Report
Status of the validation of ELISA and auto-analyser antibody 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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Star-SHL, Etten-Leur and Rotterdam
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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

Version dated 30 April 2020: first version

Version dated 5 May 2020: second version:

- Status of ELISA validation and auto-analyser tests in the Netherlands, updated after 5 May (Chapter 2)
- Evaluation supplemented with data from the following medical microbiology laboratories: LabMicTA, ; Reinier Haga - MDC; UMC Utrecht; Centre for Infectious Diseases Friesland - Izore; Central Bacteriological and Serological Laboratory; Isala (chapters 2 and 3)

Version dated 19 May: third version

- Status of ELISA validation and auto-analyser tests in the Netherlands, updated after 19 May (Chapter 2)
- Sensitivity for all tests calculated over the total number of samples from various laboratories (Section 3.2)
  Evaluation supplemented with data or additional data from the following medical microbiology laboratories: LabMicTA; Centre for Infectious Diseases Friesland - Izore; LUMC, OLVG, Jeroen Bosch Hospital; PAMM; Saltro; Radboudumc; HagaZiekenhuis; Star-SHL; RIVM-IDS; UMCU; Meander Medical Center; COMICRO; Noordwest Ziekenhuisgroep; UMCG; Amsterdam UMC (Chapter 3). This involved adding new or additional data for the following tests (Section 3.2): Wantai SARS-CoV-2 Ab ELISA; Wantai SARS-CoV-2 IgM ELISA; EUROIMMUN SARS-CoV-2 IgG (S1 protein); EDI Novel Coronavirus COVID-19 IgG; EDI Novel Coronavirus COVID-19 IgM; RecomWell SARS-CoV-2 IgG; Vircell COVID-19 ELISA IgG; Vircell COVID-19 ELISA IgM+IgA; Creative Diagnostics SARS-CoV-2 IgG ELISA; Creative Diagnostics SARS-CoV-2 IgM ELISA; Platelia SARS-CoV-2 Total Ab; NovaLisa® SARS-CoV-2 IgG; NovaLisa® SARS-CoV-2 IgM; NovaLisa® SARS-CoV-2 IgA; LIAISON® SARS-CoV-2 IgG; ARCHITECT SARS-CoV-2 IgG assay; COVID-19 VIRCLIA® SARS-CoV-2 IgG MONOTEST; COVID-19 VIRCLIA® SARS-CoV-2 IgM+IgA MONOTEST; Elecsys® Anti-SARS-CoV-2.

Version dated 26 May: fourth version

- Copyright updated (Acknowledgements)
- Status of the validation of ELISA and auto-analyser tests in the Netherlands, updated after 26 May (Chapter 2)
- Evaluation supplemented with data or additional data from the following medical microbiology laboratories: Zuyderland MC; Canisius-Wilhelmina Hospital; CBSL; Star-SHL; Jeroen Bosch Hospital (Chapter 3). This involved adding new or additional data for the following tests (Section 3.2): Wantai SARS-CoV-2 Ab ELISA, Wantai SARS-CoV-2 IgM ELISA, RecomWell SARS-CoV-2 IgG, Platelia SARS-CoV-2 Total Ab, LIAISON® SARS-CoV-2 IgG.

Version dated 2 June: fifth version

- Status of the validation of ELISA and auto-analyser tests in the Netherlands, updated after 2 June (Chapter 2)
- Evaluation supplemented with data or additional data from the following medical microbiology laboratories: Comicro, Star-SHL, Diakonessenhuis, Gelre Hospitals, Gelderse Vallei hospital, Alrijne hospital (Chapter 3). This involved adding new or additional data for the following tests (Section 3.2): Wantai SARS-CoV-2 Ab ELISA, EUROIMMUN SARS-CoV-2 IgG (S1 proteïne), EUROIMMUN SARS-CoV-2 IgA, RecomWell SARS-CoV-2 IgG, LIAISON® SARS-CoV-2 IgG, ARCHITECT SARS-CoV-2 IgG assay, Elecsys® Anti-SARS-CoV-2.

Version dated 16 June: sixth version

- Status of the validation of ELISA and auto-analyser tests in the Netherlands, updated after 16 June (Chapter 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 sensitivities are recalculated for a, b and c (Section 3.2).
- All data from control groups for specificity of which data was collected during 2020 (during pandemic) was removed, and specificity for all tests was recalculated.
- Evaluation supplemented with data or additional data from the following medical microbiology laboratories: OLVG, HagaZiekenhuis, LabMicTA, CWZ, MH-mdc, Groene Hart Hospital, Medlon, Ikazia Hospital, Comicro, Deventer hospital (Chapter 3).

