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External Quality Assessment of Sites Performing SARS-CoV-2 Antigen Test Diagnostics for the Dutch Population, April 2021

Colophon

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Contents

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Errata:
One workflow was in duplo in version 1; 11-05-2021. The duplo workflow has been removed and corresponding analyses have been corrected in version 2. The number of participating sites is corrected in version 2.

Addendum:
Clarification regarding the specificity aspect has been added to page 19 and 22.

Summary

Background

Since November 2020 lateral flow-based Rapid Antigen Tests (RAT) and Chemiluminescence Immunoassay-based high-throughput antigen tests for diagnostics of SARS-CoV-2 were implemented in the Netherlands. It is considered important by the COVID-19 WHO reference laboratories at RIVM and Erasmus Medical Centre (Erasmus MC) and the Dutch Ministry of Health, Welfare and Sport ('Dienst Testen') to obtain an overview of the performance of SARS-CoV-2 antigen tests in the Netherlands.

Objective

The goal of this National External Quality Assessment (EQA) program for antigen tests (Landelijk EQA Antigen; LEQA-Ag) is to inventorize the quality of the Dutch SARS-CoV-2 antigen diagnostics field. Sites performing SARS-CoV-2 antigen diagnostics in the Dutch network were asked to conduct antigen detection of SARS-CoV-2 in simulated clinical samples according to their workflows normally used for SARS-CoV-2 diagnostics. Participating sites can use this quality assessment to compare their results with the national average and, when necessary, take actions to improve their performance.

Materials and Methods

The LEQA1 SARS-CoV-2 antigen panel was produced at the RIVM and consisted of 15 simulated clinical samples containing Triton X-100 inactivated SARS-CoV-2: a dilution series of five samples containing wildtype virus for sensitivity determination, three dilutions of three variant viruses among which variant of concern (VOC) B.1.1.7; 20B/501Y.V1 and a sample without virus, in random order. The panel was distributed in the second week of March 2021 and could be used according to two methods; either dipping a swab in the sample or pipetting the sample. Participating sites reported their results via an online form. Samples of the panel were designated as core sample if >90% of the reported results for each method (dipping or pipetting) reported the correct result. The panel contained 9 core samples and 6 educational samples for the dipping method (DIP) and 13 core samples and 2 educational samples for the pipetting method (PIP). Workflows were given 1 point for each correct results and 0.5 points for an "Inconclusive" result for the 15 panel samples.

Results

There were 25 workflows using the 'dipping of a swab method' and 19 workflows using the 'pipetting method' reported by 35 participating sites. Ten different test brands were used: 8 RATs and 2 high throughput tests. Out of the 44 workflows reported, 43 obtained a 100% correct score for the core samples. There was no indication that the current antigen tests used in the Netherlands had problems detecting the VOC compared to the other strains in the panel. Furthermore, a significant amount of workflows - 80% (20/25) workflows using DIP and 37% (7/19) workflows using PIP - were also able to correctly identify educational samples of the panel containing SARS-CoV-2 concentrations around the Limit of Detection (LOD) of used tests. The results of the educational samples show that the reported Lowest Detected Concentration (LDC) of SARS-CoV-2 can vary between workflows, even when the same test is used.

Conclusions

Overall, the antigen test workflows used for SARS-CoV-2 diagnostics in the Netherlands perform well and that the currently used SARS-CoV-2 antigen tests are a reliable diagnostic tool. The variation in reported LDC between the workflows using visual interpretation of colloidal gold-based lateral flow assays indicate that probably training of personnel to interpret results is important for consistent interpretation of samples containing relatively low concentrations of SARS-CoV-2 virus.

1. Introduction

Since November 2020 lateral flow-based Rapid Antigen Tests (RATs) and Chemiluminescence Immunoassay-based high-throughput antigen tests for diagnostics of SARS-CoV-2 were implemented in the Netherlands. It is considered important by the COVID-19 WHO reference laboratories at RIVM and Erasmus Medical Centre (Erasmus MC) and the Dutch Ministry of Health, Welfare and Sport ('Dienst Testen') to obtain an overview in the performance of SARS-CoV-2 antigen tests in the Netherlands. In collaboration with various international partners (including the RIVM), the World Health Organisation (WHO) is developing a WHO International Standard for SARS-CoV-2 antigen. It is however not yet known when this standard will be available. For these reasons, a National External Quality Assessment (EQA) program for antigen tests (Landelijk EQA Antigen; LEQA-Ag) has been developed. In the second week of March 2021 the LEQA1 SARS-CoV-2 antigen panels were distributed to sites performing SARS-CoV-2 antigen test diagnostics. This panel consisted of 15 simulated clinical samples, containing Triton X-100 inactivated SARS-CoV-2, including one variant of concern (VOC) B.1.1.7; 20B/501Y.V1. that has become the major strain in circulation by week 9/2021 in The Netherlands [1]. Each of the sites was asked to conduct antigen detection of SARS-CoV-2 according to their workflows normally used for SARS-CoV-2 diagnostics. All data had to be reported back to the RIVM using an online reporting form.

2 Materials and methods

2.1 Approach

The LEQA1 SARS-CoV-2 antigen panel was produced and pre-tested on 6 different lateral flow-based RATs at the RIVM. The laboratory of Elizabeth Tweesteden Ziekenhuis in Tilburg pre-tested the LEQA panel on a Chemiluminescence Immunoassay-based high-throughput antigen assay. The LEQA panel was tested by two methods; either dipping a swab in the sample and subsequently putting the swab in the assay buffer or pipetting 350 µl sample directly in the assay buffer. The dipping method (DIP) resembles the original workflow more closely than the pipetting method (PIP). However, DIP can introduce more variation in the workflow, since the volume of sample a swab is absorbing ranges from 30-50 µl (depending on the type of swab used and the accuracy of 'dipping'). For DIP pre-testing at the RIVM, one type of swab and strict instructions were used, which are reflected in the instruction for use (Appendix I, in Dutch). With PIP, the sample volume is fixed. With DIP the sample is approximately 10-fold more diluted in the assay buffer compared to PIP. All SARS-CoV-2 antigen pre-tests were executed in 3-fold per panel sample to obtain the 'expected results' per sample (Appendix II, in Dutch). The Lowest Detected Concentration (LDC) of the SARS-CoV-2 B.11 19A (WT) dilution series (LEQA1_Ag21-15, -02, -13, -04 and -11) was determined for the PIP method between 75 and 750 TCID50/ml for RATs and high-throughput assays. For the DIP method LDC was between 750 and 7499 TCID50/ml for RATs and high-throughput assays. All sites performing SARS-CoV-2 diagnostics using antigen tests in the Dutch network were contacted and notified of the distribution of the panel in the second week of March 2021. Each of the sites was asked to conduct antigen detection of SARS-CoV-2 according to their workflows normally used for SARS-CoV-2 diagnostics using the dipping and/or pipetting method (Appendix I, in Dutch). Participating sites were asked to report their results using an online form (Formdesk software; Wassenaar, The Netherlands) before the 26th of March. Sites that had not reported their results by the 26th of March, were given one week grace time to report their results, after which the submission was closed on the 2nd of April 2021. The expected results (Appendix II, in Dutch) of the LEQA1 panel was communicated to the participating sites on the 6th of April 2021.

