet been evaluated and no statements can therefore be made about them.

    b. The IgM/IgG sensitivity combined reported only is 72.0% (n=25) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

    c. The IgM/IgG sensitivity combined reported only (77.8%, n=9) does not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Because this percentage is based on a very limited set of samples, the sensitivity in this population must be determined with higher confidence with a larger number of samples.

    d. The IgM/IgG specificity combined reported only (90.9%, n=22) does not meet the predetermined criteria. Because this percentage is based on a limited set of samples, the specificity must be determined with higher confidence with a larger number of samples.

AFIAS COVID-19 Ab, IgM/IgG (1 lab; total panel sensitivity n=47; specificity n=279)

    a. The IgM and IgG sensitivities (0% and 85.7%, n=7) do not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected >14 days after onset of illness. Confirmation with a larger number of samples is needed.

    b. The IgM and IgG sensitivities are 0% and 50% (n=4) for patients where samples were collected ≤14 days after onset of illness. Confirmation with a larger number of samples is needed.

    c. The IgM and IgG sensitivities (0% and 91.7%, n=36) do not meet all predetermined criteria for diagnosis in populations with mild symptoms or asymptomatic infections where samples were collected >14 days after onset of illness. Because these percentage are based on a limited set of samples, the sensitivity in this population must be determined with higher confidence with a larger number of samples.

    d. The IgM and IgG specificities (99.6 and 98.2%, n=279) meet the predetermined criteria.

Absea non-invasive MEGA test of SARS-CoV-2 (1 lab; total panel sensitivity n=10; specificity n=18)

    The test did not meet any of the four abovementioned points. No antibodies could be detected in the serum of any of the PCR confirmed patients. The uninfected GLY and E-swab Amies media, both widely used in the transport and conservation of viruses, are consequently positive in this test. These were looked at because according to the manufacturer these tests could also be used on swabs.

3.3   Correlation with the presence of neutralising antibodies

Depending on the reason why serology is being performed, it may be essential to establish the reliability of routine serology tests for the detection of the presence of neutralising antibodies. For eight POCTs, the correlation with the presence of neutralising antibodies was also examined (Erasmus MC and RIVM have testing capacity, probably non-exhaustive). The presence of neutralising antibodies is a possible indicator of immunity.

When comparing the IgG detection of 7 POCTs against the Wantai ELISA (for which national stocks are held) and specifically for the serums that tested positive in both the Wantai ELISA and in the virus neutralisation test, it was observed that the InTec test scored highest with IgG detection in 14 out of 16 serum samples with a neutralising titre (87.5%). For the Biomerica and Acro Biotech tests, this was

13 out of 15 (86.7%) serum samples with neutralising antibodies. For Cellex it was 12 out of 16 (75%), for Dynamiker 11 out of 16 (68.7%), for Diagnosure 8 out of 15 (53%) and for Shanghai Liangrun 6 out of 15 (40%). In the second study, a good correlation was found with a neutralisation test for the Zhejiang Orient Gene/Healgen (IgM/IgG combined reported only: 100%, n=130). For InTec this correlation with neutralisation tests was IgG 88% (n=131), and for Cellex 87% (n=131).

3.4   Summary of the initial laboratory findings

The various POCTs vary widely in how well they perform. *Due to the limited availability of tests, all findings are provisional and must be confirmed with a larger number of samples. The number of samples is too small for definitive conclusions regarding the use for tests that still have implementation potential for certain contexts after this initial validation round.*