Version dated 2 July: seventh version

- Report is translated in English
- Status of the validation of ELISA and auto-analyser tests in the Netherlands, updated after 2 July (Chapter 2)
- Evaluation supplemented with data or additional data from the following medical microbiology laboratories: Star-SHL, Maastricht UMC+, Medlon, LUMC, Franciscus, PAMM, RIVM-IDS, Jeroen Bosch Hospital, Saltro, Diakonessenhuis, OLVG, Atalmedial, Groene Hart, St Jansdal Hospital (Chapter 3). This involved adding new or additional data for all tests in this document (Section 3.2).

Version dated 15 July: eight version

- Status of the validation of ELISA and auto-analyser tests in the Netherlands, updated after 15 July (Chapter 2)
- Evaluation supplemented with data or additional data from the following medical microbiology laboratories: LabMicTA, RIVM-IDS, MH-MDC, UMCG, Jeroen Bosch Hospital, Noordwestziekenhuisgroep, Amsterdam UMC, Groene Hart Hospital, LUMC, Gelre Hospital, COMICRO, Deventer Hospital (Chapter 3). This involved adding new or additional data for the following tests (Section 3.2):
  - Wantai SARS-CoV-2 Ab
  - Wantai SARS-CoV-2 IgM
  - EUROIMMUN SARS-CoV-2 IgG (protein S1)
  - EUROIMMUN SARS-CoV-2 IgA
  - EDI Novel Coronavirus COVID-19 IgG
  - EDI Novel Coronavirus COVID-19 IgM
  - recomWell SARS-CoV-2 IgG
  - Vircell COVID-19 ELISA IgG
  - Vircell COVID-19 ELISA IgM+IgA
  - LIAISON® SARS-CoV-2 IgG
  - Architect® SARS-CoV-2 Assay
  - Vircell COVID-19 VIRCLIA IgG monotest
  - Vircell COVID-19 VIRCLIA IgM+IgA monotest
  - Elecsys® Anti-SARS-CoV-2
  - Siemens SARS-CoV-2 total antibody test
  - Maglumi 2019-nCoV IgG (CLIA)
  - Maglumi 2019-nCoV IgM (CLIA)

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1 Introduction and considerations

The report you are reading is "Status of the validation of ELISA and auto-analyser antibody 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 thirty-nine 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 at least partial 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:

- Investigating whether someone has had an infection (recently or in the past)
- Testing 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. The World Health Organization

(WHO) therefore issued a warning on 24 April against making assumptions based on antibody detection with respect to protection against a second infection and basing measures specific to the individual on that [1].

In response to the growing need for test capacity worldwide during the COVID-19 pandemic, enzyme-linked immunosorbent assays (ELISA) and auto-analyser antibody tests are being offered by various manufacturers. These tests can be used in a laboratory setting to determine the presence of antibodies against SARS-CoV-2 in patients' serum to investigate whether an individual has COVID-19 or has had it in the recent past.

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. Provisional results show that ELISA tests can correlate well with virus-neutralising antibodies against SARS-CoV-2 [2].

This report describes considerations relating to antibody tests. It also carries out an initial comparative study of ELISA and auto-analyser tests for detecting antibodies to SARS-CoV-2 evaluated in Dutch laboratories and shares the provisional results and conclusions. This report will be updated weekly 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 structure of the tests is 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 separately before being used.
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 [3]. 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 false-positive test results.
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 [4]. This cannot be excluded and research will have to make this clear over the coming years. On 24 April, the World Health Organization stated that there is insufficient evidence that the presence of antibodies against SARS-CoV-2 protects against a second infection [1]. This is the reason why it is not safe to let people with antibodies care for COVID-19 patients without protective measures.

Limitations of antibody tests:

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 (false-positive test results) or the sensitivity of tests in people who have had a mild infection or remained asymptomatic (false-negative test results).
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 false negatives.

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 false positives 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.
6) There is often only limited information available about the patients whose serum was used for characterising the test's performance. Relevant information that is missing includes (1) the relationship between the moment of sampling and the first day of illness, (2) how severely ill the patients were, (3) the patient characteristics for the negative samples and (4) whether cross-reactivity with antibodies against other human coronaviruses was examined. Points (1) and (2) are determinants of the test's sensitivity; points (3) and (4) are determinants of the specificity. Because the information about these points is often missing, the ELISA and auto-analyser tests need to be accurately assessed to make it possible to define what populations they can be used in and at what time after infection.

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 is limited or virtually non-existent. Before a test can be used, its performance characteristics have to be investigated properly. 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 currently 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 mostly been developed to show infections among people who have or have recently had significant symptoms. 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.

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 exceptionally good test characteristics for a serological test. Many antibody tests at an advanced stage of development that are used in hospitals on a daily basis, where the a priori likelihood of the condition is high, 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.