2.2 Contents of LEQA1 panel

The LEQA1 SARS-CoV-2 Antigen panel consisted of 15 simulated clinical samples (1ml) containing Triton X-100 inactivated SARS-CoV-2 viruses or no virus in virus transport medium GLY. SARS-CoV-2 was isolated from clinical specimens on VERO E6 cells and inactivated by Triton X-100 treatment at room temperature for two hours. The TCID50 of each virus stock before Triton X-100 treatment as proxy for detectable antigen was determined on VERO E6 monolayers in 96-well plates. The panel contained the following SARS-CoV-2 strains: hCoV-19/Netherlands/NoordBrabant_10003/2020 (B.11, 19A), hCoV-19/Netherlands/GE-RIVM-20300/2020 (B.1.177, 20AEU1), hCoV-19/Netherlands/NH-RIVM-20432/2020 (B.1.1.7, 20B/501Y.V1) and hCoV-19/Netherlands/NB-RIVM-20274/2020 (B.1.5, 20A), and a sample without any virus. hCoV-19/Netherlands/NoordBrabant_10003/2020 (B.11, 19A) was included as 10-fold dilution series to assess the sensitivity, aka the LDC, of each workflow. Of the other viruses a low, mid and high concentration sample at 10-fold difference from each other were included. All samples were submitted randomized. Table 1 shows the composition of the LEQA1 SARS-CoV-2 antigen panel.

2.3 Scoring the workflows

The performance of each reported workflow was evaluated after which they were scored on a scale from 0 to 15, with 15 being the best score. A distinction is made between core samples and educational samples. Samples of the panel were designated as core sample if >90% of the reported results for each method (dipping or pipetting) reported the correct result; i.e. %Positive plus %Weak positive for SARS-CoV-2 containing samples. Since with the dipping method (DIP) the sample is approximately 10-fold more diluted than with the pipetting method (PIP), there were more samples in the panel around the limit of detection (LOD) of the used tests for DIP compared to PIP. All samples except LEQA1_Ag21-04, -05, -08, -11, -13 and -14, containing the lowest and second lowest concentration of the dilution series of SARS-CoV-2 or the lowest concentration of the three variant viruses, were deemed core samples for DIP (Table 1 and 2, Figure 3A). All samples except LEQA1_Ag21-4 and LEQA1_Ag21-11, containing the lowest and second lowest concentration of the dilution series of SARS-CoV-2, were deemed core samples for PIP (Table 1 and 2, Figure 3B). Thus, the panel contained 9 core- and 6 educational samples for DIP and 13 core samples and 2 educational samples for PIP. The sites were given the option to evaluate samples with the following conclusions: Positive, Weak positive, Negative, or Inconclusive. Workflows were given 1 point for each correct results and 0.5 points for an “Inconclusive” result for the 15 panel samples.

A 100% correct score for the core samples results in 9 points for workflows using DIP and 13 points for workflows using PIP and performs according to the national average. Workflows scoring less than 9 or 13 points based on the core samples, depending on the method used, could indicate that actions should be taken to improve their performance. Furthermore, correct identification of educational samples was also scored to identify workflows that perform better than the national average.

Table 1: Composition of LEQA1 SARS-CoV-2 Antigen panel for SARS-CoV-2 detection

Panel coding	SARS-CoV-2 variant	Concentration TCID50/ml	qRT-PCR Ct value[1]			Qualification based on reported results (core >90% correct; Table 2 and Figure 3)	
			E-gene	RdRP-gene	N-gene	Dipping method	Pipetting method
LEQA1_Ag21-01	B.1.177 20A.EU1	1334	31.4	23.2	25.6	Core positive	Core positive
LEQA1_Ag21-02	B.11; 19A; wildtype	7499	29.1	21.1	23.7	Core positive	Core positive
LEQA1_Ag21-03	B.1.1.7 20B/501Y.V1	23714	30.7	24.4	26	Core positive	Core positive
LEQA1_Ag21-04	B.11; 19A; wildtype	75	35.3	28.1	30.4	Educational	Educational
LEQA1_Ag21-05	B.1.1.7 20B/501Y.V1	237	36.1[2]	30.8	32.7	Educational	Core positive
LEQA1_Ag21-06	B.1.177 20A.EU1	13335	28.1	19.9	22.5	Core positive	Core positive
LEQA1_Ag21-07	B.1.5 20A	2371	32.1	23.9	26.9	Core positive	Core positive
LEQA1_Ag21-08	B.1.177 20A.EU1	133	33.7	26.7	29.1	Educational	Core positive
LEQA1_Ag21-09	No virus	0	Negative	Negative	Negative	Core negative	Core negative
LEQA1_Ag21-10	B.1.1.7 20B/501Y.V1	2371	34.3	27.7	29.1	Core positive	Core positive
LEQA1_Ag21-11	B.11; 19A; wildtype	7	Negative	31.2	33.6	Educational	Educational
LEQA1_Ag21-12	B.1.5 20A	23714	28.9	20.6	23.6	Core positive	Core positive
LEQA1_Ag21-13	B.11; 19A; wildtype	750	32.4	24.7	27.2	Educational	Core positive
LEQA1_Ag21-14	B.1.5 20A	237	35.4	27.4	30.1	Educational	Core positive
LEQA1_Ag21-15	B.11; 19A; wildtype	74989	26.1	18.1	20.9	Core positive	Core positive

[1] The Ct values shown in this table are based on RT-qPCR tests performed on the panel samples using the routinely used RIVM in-house assays. The in-house real-time RT-qPCRs have been performed using the following reagents and volumes: ThermoFisher TaqMan® Fast Virus 1-Step Master Mix after extraction of 200 µl sample on Roche MagNA Pure 96 instrument with Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit, elution in 50 µl and 5 µl extract per RT-qPCR reaction on Roche LightCycler 480 mark I or II. Extractions and subsequent RT-qPCRs were performed in 3-fold. SARS-CoV-2 E-gene Sarbeco specific primers and probes are those published by Corman et al.; the RdRP primers and probes are modified from those published by Corman et al. to become SARS-CoV-2 specific and similar in LOD95 compared to the E-gene Rt-qPCR.

[2] Target gene tested positive in 2 out of 3 replicates of the RT-qPCR.

3. Results

3.1 Overview antigen tests used in the Netherlands

In total, 44 workflows have been reported via the online reporting form by 35 participating sites, which were linked to 24 responsible laboratories. Four test locations reported multiple workflows due to another type of SARS-CoV-2 antigen test or a different testing method (dipping a swab in the sample or pipetting the sample). Ten types of SARS-CoV-2 antigen tests have been reported, which can be divided in lateral flow-based Rapid Antigen Tests (RAT) including Abbott - Panbio COVID-19-Ag rapid test (n=19), Roche - SARS-CoV-2 Rapid Antigen Test (n=9), Becton Dickinson - BD Veritor COVID test (n=2), Biozek - Covid-19 antigen rapid test cassette (n=2), Siemens Healthineers - Clinitest Rapid COVID-19 Antigen test (n=1), SD Biosensor - Standard F-Covid-19 Ag (n=1), GR Biotech - Saliva test (Shenzhen Lvshiyuan Biotechnology Co., Ltd.) (n=1) and GR Biotech - Rapid Test Swab (Shenzhen Lvshiyuan Biotechnology Co., Ltd.) (n=1), and Chemiluminescence Immunoassay-based high-throughput antigen tests including Diasorin - Liaison SARS-CoV-2 Ag (n=7) and Siemens Healthineers - Atellica IM CoV2Ag (n=1) (Figure 1A). All reported SARS-CoV-2 antigen tests target the N-protein (nucleocapsid protein) of the virus. The results of the Chemiluminescence Immunoassay-based high-throughput antigen tests Diasorin and Siemens Healthineers - Atellica and the fluorescent-based lateral flow assay of SD Biosensor are interpreted by a reader. The results of the colloidal gold-based lateral flow assay Becton Dickinson (BD), is read both by eye (n=1) and by reader (n=1). Other antigens tests are colloidal gold-based lateral flow assays and are visually interpreted by 1, 2 or 3 individual technicians, depending on the site. An overview of the antigen tests and numbers used in the reported workflows is shown in Figure 1A. The most common SARS-CoV-2 antigen test reported is the Panbio COVID-19-Ag rapid test from Abbott. A distinction is made in the type of testing method dipping (DIP) and pipetting (PIP) with the corresponding SARS-CoV-2 antigen tests, as shown in Figure 1B and C. Within the dipping method a relatively large amount of workflows using the Abbott antigen test have been reported.