At the moment, the following tests meet the predetermined criterion of specificity >98% for both IgM and IgG when all specification panels of the various laboratories are bundled together.
  – Cellex qSARS-CoV-2 IgG/IgM cassette Rapid test (IgM: 99.1%; IgG: 98.2, n=112)*
  – DiagnoSure COVID-19 IgG/IgM rapid test cassette (IgM and IgG both:100%, n=25)*
  – Shanghai LiangRun Diagnostic kit for antibody IgM/IgG of Novel Coronavirus COVID-19 (IgM and IgG both 100%, n=25)*
  – VivaDiag COVID-19 IgM/IgG Rapid Test (IgM/IgG combined: 100%, n=10)*
  – Wantai SARS-CoV-2 Ab rapid test (IgM/IgG combined:100%, n=9)*
  – Surescreen COVID-19 Coronavirus Rapid test Cassette (IgM/IgG combined: 98.2%, n=56)*
  – AFIAS COVID-19 Ab, IgM/IgG (IgM: 99.6%; IgG: 98.2%, n=279)
Six (*) of these seven tests that meet the predetermined criteria for specificity of IgM and IgG both, have been evaluated with a total of < 200 samples and therefore require further testing with a larger number of sample sets. The Prometheus 2019-nCov IgG/IgM, PRIMA COVID-19 IgG/IgM Rapid Test and Wondfo SARS-CoV-2 Antibody Test have not yet been evaluated for specificity.

The following tests need further assessment for applicability in diagnostics because they do have a specificity of > 98% for IgG but do not meet this criterion for IgM or for IgM/IgG combined:
  – Zhejiang Orient Gene/Healgen COVID-19 IgM/IgG Rapid Test Cassette (IgM: 92.6%; IgG: 98.6%, n=136 and IgM/IgG: 97.3%, n=73)*
  – BIOSYNEX COVID-19BSS (IgM: 90.6; IgG: 100%, n=53)*
  – BIOZEK Corona virus COVID rapid test (IgM: 85.9%; IgG: 98.2%, n=489)
  – Acro Biotech COVID-19 Rapid POC test (IgM: 96.0%; IgG: 98.0%, n=50)*
  – Biomerica COVID-19 IgG/IgM Rapid test (IgM: 96.0%: IgG:100%, n=25)*

Albeit in evaluations with a very limited number of samples, the following tests meet the predetermined criterion of sensitivity > 95% for combined IgG/IgM potential use as an **addition to** the preferred diagnostics in **seriously ill patients, from 14 days after onset of illness.** However, the standard for diagnostics in this setting is RT-PCR. Serology may have diagnostic value in this group of patients, where the clinical picture (based for instance on a CT scan) suggests there is a strong suspicion of a SARS-CoV-2 infection, but the PCR is repeatedly negative. The preference is however for ELISA tests, where higher sensitivity and specificity can be achieved than with POCT (see document preconditions for SARS-CoV-2 diagnostics). This will have to be investigated further.
  – DiagnoSure COVID-19 IgG/IgM rapid test cassette (IgM and IgG both:100%, n=5)
  - VivaDiag COVID-19 IgM/IgG Rapid Test (IgM/IgG combined: 100%, n=8)
  - Vomed COVID-19 IgG/IgM Rapid Test Cassette (IgM/IgG combined: 100%, n=13)

Of these tests, Diagnosure and VivaDiag also meet the predetermined criteria for specificity. All tests used a very limited number of samples; further examination is therefore required for all tests.

The following tests need further assessment for applicability in diagnostics in **patients with severe infections who are hospitalised** and where samples are collected **>14 days after onset of illness** because they do have a sensitivity of > 95% for IgG but do not meet the sensitivity criterion of > 95% for IgM.
- Cellex qSARS-CoV-2 IgG/IgM cassette Rapid test (IgG: 100%; IgM: 47.2%, n=36)
- InTec Rapid SARS-CoV-2 antibody (IgM/IgG) Test (IgG: 100%; IgM 83.3% n=36)
- Zhejiang Orient Gene/Healgen COVID-19 IgM/IgG Rapid Test Cassette (IgG:100%; IgM: 88.9%, n=63)
- BIOSYNEX COVID-19 BSS (IgG: 96.6%; IgM: 93.1%, n=58)
- BIOZEK Corona virus COVID rapid test (IgG: 95.4%; IgM: 53.8%, n=130)
- Biomerica COVID-19 IgG/IgM Rapid test (IgG: 100%; IgM: 40.0%, n=5)
- BOSON 2019-nCoV IgM/IgG combo test (IgG: 96.8%; IgM: 61.3%, n=62)
- Shanghai LiangRun Diagnostic kit for antibody IgM/IgG of Novel Coronavirus COVID-19 (IgG:100%; IgM:0%)

Of these tests, Cellex, and Shanghai Liangrun also meet the predetermined criteria for specificity. These are all observations based on a limited set of samples. The Prometheus, Surescreen and Medea Medical POC tests still need to be tested in patients with severe infections where samples are collected >14 days after onset of illness.