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 COVID-19 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.

Chapters 2 and 3 below share the provisional results of evaluations in the Netherlands of the possible applications of ELISA and serological tests with auto-analysers.

2 Status of the validation of ELISA and serology auto-analysers

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 ELISA or auto-analyser tests (IgM and IgG) from 16 different manufacturers were at various stages of validation in the Netherlands on 15 July 2020. The selection of these tests by the laboratories was based on the availability and existing (in-house) platforms in the individual laboratories. A total of 122 different ELISA tests were available on the market worldwide on 13 July 2020 [5]. Table 1 shows 27 ELISA and auto-analyser tests that are at some stage of validation in the Netherlands, including two tests for which validation has not yet been started. This list was compiled based on information from the 53 laboratories that responded to the request for information and it may not be complete.

Table 1. ELISA and serological auto-analysers at various stages of validation in the Netherlands as at 15 July 2020

				Stage of evaluation (n labs)		
POCT	**Manufacturer**	**Type**	**Regulatory**	**Finished**	**Started**	**Planned**
Wantai SARS-CoV-2 Ab ELISA	Beijing Wantai Biological	ELISA	CE-IVD	25	2	0
Wantai SARS-CoV-2 IgM ELISA	Beijing Wantai Biological	ELISA	CE-IVD	11	0	0
EUROIMMUN SARS-CoV-2 IgG (protein S1)	EUROIMMUN AG	ELISA	CE-IVD	13	0	1
EUROIMMUN SARS-CoV-2 IgA	EUROIMMUN AG	ELISA	CE-IVD	7	0	2
EDI™ Novel Coronavirus COVID-19 ELISA IgG	Epitope Diagnostics Inc	ELISA	CE-IVD	8	0	0
EDI™ Novel Coronavirus COVID-19 ELISA IgM	Epitope Diagnostics Inc	ELISA	CE-IVD	7	0	0
recomWell SARS-CoV-2 IgG	Mikrogen Diagnostik	ELISA	CE-IVD	8	0	0
COVID-19 ELISA IgG	Vircell S.L.	ELISA	CE-IVD	6	0	0
COVID-19 ELISA IgM+IgA	Vircell S.L.	ELISA	CE-IVD	5	0	0
SARS-CoV-2 IgG ELISA kit	Creative Diagnostics	ELISA	RUO	1	0	0
SARS-CoV-2 IgM ELISA kit	Creative Diagnostics	ELISA	RUO	1	0	0
Platelia SARS-CoV-2 Total Ab	Bio-Rad Laboratories	ELISA	CE-IVD	3	0	0
NovaLisa® SARS-CoV-2 IgG	NovaTec Immundiagnostica GmbH	ELISA	CE-IVD	1	0	0
NovaLisa® SARS-CoV-2 IgM	NovaTec Immundiagnostica GmbH	ELISA	CE-IVD	1	0	0
NovaLisa® SARS-CoV-2 IgA	NovaTec Immundiagnostica GmbH	ELISA	CE-IVD	1	0	0
LIAISON® SARS-CoV-2 IgG	Diasorin	AA	CE-IVD	18	4	0
ARCHITECT SARS-CoV-2 IgG assay	Abott core laboratory	AA	CE-IVD	7	2	3
COVID-19 VIRCLIA® IgG monotest	Vircell S.L.	AA	CE-IVD	3	0	1
COVID-19 VIRCLIA® IgM+IgA monotest	Vircell S.L.	AA	CE-IVD	2	0	1
Elecsys® Anti-SARS-CoV-2	Roche Diagnostics Inc	AA	CE-IVD	7	1	1
SARS-CoV-2 total antibody test, voor Centaur en Atellica	Siemens Healthineers	AA	CE-IVD	5	2	1
VIDAS® anti-SARS-CoV-2 IgG	BioMérieux	AA	CE-IVD	1	0	1
VIDAS® anti-SARS-CoV-2 IgM	BioMérieux	AA	CE-IVD	1	0	1
MAGLUMI 2019-nCoV IgG (CLIA)	Snibe Co. Ltd.	AA	CE-IVD	1	0	0
MAGLUMI 2019-nCoV IgM (CLIA)	Snibe Co. Ltd.	AA	CE-IVD	1	0	0
Access SARS-CoV-2 IgG assay	Beckman Coulter Inc.	AA	CE-IVD	0	0	1

COVID-19 antibody test (IgG, IgM, IgA) on Simoa®	Quanterix Corp.	AA	CE-IVD	0	0	1

AA=auto-analyser.