Testing of the LEQA1 SARS-CoV-2 antigen panel has been conducted on 13 different types of testing sites as reported. The testing sites are subdivided as follows: Laboratory (n=25) includes laboratory (n=23), hospital laboratory used for health care workers (n=1) and hospital laboratory used for screening of non-suspect patients (n=1). Municipal Health Services (GGD) testing sites (n=10) includes on site GGD-(X)L drive or walk through testing site (n=6), on site GGD-L testing site (n=2), on site pop-up laboratory connected to GGD XL walk through testing site (n=1) and on site at GGD and at nursing homes, care homes and home care (VVT) (n=1). Other testing sites (n=9) includes any testing site which is not listed above, including hospital employee test site (n=6). A distinction is made between the type of testing method dipping (DIP) and pipetting (PIP) with the corresponding testing sites, as shown in Figure 2A and B. Within the pipetting method a relatively large amount of tests has been conducted in a laboratory.

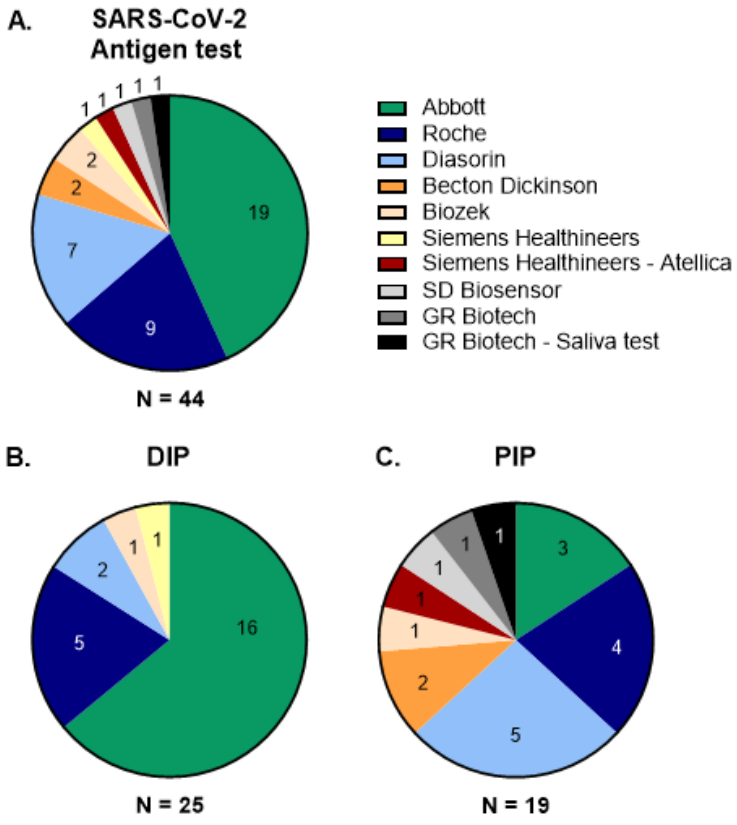


Figure 1. (A) Overview of all SARS-CoV-2 antigen tests reported by the participating testing sites used in their workflows (n=44). The color of each antigen test is shown in the legend. A distinction is made between the types of testing methods, either (B) dipping a swab in the sample (DIP; n=25) or (C) pipetting the sample (PIP; n=19).

Abbott - Panbio COVID-19-Ag rapid test; Roche - SARS-CoV-2 Rapid Antigen Test; Diasorin - Liaison SARS-CoV-2 Ag; Becton Dickinson - BD Veritor COVID test; Biozek - Covid-19 antigen rapid test cassette; Siemens Healthineers - Clinitest Rapid COVID-19 Antigen test; Siemens Healthineers - Atellica IM CoV2Ag; SD Biosensor - Standard F-Covid-19 Ag; GR Biotech - Rapid Test Swab (Shenzhen Lvshiyuan Biotechnology Co., Ltd.); GR Biotech - Saliva test (Shenzhen Lvshiyuan Biotechnology Co., Ltd.)

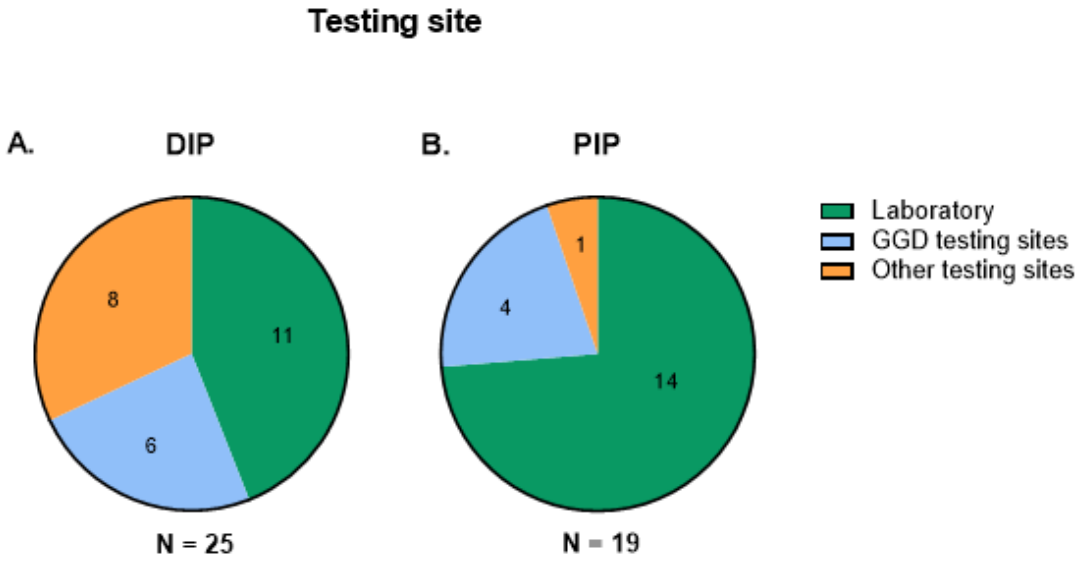


Figure 2. (A) Overview of testing sites of the reported workflow (n=44). The color of each type of testing site is shown in the legend. A distinction is made between the types of testing methods, either (A) dipping a swab in the sample (DIP; n=25) or (B) pipetting the sample (PIP; n=19).