None of the evaluated rapid tests meet the predetermined criterion (in an evaluation with a limited number of samples) of sensitivity > 95% for diagnostics in a population of **patients with mild symptoms or with asymptomatic infections** where sample material was collected **>14 days after onset of symptoms**.

The IgG sensitivity of the Zhejiang Orient Gene/Healgen COVID-19 IgM/IgG Rapid Test Cassette (93,2%, n= 74), the BIOZEK Corona virus COVID rapid test (85.7%, n=21), ACRO Biotech COVID-19 Rapid POCT test (90.9%, n=12) and the AFIAS COVID Ab (91.7%, n=36) in a population with mild symptoms meet the criterion of > 85% for sensitivity. Although all based on a small number of samples, they may possibly be suitable for testing subpopulations and for seroprevalence studies when sample collection is done **>14 days after onset of symptoms.** This could be sensible once national and international research has given a better understanding of how the presence of antibodies can be an indication for protective immunity against reinfection (and possibly for reduced contagiousness), but is not applicable yet. However, ascertaining this first in larger cohorts is imperative.

3.5   Preliminary conclusion based on initial laboratory findings

The following preliminary conclusions can be drawn based on the initial results:

1. None of the 22 investigated POC antibody tests meet the predetermined criteria for IgM and IgG sensitivity and IgG specificity based on adequate validation including a sufficient amount of diagnostic samples. Three of the 22 investigated POC antibody tests meet the predetermined criteria for diagnostics in severe infections, where samples were collected >14 days after onset of illness, but on very low sample numbers. However, the relevance and added value compared to other diagnostics are unclear because this is a group who were hospitalised and are usually diagnosed using PCR, so there is no benefit to carrying out a rapid

test compared to a routine ELISA. For patients with a negative SARS-CoV-2 PCR and a persistent strong suspicion, an antibody determination after >14 days can have added value, provided that the sensitivity is high. Investigations are still needed into whether these tests are suitable for severely ill patients in home or nursing home situations where a decision has been taken for other reasons to not hospitalise the patient. There seems as yet to be no added value from use in GP practices where patients with mild to moderate symptoms are seen (see next point). There is insufficient data for a definitive conclusion.

2. Of the POC antibody tests that were evaluated in populations with mild symptoms or asymptomatic infections where the material was collected after >14 days, none meet the predetermined criteria. For the time being, this indicates that the use of rapid tests in primary care is not recommended.

The POCTs that were evaluated in this report are tests that could be used outside laboratory conditions with whole blood from a finger prick as input material. This report only describes results and conclusions obtained in laboratory conditions, mostly with serum as the input material. Performance will be lower than reported here when the tests are used outside laboratories with finger-prick blood. Additionally, POCTs may be used outside a professional laboratory setting by people who have little experience, in which case vague bands or unclear instructions can lead to incorrect conclusions. This happens out of sight of the quality assurance process.

Finally, it must be noted once again that the World Health Organization (WHO) made a statement on 8 April 2020 about the use of POCT tests, advising that these tests should only be used for research purposes. They should not be used for any other purpose, such as clinical diagnostics or underlying evidence for policymaking, until more evidence has been provided and collected on the use for specific indications (https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19).).

4   Plan for the near future

This is a bundled report covering various datasets collected by Dutch medical microbiological laboratories, all ISO 15189 accredited with a flexible scope in the fields Medical Microbiology or Medical Immunology with relevant elements. The report will be updated as part of the regular response structure to COVID-19 when new validation data is obtained. The specifics and frequency of publishing reports are yet to be determined. However, until further notice, data relating to performance characteristics of tests to share with colleague laboratories can still be sent through the email address taskforce.serologie@rivm.nl.