The implementation of the plans for further research into these ELISA and auto-analyser tests and subsequent validation of such tests depends on the availability and delivery of the kits. Various laboratories have stated that there are problems with the delivery of some of the kits listed above. This resulted in some validations being carried out less extensively than would normally be desired. This emphasises the importance of coordinated collection of data about the tests from different laboratories, as in this report (which can partially address the issue).

3 Results and conclusions of ELISA validation in Dutch laboratories

3.1 Scope and criteria

Status as at 15 July 2020

The available results from validations of ELISA and auto-analysers for SARS-CoV-2 as at 15 July 2020 are the outcomes of validation processes that are sometimes limited because some kits are not available in large quantities. The data in this report can therefore for some tests be seen as an initial screening by Dutch laboratories. There are also publications on the evaluation of commercially available ELISA tests and auto-analyser antibody tests for SARS-CoV-2 [2, 6-24].

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. This will change as the circulation of the virus continues and people might get exposed/infected multiple times in which case IgM/IgA responses might become important markers for a recent infection. The main application of serology is in patient care. The criteria that antibody tests must meet differ depending on where the test is to be applied. In this initial screening of ELISA and auto-analyser tests, the following criteria were used (expert opinion):

- For individual patient diagnostics: IgG and IgM antibodies: both *separately*, with a specificity of >98% and sensitivity of >95% from 14 days[1] after symptoms appear
- Once national and international research has given a better understanding of how the presence of antibodies can be an indication for full or partial 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 to inform person-specific measures: Only IgG: specificity >98%, sensitivity >85% from 14 days[1] after symptoms appear.
- Epidemiological and serological prevalence studies: Only IgG: specificity >98%, sensitivity >95%

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 local experts in each situation.

[1] 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.

3.2 Results and conclusions for each ELISA or auto-analyser

Status as at 15 July 2020

The results and conclusions for each ELISA for detecting antibodies are described below, stating four points consecutively each time:

a. sensitivity in patients (confirmed positive by RT-PCR) with severe symptoms in hospital and with serum samples taken > 14 days after onset of illness.
b. sensitivity in patients (confirmed positive by RT-PCR) with severe symptoms in the hospital and with serum samples taken < 14 days of onset of illness. 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. That is why no criteria have been drawn up for this.
c. sensitivity in populations (confirmed positive by RT-PCR) with no symptoms or mild symptoms. It should be noted that the sensitivity of a test in this category cannot be assessed properly where serum samples were taken < 14 days after onset of illness.
d. sensitivity in patients with a positive neutralisation titre (PRNT/VNT50; VNT90)
e. specificity.

Where multiple laboratories have evaluated the same test in patients from the same group, the results are aggregated 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.

3.2.1 Results of ELISA tests

Wantai SARS-CoV-2 Ab ELISA (25 labs; total panel sensitivity n=1517, specificity n=1334)

a. The sensitivity (97.5%, n=646) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected > 14 days after onset of illness.
b. The sensitivity is 78.2% (n=459) in patients with severe infections where samples were collected ≤14 days after onset of illness.
c. The sensitivity (95.4%, n=372) meets the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected > 14 days after onset of illness. The sensitivity is 67.5% (n=40) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness.
d. Good correlation with neutralising antibodies with a sensitivity of 100% (n=155) for titres in VNT50%, 99% (n=155) for titres in VNT90%, and 98% (n=200) for titres in PRNT50.
e. The specificity is 99.6% (n=1334) and meets the predetermined criterion.

Wantai SARS-CoV-2 IgM ELISA (11 labs; total panel sensitivity n=420, specificity n=375)

a. The sensitivity (93.3%, n=149) 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 is 74.1% (n=166) in patients with severe infections where samples were collected ≤14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (79.0%, n=81) does not meet the defined criteria in populations with mild symptoms or asymptomatic infections where samples were collected > 14 days after onset of illness. The sensitivity is 41.7% (n=24) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these

percentages are based on limited sets of samples, confirmation with a larger number of samples is needed.

d. Limited correlation with neutralising antibodies with a sensitivity of 89% (n=198) for titres in PRNT50.
e. The specificity is 99.2% (n=375) and meets the predetermined criterion.

EUROIMMUN SARS-CoV-2 IgG, S1 protein (13 labs, total panel sensitivity n=632; specificity n=652)

a. The sensitivity (96.1%, n=229) 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 sensitivity is 46.6% (n=251) in patients with severe infections where samples were collected ≤ 14 days after onset of illness.
c. The sensitivity (76.2%, n=130) 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 sensitivity is 54.4% (n=22) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with larger numbers of samples is needed.
d. The correlation with neutralising antibodies is variable with a sensitivity of 97% (n=35) for titres in VNT50%, 100% (n=14) for titres in VNT90% and 81% (n=75) for titres in PRNT50.
e. The specificity is 98.3% (n=652) and meets the predetermined criterion.