Laboratory includes laboratory, hospital laboratory used for health care workers and hospital laboratory used for screening of non-suspect patients. GGD testing sites includes on site GGD-(X)L drive or walk through testing site, on site GGD-L testing site, on site pop-up laboratory connected to GGD XL walk through testing site and POCT (on side) at GGD and at VVT. Other testing sites includes any testing site which is not listed above

Table 2: Aggregated overview of workflow conclusions by LEQA1 SARS-CoV-2 antigen panel sample

Panel coding	SARS-CoV-2 variant	Concentration TCID50/ml	Cumulative results dipping (n=25)				Cumulative results pipetting (n=19)			
			Positive	Weak positive	Inconclusive	Negative	Positive	Weak positive	Inconclusive	Negative
LEQA1_Ag21-03	B.1.1.7 20B/501Y.V1	23714	25 (100%)	0	0	0	19 (100%)	0	0	0
LEQA1_Ag21-10	B.1.1.7 20B/501Y.V1	2371	22 (88%)	2 (8%)	0	1 (4%)	19 (100%)	0	0	0
LEQA1_Ag21-05[1]	B.1.1.7 20B/501Y.V1	237	5 (20%)	10 (40%)	1 (4%)	9 (36%)	16 (84%)	3 (16%)	0	0
LEQA1_Ag21-06	B.1.177 20A.EU1	13335	25 (100%)	0	0	0	19 (100%)	0	0	0
LEQA1_Ag21-01	B.1.177 20A.EU1	1334	22 (88%)	2 (8%)	0	1 (4%)	19 (100%)	0	0	0
LEQA1_Ag21-08[1]	B.1.177 20A.EU1	133	6 (24%)	13 (52%)	2 (8%)	4 (16%)	16 (84%)	3 (16%)	0	0
LEQA1_Ag21-12	B.1.5 20A	23714	24 (96%)	1 (4%)	0	0	19 (100%)	0	0	0
LEQA1_Ag21-07	B.1.5 20A	2371	22 (88%)	2 (8%)	0	1 (4%)	19 (100%)	0	0	0
LEQA1_Ag21-14[1]	B.1.5 20A	237	7 (28%)	10 (40%)	1 (4%)	7 (28%)	17 (89%)	2 (11%)	0	0
LEQA1_Ag21-15	B.11; 19A; wildtype	74989	25 (100%)	0	0	0	19 (100%)	0	0	0
LEQA1_Ag21-02	B.11; 19A; wildtype	7499	21 (84%)	3 (12%)	0	1 (4%)	19 (100%)	0	0	0
LEQA1_Ag21-13[1]	B.11; 19A; wildtype	750	4 (16%)	13 (52%)	2 (8%)	6 (24%)	17 (89%)	2 (11%)	0	0
LEQA1_Ag21-04[1, 2]	B.11; 19A; wildtype	75	0	0	0	25 (100%)	3 (16%)	4 (21%)	0	12 (63%)
LEQA1_Ag21-11[1, 2]	B.11; 19A; wildtype	7	0	0	0	25 (100%)	0	1 (5%)	0	18 (95%)
LEQA1_Ag21-09	Negative		0	0	0	25 (100%)	0	0	0	19 (100%)

All samples are sorted on strain and SARS-CoV-2 concentration rather than their respective panel number.

[1] These samples have in general a concentration of SARS-CoV-2 around the lowest detected concentration (LDC) for the dipping method (DIP) and deemed as an educational sample.

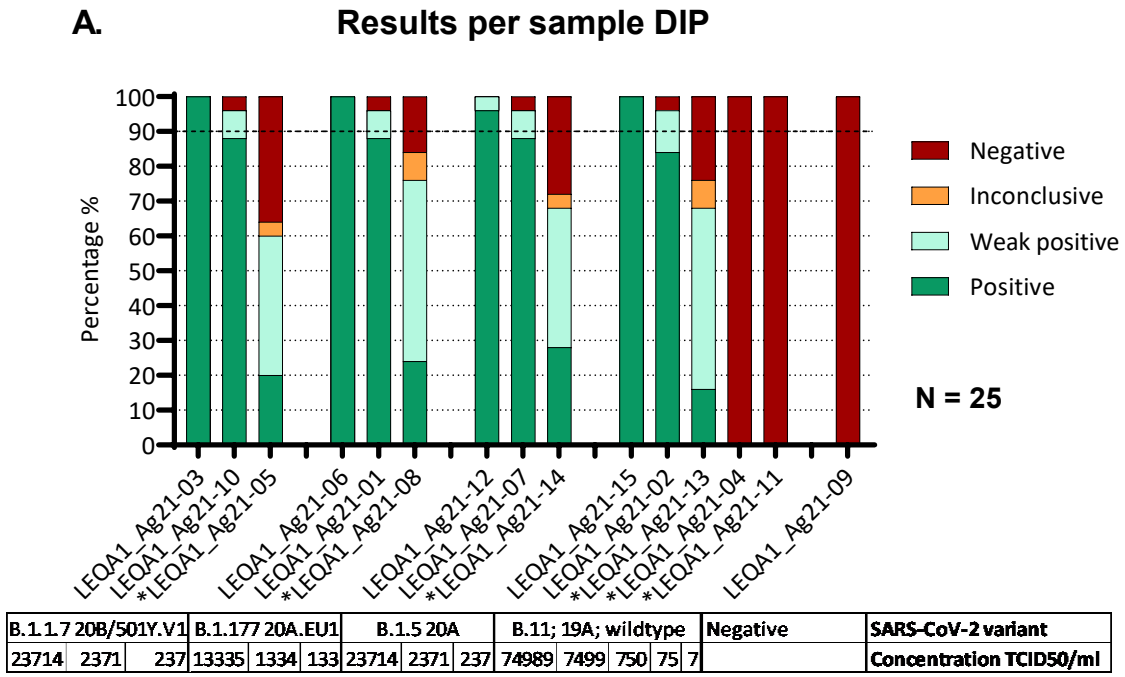
[2] These samples have in general a concentration of SARS-CoV-2 around the lowest detected concentration (LDC) for the pipetting method (PIP) and deemed as an educational sample.

3.2 Performance antigen testing sites in the Netherlands

The LEQA1 SARS-CoV-2 antigen panel consisted of 15 simulated clinical samples including four dilution series of SARS-CoV-2 variants: B.1.1.7 20B/501Y.V1 (n=3; variant of concern; VOC), B.1.177 20A.EU1 (n=3), B.1.5 20A (n=3), B.11 19A (n=5; wildtype; WT) and a sample without any virus (n=1; Negative). Panel coding numbers with corresponding SARS-CoV-2 variant, concentration TCID50/ml are shown in Table 2 and are sorted on strain and concentration rather than their respective panel number.

The participating sites have reported their results for each sample with the following interpretations: Positive, Weak positive, Negative, or Inconclusive. It should be noted that not all sites agreed with the term “Weak positive” and reported ‘’Positive’’ regardless of the intensity of the signal. Therefore, indications of ‘’Weak positive’’ results are only educational and serve merely as a rough indication whether the result was easy or more difficult to assess. The cumulative results are listed in Table 2 and are visually shown in bar graphs in Figure 3. A clear correlation in viral load and correct test result is visible, as mainly the samples with low SARS-CoV-2 virus concentration were indicated ‘’Negative’’ or ‘’Inconclusive’’. The results between the SARS-CoV-2 variants are quite similar, meaning there is no difference in sensitivity of antigen tests in general between the variants, including the VOC. The negative core sample was reported “Negative” by all sites.

A distinction was made between the type of testing method dipping and pipetting, because pipetting method dilutes the sample less in the assay buffer than the dipping method (approximately 1:1 vs 1:10, respectively). There were more samples in the panel around the LOD (educational samples, indicated by *) for DIP compared to PIP (Figure 3A and B). Due to this difference, analysis of the dipping method provided more insight on the different interpretations of educational samples by various workflows. For example all possible interpretations have been reported for the samples LEQA1_Ag21-05, -08, -14 and -13, containing 133-750 TCID50/ml SARS-CoV-2. In these educational samples “Weak positive” was frequently reported and also on the photos of the RAT results the sites provided, very faint or no signal could be seen. This indicates that interpretation of the results with the educational samples for DIP are challenging, consequently resulting in correct “Positive” or “Weak Positive” interpretation, but also “Inconclusive” and “Negative” interpretations.