EUROIMMUN SARS-CoV-2 IgA (7 labs, total panel sensitivity n=307; specificity n=367)

a. The sensitivity (96.0%, n=99) 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 sensitivity is 78.8% (n=137) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (63.6%, n=66) 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 sensitivity is 40.0% (n=5) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Confirmation with larger numbers of samples is needed.
d. The correlation with neutralising antibodies has a sensitivity of 97% (n=75) for titres in PRNT50.
e. The specificity is 90.2% (n=367) and does not meet the predetermined criterion.

EDI Novel Coronavirus COVID-19 ELISA IgG (8 labs, total panel sensitivity n=313; specificity n=257)

a. The sensitivity (96.6%, n=97) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected > 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
b. The sensitivity is 67.0% (n=109) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (78.5%, n=93) does not meet the predetermined criteria for diagnosis in patients with mild or asymptomatic infections where samples were collected > 14 days after

onset of illness. The sensitivity is 35.7% (n=14) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based limited sets of samples, confirmation with a larger number of samples is needed.

d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 95.3% (n=257) and does not meet the predetermined criterion.

EDI Novel Coronavirus COVID-19 ELISA IgM (7 labs, total panel sensitivity n=281; specificity n=221)

a. The sensitivity (78.4%, n=97) does not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected > 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
b. The sensitivity is 60.4% (n=111) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (31.7%, n=63) 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 sensitivity is 20.0% (n=10) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 98.6% (n=221) and meets the predetermined criterion.

RecomWell SARS-CoV-2 IgG, Mikrogen Diagnostik (8 labs, total panel sensitivity n=324; specificity n=330)

a. The sensitivity (96.3%, n=108) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected ≥ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
b. The sensitivity is 65.3% (n=95) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (86.8%, n=91) does not meet all predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected ≥ 14 days after onset of illness. The sensitivity is 30.0% (n=30) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 96.4% (n=330) and does not meet the predetermined criterion.

Vircell COVID-19 ELISA IgG (6 labs, total panel sensitivity n=262; specificity n=265)

a. The sensitivity (96.7%, n=91) 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 sensitivity is 79.1% (n=129) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (89.2%, n=37) does not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected >14 days days after onset of illness. The sensitivity is 40.0% (n=5) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with larger numbers of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 93.6% (n=265) and does not meet the predetermined criterion.

Vircell COVID-19 ELISA IgM+IgA (5 labs, total panel sensitivity n=236; specificity n=178)

a. The sensitivity (96.7%, n=91) 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 sensitivity is 70.9% (n=103) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (70.3%, 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. The sensitivity is 20.0% (n=5) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 82.0% (n=178) and does not meet the predetermined criterion. Confirmation with a larger number of samples is needed.

SARS-CoV-2 IgG ELISA kit, Creative Diagnostics (1 lab, total panel sensitivity n=102; specificity n=78)

a. The sensitivity (75.0%, n=24) 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 is 28.1% (n=32) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (52.2%, n=46) 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, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.

e. The specificity is 98.7% (n=78) and meets the predetermined criterion. Confirmation with a larger number of samples is needed.

SARS-CoV-2 IgM ELISA kit, Creative Diagnostics (1 lab, total panel sensitivity n=102; specificity n=78)

a. The sensitivity (83.3%, n=24) 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 is 56.3% (n=32) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (54.3%, n=46) 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, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 97.4% (n=78) and does not meet the predetermined criterion. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.

Platelia SARS-CoV-2 Total Ab (2 labs, total panel sensitivity n=185; specificity n=122)

a. The sensitivity (92.6%, n=27) 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 is 62.5% (n=72) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (89.2%, n=83) 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 sensitivity is 66.7% (n=3) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 94.3% (n=122) and does not meet the predetermined criterion.

NovaLisa® SARS-CoV-2 IgG (1 lab, total panel sensitivity n=72; specificity n=72)

a. The sensitivity (90.3%, n=31) 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 is 44.4% (n=36) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (100%, n=5) 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 a very limited set of samples, confirmation with a larger number of samples is needed.

d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 95.8% (n=72) and does not meet the predetermined criterion. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.

NovaLisa® SARS-CoV-2 IgM (1 lab, total panel sensitivity n=72; specificity n=72)

a. The sensitivity (58.1%, n=31) 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 is 27.8% (n=36) 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 sensitivity (0%, n=5) does not meet the predetermined criteria in populations with mild symptoms or asymptomatic infections where the samples were collected >14 days after onset of illness. Because this percentage is based on a very limited set of samples, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 98.6% (n=72) and meets the predetermined criterion. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.