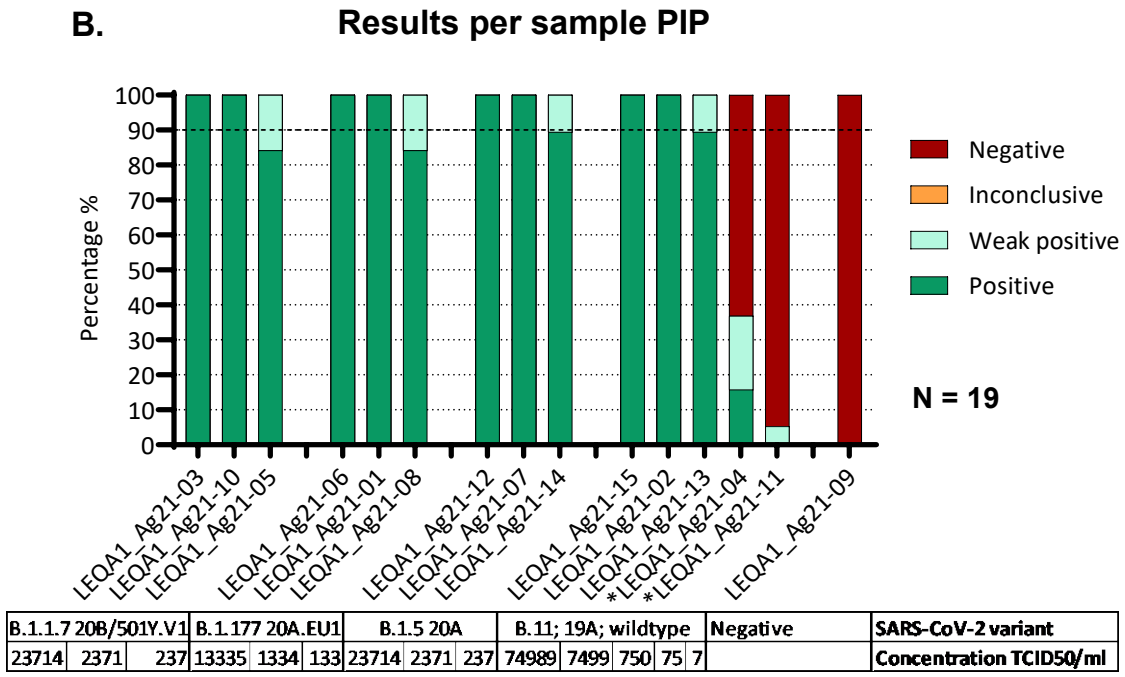
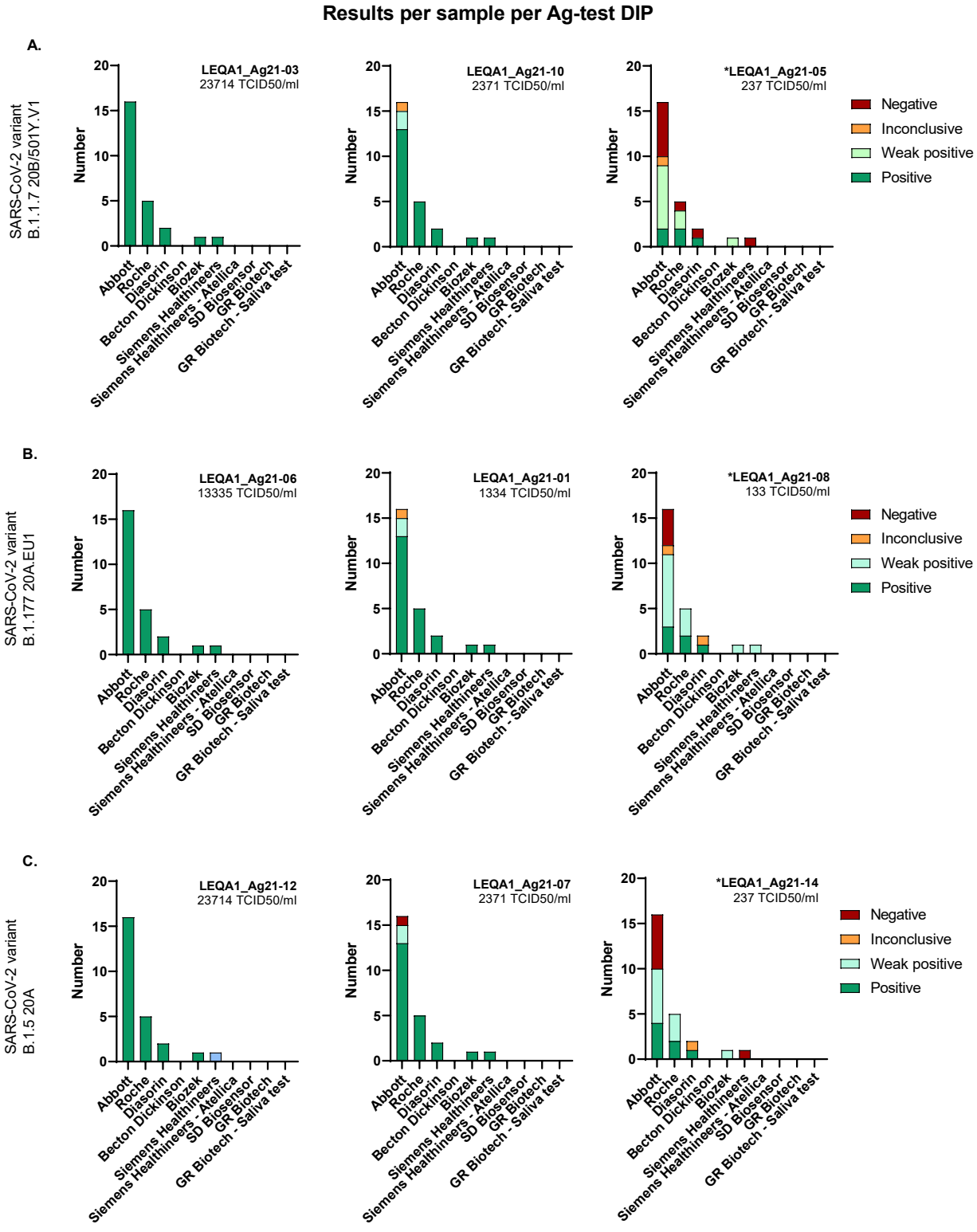


Figure 3. An overview of the reported results for each sample (n=44). The sites were given the option to evaluate samples with the following conclusions: Positive, Weak positive, Negative, or Inconclusive. The color of the scoring is shown in the legend. Beneath the sample coding number, the SARS-CoV-2 variant and concentration SARS-CoV-2 in TCID50/ml of each sample is given. A distinction is made between the types of testing methods, either (A) dipping a swab in the sample (DIP; n=25) or (B) pipetting the sample (PIP; n=19) of which the results are given in percentages. Samples of the panel were designated as core sample if >90% of the reported results for each method reported the correct result.
** No core (aka educational) samples*

An overview containing the results obtained per test per panel sample using the dipping or pipetting method is shown in Figure 4 and 5, respectively. The number of reported workflows per test brand is too limited to draw strong conclusions on the performance of individual brands. Figure 4 shows that results with the Abbott test and DIP are relatively often reported as ''Weak positive'', suggesting a more challenging visual interpretation of the signal (test line) with samples containing low concentrations of virus (LEQA1_Ag21-05, -08, -14, -13) and consequently are more likely to assess these samples as "Inconclusive" (doubting whether a test line is visible) or "Negative". For example for LEQA1_Ag21-10 (Figure 4A), the Abbott workflows reported 18.8% (3/16) "Weak positive" or "Inconclusive", compared to none (0/5) for Roche. At a 10-fold lower concentration SARS-CoV-2 (LEQA1_Ag21-05), the workflows using Abbott reported 87.5% (14/16) "Weak positive", "Inconclusive" and "Negative" conclusions, while the workflows using Roche reported 60.0% (3/5) "Weak-positive" and "Negative" conclusions (Figure 4A). It should be noted that the number of reported workflows using Roche is limited compared to Abbott. Other brands cannot be properly assessed for this question due to the low number of reported results per test brand. With PIP (Figure 5), the brands seem to perform more comparable. Variation in DIP caused by differences in the volume that is absorbed by the swab, usage of different swabs or suboptimal mixing of the sample (less likely to occur when using a pipet), cannot be excluded. The (only) workflow using the Siemens Healthineers - Atellica IM CoV2Ag test, could also correctly identify the sample (LEQA1_Ag21-11) with the lowest SARS-CoV-2 concentration in the B.11 19A (WT) dilution series with the pipetting method (Figure 5D). Overall, all reported test brands are able to correctly identify SARS-CoV-2 in the core samples for either DIP or PIP (Figure 4 and 5). Furthermore, there appears to be no difference in sensitivity (based on the dilution series of the four SARS-CoV-2 strains) of the tests between the SARS-CoV-2 variants, including the VOC.


Results per sample per Ag-test DIP
A.
SARS-CoV-2 variant
B.1.1.7 20B/501Y.V1
LEQA1_Ag21-03
23714 TCID50/ml
LEQA1_Ag21-10
2371 TCID50/ml
*LEQA1_Ag21-05
237 TCID50/ml
B.
SARS-CoV-2 variant
B.1.177 20A.EU1
LEQA1_Ag21-06
13335 TCID50/ml
LEQA1_Ag21-01
1334 TCID50/ml
*LEQA1_Ag21-08
133 TCID50/ml
C.
SARS-CoV-2 variant
B.1.5 20A
LEQA1_Ag21-12
23714 TCID50/ml
LEQA1_Ag21-07
2371 TCID50/ml
*LEQA1_Ag21-14
237 TCID50/ml
Number
Negative
Inconclusive
Weak positive
Positive
Abbott
Roche
Diasorin
Becton Dickinson
Biozek
Siemens Healthineers
Siemens Healthineers - Atellica
SD Biosensor
GR Biotech
GR Biotech - Saliva test

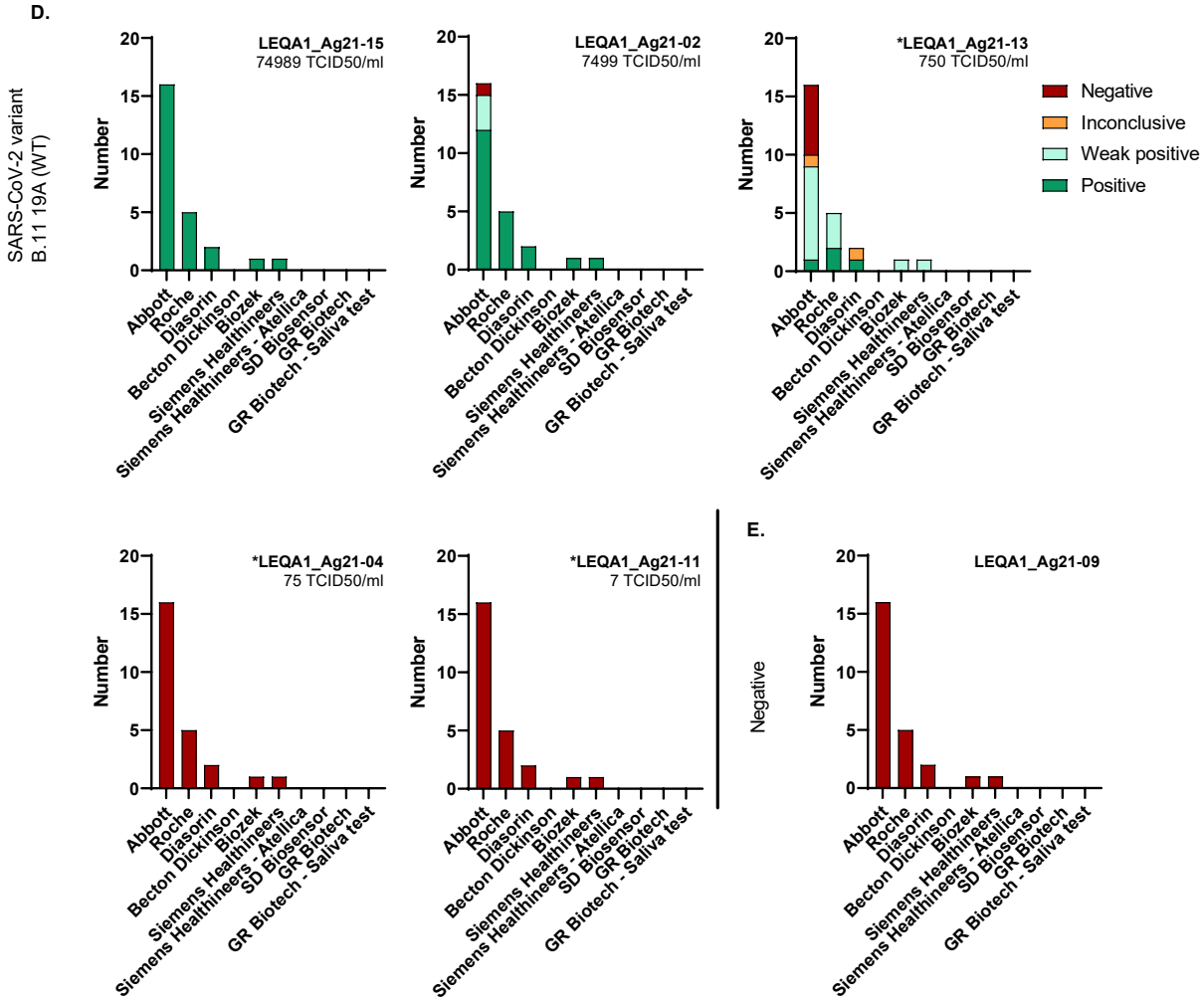
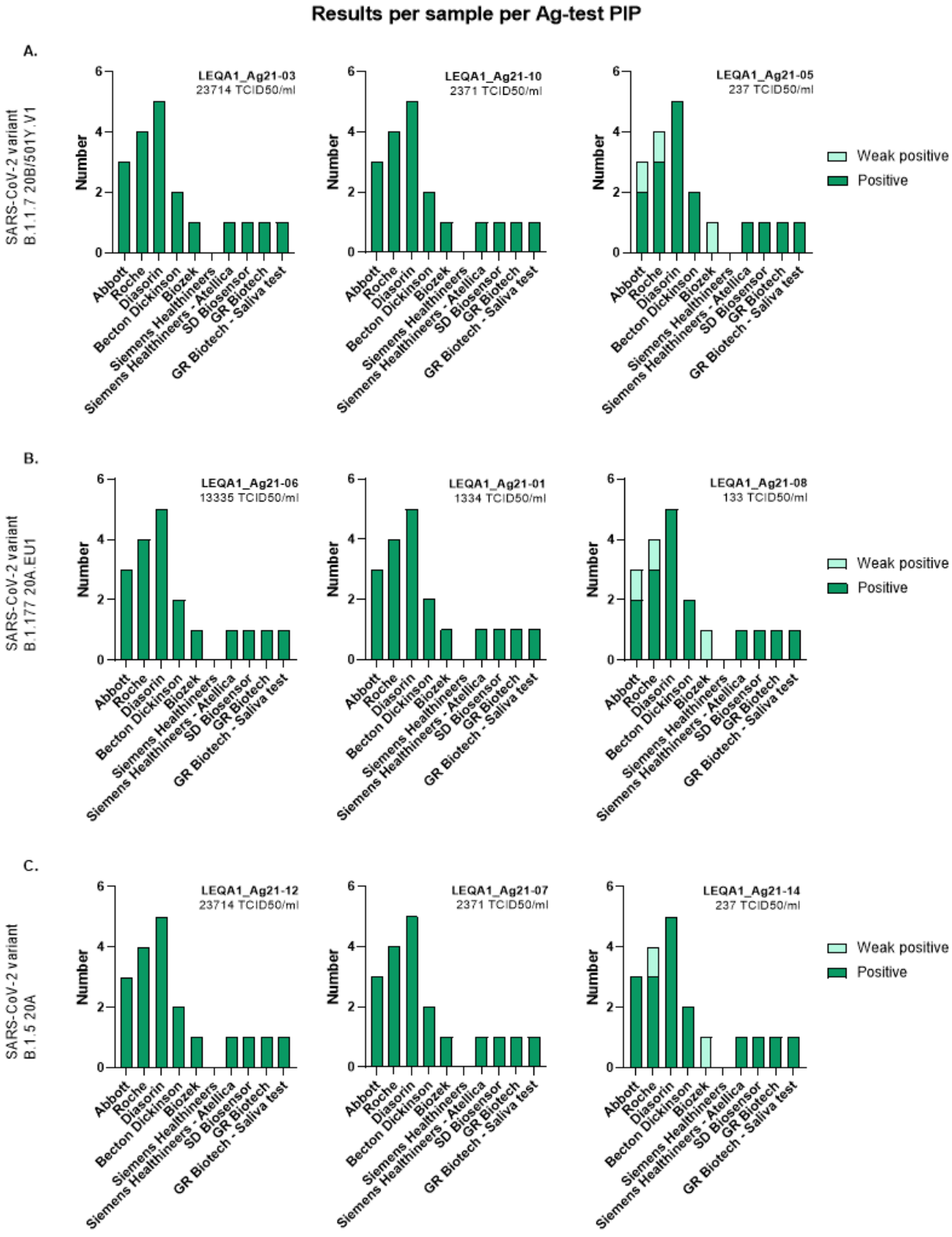