NovaLisa® SARS-CoV-2 IgA (1 lab, total panel sensitivity n=72; specificity n=72)

a. The sensitivity (90.3%, n=31) 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 is 66.7% (n=36) in patients with severe infections where samples were collected ≤ 14 days after onset of illness. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
c. The sensitivity (40%, n=5) 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, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with these ELISA tests and no statements can therefore be made about them.
e. The specificity is 88.9% (n=72) and does not meet the predetermined criterion. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.

3.2.2 Results of auto-analyser antibody tests

LIAISON SARS-CoV-2 IgG (18 labs, total panel sensitivity n=827; specificity n=946)

a. The sensitivity (94.3%, n=366) does not meet the predetermined criteria for diagnosis in patients with severe infections where samples were collected > 14 days after onset of illness.
b. The sensitivity is 20.0% (n=275) 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 sensitivity (81.2%, n=165) 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 sensitivity is 66.7% (n=21) for diagnosis in patients with mild or asymptomatic infections where the samples were collected ≤ 14 days after onset of illness. Because this latter percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.
d. The correlation with neutralising antibodies is limited, with a sensitivity of 81% (n=165) in serum samples with titres in PRNT50.
e. The specificity is 97.3% (n=946) and does not meet the predetermined criterion.

ARCHITECT SARS-CoV-2 IgG assay (7 labs, total panel sensitivity n= 340; specificity n=224)

a. The sensitivity (94.0%, n=117) 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 is 36.2% (n=127) 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 sensitivity (89.2%, n=83) does not meet all predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected > 14 days after onset of illness. The sensitivity is 7.7% (n=13) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with a larger number of samples is needed.
d. The correlation with neutralising antibodies has a sensitivity of 96% (n=13) for titres in PRNT50. Confirmation with a larger number of samples is needed.
e. The specificity is 100% (n=224) and meets the predetermined criterion.

COVID-19 VIRCLIA® IgG monotest (3 labs, total panel sensitivity n=158; specificity n=135)

a. The sensitivity (86.7%, n=45) 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 is 57.5% (n=87) 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 sensitivity (96.2%, n=26) 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 a limited set of samples, confirmation with a larger number of samples is needed. The samples in this tested cohort with mild symptoms are predominantly from healthcare workers, potentially, their samples were taken much later after onset of disease.
d. Neutralisation tests have not yet been carried out in correlation with this auto-analyser test and no statements can therefore be made about this aspect.
e. The specificity is 96.3% (n=135) and does not meet the predetermined criterion.

COVID-19 VIRCLIA® IgM+IgA monotest (2 labs, total panel sensitivity n=78; specificity n=75)

a. The sensitivity (96.8%, n=31) 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 sensitivity is 80.5% (n=41) 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 sensitivity (66.7%, n=6) 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, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with this auto-analyser test and no statements can therefore be made about this aspect.
e. The specificity is 90.7% (n=75) and does not meet the predetermined criterion. Because this percentage is based on a limited set of samples, confirmation with a larger number of samples is needed.

Elecsys® Anti-SARS-CoV-2 (7 labs, total panel sensitivity n=495; specificity n=472)

a. The sensitivity (94.4%, n=198) 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 is 46.5% (n=157) 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 sensitivity (89.2%, n=120) 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 sensitivity is 75.0% (n=20) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with this auto-analyser test and no statements can therefore be made about this aspect.
e. The specificity is 99.8% (n=472) and meets the predetermined criterion.

Siemens Healthineers SARS-CoV-2 total antibody test, Centaur and Atellica (5 labs, total panel sensitivity n=227; specificity n=185)

a. The sensitivity (92.6%, n=108) 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 is 55.1% (n=69) 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 sensitivity (84.0%, n=50) 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 number of samples, confirmation with a larger number of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with this auto-analyser test and no statements can therefore be made about this aspect.
e. The specificity is 98.9% (n=185) and meets the predetermined criterion. Confirmation with a larger number of samples is needed.

VIDAS® anti-SARS-CoV-2 IgG (1 lab, total panel sensitivity n= 28; specificity n=20)

a. The sensitivity (100%, n=22) 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 sensitivity is 33.3% (n=6) 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 characteristics in mild SARS-CoV-2 infections have not yet been evaluated and no statements can therefore be made about them.
d. Neutralisation tests have not yet been carried out in correlation with this auto-analyser test and no statements can therefore be made about this aspect.
e. The specificity is 90% (n=20) and does not meet the predetermined criterion. Because this percentage is based on a limited number of samples, confirmation with a larger number of samples is needed.