Figure 4. Overview performance SARS-CoV-2 antigen tests for testing method dipping a swab in the sample (DIP). Panel coding numbers are sorted on SARS-CoV-2 strain (A) B.1.1.7 20B/501Y.V1, (B) B.1.177 20A.EU1, (C) B.1.5 20A, (D) B.11 19A and (E) no virus and concentration (TCID50/ml). The number of reported workflows per test are indicated at the Y-axis. Samples of the panel were designated as core sample if >90% of the reported results for each method reported the correct result.
** No core (aka educational) samples*

Abbott - Panbio COVID-19-Ag rapid test; Roche - SARS-CoV-2 Rapid Antigen Test; Diasorin - Liaison SARS-CoV-2 Ag; Becton Dickinson - BD Veritor COVID test; Biozek - Covid-19 antigen rapid test cassette; Siemens Healthineers - Clinitest Rapid COVID-19 Antigen test; Siemens Healthineers - Atellica IM CoV2Ag; SD Biosensor - Standard F-Covid-19 Ag; GR Biotech - Rapid Test Swab (Shenzhen Lvshiyuan Biotechnology Co., Ltd.); GR Biotech - Saliva test (Shenzhen Lvshiyuan Biotechnology Co., Ltd.)


Results per sample per Ag-test PIP
A.
SARS-CoV-2 variant
B.1.1.7 20B/501Y.V1
LEQA1_Ag21-03
23714 TCID50/ml
LEQA1_Ag21-10
2371 TCID50/ml
LEQA1_Ag21-05
237 TCID50/ml
B.
SARS-CoV-2 variant
B.1.177 20A.EU1
LEQA1_Ag21-06
13335 TCID50/ml
LEQA1_Ag21-01
1334 TCID50/ml
LEQA1_Ag21-08
133 TCID50/ml
C.
SARS-CoV-2 variant
B.1.5 20A
LEQA1_Ag21-12
23714 TCID50/ml
LEQA1_Ag21-07
2371 TCID50/ml
LEQA1_Ag21-14
237 TCID50/ml
Number
0
2
4
6
Abbott
Roche
Diasorin
Becton Dickinson
Biozek
Siemens Healthineers
Siemens Healthineers - Atellica
SD Biosensor
GR Biotech
GR Biotech - Saliva test
Weak positive
Positive