VIDAS® anti-SARS-CoV-2 IgM (1 lab, total panel sensitivity n= 28; specificity n=18)

a. The sensitivity (100%, n=22) 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 sensitivity is 66.7% (n=6) 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 characteristics in mild SARS-CoV-2 infections have not yet been evaluated and no statements can therefore be made about them.
d. Neutralisation tests have not yet been carried out in correlation with this auto-analyser test and no statements can therefore be made about this aspect.
e. The specificity is 88.9% (n=18) and does not meet the predetermined criterion. Because this percentage is based on a limited number of samples, confirmation with a larger number of samples is needed.

MAGLUMI 2019-nCoV IgG, CLIA (1 lab, total panel sensitivity n=59; specificity n=62)

a. The sensitivity (100%, n=24) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected > 14 days after onset of illness. Because this percentage is based on a limited number of samples, confirmation with a larger number of samples is needed.
b. The sensitivity is 100% based on just one sample 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 sensitivity (86.4%, n=22) does not meet all predetermined criteria in populations with mild symptoms or asymptomatic infections where samples were collected > 14 days after onset of illness. The sensitivity is 83.3% (n=12) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with larger numbers of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with this auto-analyser test and no statements can therefore be made about this aspect.
e. The specificity is 96.8% (n=62) and does not meet the predetermined criterion. Because this percentage is based on a limited number of samples, confirmation with a larger number of samples is needed.

MAGLUMI 2019-nCoV IgM, CLIA (1 lab, total panel sensitivity n=59; specificity n=62)

a. The sensitivity (95.8%, n=24) meets the predetermined criteria for diagnosis in patients with severe infections where samples were collected > 14 days after onset of illness. Because this percentage is based on a limited number of samples, confirmation with a larger number of samples is needed.
b. The sensitivity is 100% based on just one sample 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 sensitivity (68.2%, n=22) 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 sensitivity is 91.7% (n=12) for diagnosis in patients with mild or asymptomatic infections where samples were collected ≤ 14 days after onset of illness. Because these percentages are based on limited sets of samples, confirmation with larger numbers of samples is needed.
d. Neutralisation tests have not yet been carried out in correlation with this auto-analyser test and no statements can therefore be made about this aspect.
e. The specificity is 96.8% (n=62) and does not meet the predetermined criterion. Because this percentage is based on a limited number of samples, confirmation with a larger number of samples is needed.

3.3 Correlation of positive results in ELISA 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 as a proxy for the presence of neutralising antibodies. Erasmus MC and RIVM have submitted data on the presence of neutralising antibodies in relation to routine serology test outcomes. The presence of neutralising antibodies is a possible indicator of protective immunity. The sensitivity of the tests from this report (calculated with a neutralisation test as the reference) has already been mentioned under point “d”.

In a cohort of 47 patients with mild symptoms who tested positive for the presence of Ab with the Wantai Ab ELISA, the presence of neutralising antibodies was found in a VNT_{50} test in 34/47 (72%) patients, and in 12/47 (26%) patients at a cut-off of 90% inhibition.

In a cohort of n=111 patients with severe symptoms who tested positive for the presence of Ab with the Wantai Ab ELISA, the presence of neutralising antibodies was found in a VNT50 test in 102/111 (92%) patients, and in 95/111 (86%) patients at a cut-off of 90% inhibition. This data seems to be an initial indication of a poor correlation between positivity in the Wantai total Ab ELISA and the presence of neutralising antibodies in cohorts with mild symptoms (patients who were not hospitalised).

3.4 Summary of the initial laboratory findings

The ELISA tests and auto-analysers vary in how well they perform. *In certain groups, the sample numbers are too small for solid conclusions to be drawn about use; these still need to be confirmed with a larger number of samples. For that reason, it is important that laboratories include the underlying data in sections 3.1 to 3.3 in their decision-making.*

At the moment, the following tests meet the predetermined criterion of specificity > 98% when all specification panels of the various laboratories are bundled together:

- Wantai SARS-CoV-2 Ab (99.6%, n=1334)

- Wantai SARS-CoV-2 IgM (99.2%, n=375)
- EUROIMMUN SARS-CoV-2 IgG (98.3%, n=652)
- EDI Novel Coronavirus COVID-19 ELISA IgM (98.6%, n=221)
- Creative Diagnostics SARS-CoV-2 IgG (98.7%, n=78)*
- NovaLisa® SARS-CoV-2 IgM (98.6%, n=72)*
- Architect SARS-CoV-2 IgG (100%, n=224)
- Elecsys® Anti-SARS-CoV-2 (99.8%, n=472)
- Siemens SARS-CoV-2 total antibody (98.9%, n=185)*

Three (*) of these nine tests that meet the predetermined criteria for specificity have been evaluated with a total of < 200 samples and therefore require further testing with a larger number of sample sets. Serological tests with lower specificity can be used, but follow-up testing is then needed. This must be considered by local experts for specific situations.

Albeit in evaluations with a limited number of samples for most evaluated tests, the following tests do currently meet the predetermined criterion of sensitivity > 95% for 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.

- Wantai SARS-CoV-2 Ab (97.5%, n=646)
- EUROIMMUN SARS-CoV-2 IgG (96.1%, n=229)
- EUROIMMUN SARS-CoV-2 IgA (96.0%, n= 99)
- EDI Novel Coronavirus COVID-19 ELISA IgG (96.6%, n=97)
- RecomWell SARS-CoV-2 IgG (96.3%, n=108)
- Vircell COVID-19 ELISA IgG (96.7%, n=91)
- Vircell COVID-19 ELISA IgM+IgA (96.7%, n=91)
- COVID-19 VIRCLIA® IgM+IgA monotest (96.8%, n=31)
- VIDAS® anti-SARS-CoV-2 IgG (100%, n=22)
- VIDAS® anti-SARS-CoV-2 IgM (100%, n=22)
- MAGLUMI 2019 nCoV-2 IgG (100%, n=24)
- MAGLUMI 2019 nCoV-2 IgM (95.8%, n=24)

Of these, the Wantai SARS-CoV-2 Ab and the EUROIMMMUN SARS-CoV-2 IgG are the only kits that also meet the specificity criterion of > 98%. With exception of Wantai and EUROIMMUN IgG, all tests need to be evaluated with more samples, as they are currently tested with less than 200 samples.

The test below meets the predetermined criterion of sensitivity > 95% for diagnostics in a population of **patients with mild symptoms or with asymptomatic infections** where the sample material was collected **> 14 days after the symptoms,** if any, appear. This sensitivity is also high enough for testing subpopulations and for seroprevalence tests.

- Wantai SARS-CoV-2 Ab (95.0%, n=279)
- COVID-19 VIRCLIA® IgG monotest (96.2%, n=26)

The Wantai SARS-CoV-2 Ab also meets the criterion for specificity of > 98%. The VIRCLIA® IgG monotest is tested with a limited set of samples, and needs to be confirmed with a larger sample set. The NovaLisa® SARS-CoV-2 IgG has been tested with a very limited number of samples. The values stated in this report for this last test do meet the predetermined criteria, but are not reliable because the sample size is too small and they must be evaluated further before conclusions can be drawn. The

VIDAS® anti-SARS-CoV-2 IgG and IgM antibody tests are not yet evaluated in populations with mild infections where samples were collected >14 days after onset of illness.

The tests below do not meet the predetermined criterion for **diagnostics** in a population of patients with mild symptoms or asymptomatic infections, but may possibly be suitable for testing subpopulations and for seroprevalence studies because their IgG (or total Ig) sensitivity was > 85% in samples that were collected **> 14 days after the symptoms,** if any, appeared.

- RecomWell SARS-CoV-2 IgG (86.8%, n=91)
- Vircell COVID-19 ELISA IgG (89.2%, n=37)
- Platelia SARS-CoV-2 Total Ab (89.2%, n=83)
- Architect SARS-CoV-2 IgG (89.2%, n=83)
- Elecsys® Anti-SARS-CoV-2 (89.2%, n=120)
- MAGLUMI 2019 nCoV-2 IgG (86.4%, n=22)

After the confirmation of these sensitivities using larger numbers of samples, these tests could make sense 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). The use of these tests must be assessed by local experts for each situation and problem.

3.5 Preliminary conclusion based on initial laboratory findings

Based on the results presented here, the following four preliminary conclusions can be drawn for the use of tests for patient care:

1. Not all antibody tests tested here have a specificity of > 98%. Serological tests with lower specificity can be used, but follow-up testing is then needed. This must be considered by local experts for specific situations.

2. For diagnostics in patients with severe symptoms, where the material is collected at least 14 days after onset of illness, the Wantai SARS-CoV-2 Ab and the EUROIMMMUN SARS-CoV-2 IgG meet the predetermined criteria for both sensitivity and specificity. Not all antibody tests evaluated here were sufficiently tested in this population and need further study.

3. When testing infections suffered in populations with mild symptoms or asymptomatic infections, the Wantai SARS-CoV-2 Ab ELISA meets all the predetermined criteria when sample collection is done > 14 days after onset of illness. Other antibody tests evaluated either do not meet all the criteria or their performance in this population looks promising but has not yet been sufficiently tested and needs further study.

4. There is a good correlation between positivity in the Wantai total Ab ELISA and the presence of neutralising antibodies. The correlation is however poorer in the population with mild symptoms (patients who were not hospitalised). This must be evaluated further with a larger number of samples.

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.

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