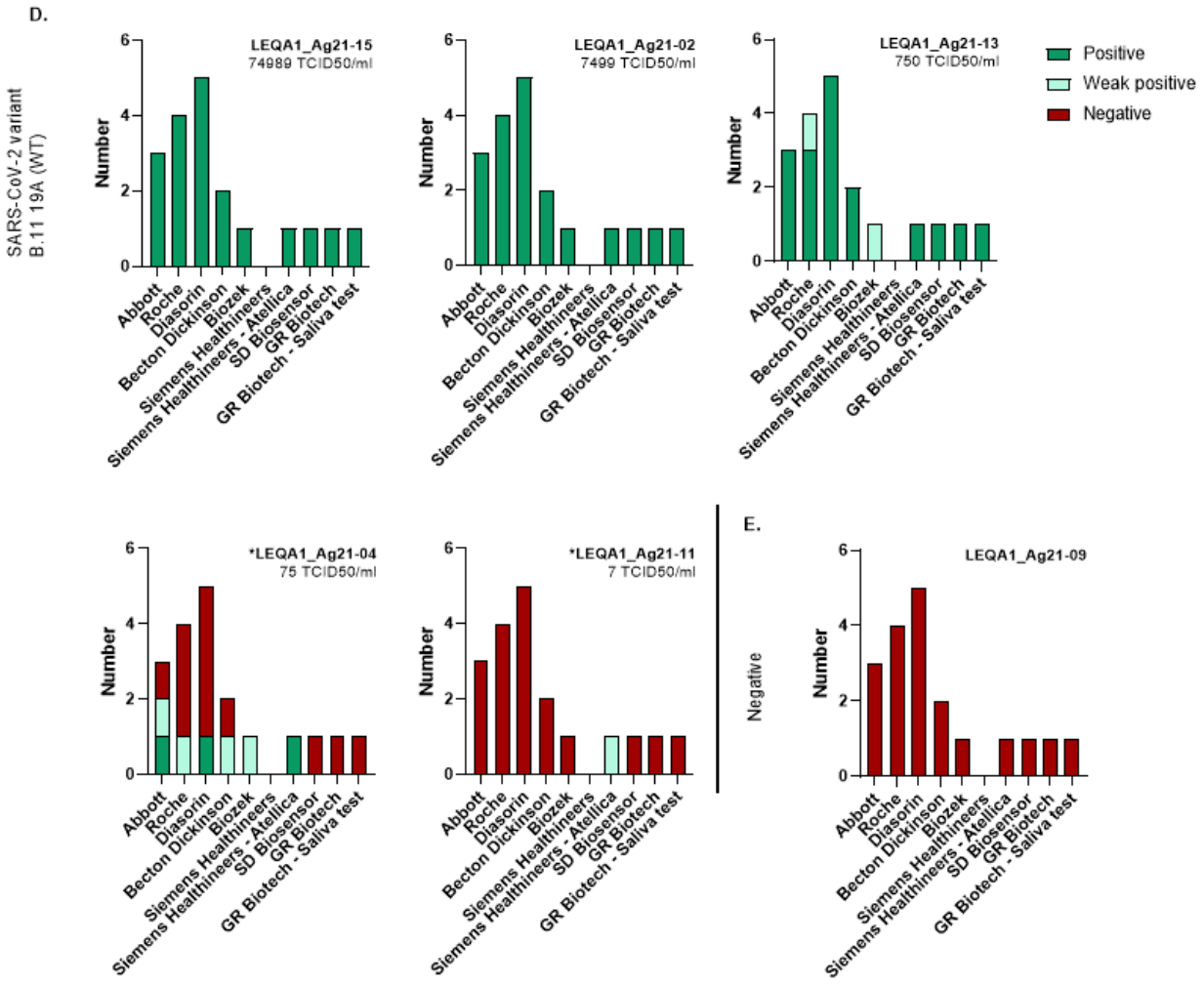


Figure 5. Overview performance SARS-CoV-2 antigen tests for testing method pipetting the sample (PIP). Panel coding numbers are sorted on SARS-CoV-2 strain (A) B.1.1.7 20B/501Y.V1, (B) B.1.177 20A.EU1, (C) B.1.5 20A, (D) B.11 19A and (E) no virus and concentration (TCID50/ml). The number of reported workflows per test are indicated at the Y-axis. Samples of the panel were designated as core sample if >90% of the reported results for each method reported the correct result.
** No core (aka educational) samples*

Abbott - Panbio COVID-19-Ag rapid test; Roche - SARS-CoV-2 Rapid Antigen Test; Diasorin - Liaison SARS-CoV-2 Ag; Becton Dickinson - BD Veritor COVID test; Biozek - Covid-19 antigen rapid test cassette; Siemens Healthineers - Clinitest Rapid COVID-19 Antigen test; Siemens Healthineers - Atellica IM CoV2Ag; SD Biosensor - Standard F-Covid-19 Ag; GR Biotech - Rapid Test Swab (Shenzhen Lvshiyuan Biotechnology Co., Ltd.); GR Biotech - Saliva test (Shenzhen Lvshiyuan Biotechnology Co., Ltd.)

The performance of each reported workflow (identification, Id) was evaluated after which they were scored on a scale from 0 to 15, with 15 being the best score (Figure 6). A distinction is made between core samples and educational samples. The panel contained 9 core- and 6 educational samples for DIP and 13 core samples and 2 educational samples for PIP. A 100% correct score for the core samples results in 9 points for workflows using DIP and 13 point for workflows using PIP and performs according to the national average. Out of the 25 workflows using DIP, 24 (96%) scored a 100% correct score for the 9 core samples (9 points) (Figure 6A). The workflow that did not correctly identified all core samples (Id26), could misdiagnose persons with low SARS-CoV-2 viral loads with the antigen test. After inquiry this site reported that all tested person receive a PCR test in addition to the antigen test. All 19 workflows using PIP scored a 100% correct score for the 13 core samples (13 points) (Figure 6B).

In total 80% (20/25) of workflows using DIP (scoring ≥ 10 points) were also able to detect educational loads of SARS-CoV-2 in the panel samples (Figure 6A and Table 2). The best reported score for the workflows using DIP was 13 points and was achieved by 60% (15/25) of the workflows. In total 37% (7/19) of workflows using PIP (scoring ≥ 14 points) were also able to detect educational loads of SARS-CoV-2 in the panel samples (Figure 6B and Table 2). The best reported score for the workflow using PIP was 15 points, thus identifying all 15 samples correctly, and was achieved by one workflow. The results obtained by the educational samples show that Lowest Detected Concentration (LDC) can vary between workflows. The visual interpretation of a RAT result is subjective and one person could be more skilled than the other. To compensate for this, the test results of the RATs are often visually interpreted by more persons. Some RATs and high-throughput assays (have to) use readers for the interpretation of the results. The test brand and/or method of read-out of the test results could influence the LDC of the workflow. Figure 7 shows the LDC of the SARS-CoV-2 B.11 19A (WT) dilution series (LEQA1_Ag21-15, -02, -13, -04 and -11) per workflow in combination with the test brand and method of test read-out used. To correct for the 10-fold dilution of workflows using DIP compared to PIP, the reported LDC was adjusted by dividing by 10 for the DIP workflows. Almost all (42 out of 44) workflows reported an (adjusted) LDC of 75 or 750 TCID50/ml.

Figure 7 shows no difference in reported LDC by workflows using the Abbott test (36.8% LDC of 750 TCID50/ml; 7 out of 19) compared to workflows using Roche (33.3% LDC of 750 TCID50/ml; (3 out of 9). Diasorin workflows reported relatively more the 'higher' LDC (71,4% LDC of 750 TCID50/ml; (5 out of 7) compared to Abbott and Roche. It should be noted that the number of reported workflows per test brand is too limited to draw strong conclusions on the performance of individual brands or type of antigen tests (colloidal gold- or fluorescent-based RATs or high-throughput assay). Abbott and Roche require the subjective trained eye of (one or more) technicians for the correct interpretation of the results. The number (1, 2 or 3) of technicians interpreting the results by eye, may partially explain the variation in reported LDC using a certain test brand. For example, workflows using the Abbott test reported the 'higher' LDC of 750 TCID50/ml in 50.0% (1 out of 2), 40.0% (6 out of 15) and 0% (0 out of 2) of the workflows with 1, 2 and 3 independent visually interpretations, respectively. However, it should be noted that workflows using the same test (e.g. Abbott or Roche), the same number of technicians to interpret the results and the same EQA method (DIP or PIP) still can report variable LDC (Figure 7), indicating that a certain person can be more skilled in correct assessment of very weak signals. The Becton Dickinson test was the only test of which results were reported using both a reader (n=1) and using visual interpretation (n=1). The LDC was 75 TCID50/ml for the workflow using the reader compared to 750 TCID50/ml for the workflow using visual interpretation (both workflows used PIP). The LDC reported by workflows using a reader, such as the Diasorin test also varied (71.4% LDC of 750 TCID50/ml; 5 out of 7). Variation in reported LDC between these workflows can occur when e.g. the cut-off is raised (as was indicated by 3 workflows, with the reason to prevent false-positive results) or pre-analytical adjustments in the volumes compared to the manufactures' instructions. It

should be noted that this LEQA1 SARS-CoV-2 antigen panel focused on the analytical sensitivity of the SARS-CoV-2 antigen test workflows. The performance of the workflows on specificity was not specifically assessed. Although the LEQA1 antigen panel samples consists of simulated clinical samples, actual clinical samples might contain more contaminants that may cause a very weak aspecific signal in the tests. Correctly discriminating between SARS-CoV-2-specific weak signals or aspecific weak signals is challenging for workflows. We could not assess whether the workflows that reported the 'lower' LDC (aka more sensitive) compared to the workflows that reported the 'higher' LDC, perform equivalent on specificity. The only negative sample in the panel was interpreted correctly negative by 100% of the workflows. Overall, the variation in reported LDC between the workflows (using the same test brand and) using read-out by eye indicate that interpretation of results is challenging when samples contain relative low concentrations of SARS-CoV-2 virus. Training of (multiple) persons to interpret results of colloidal gold-based lateral flow assays is important for consistent performance of the workflows.

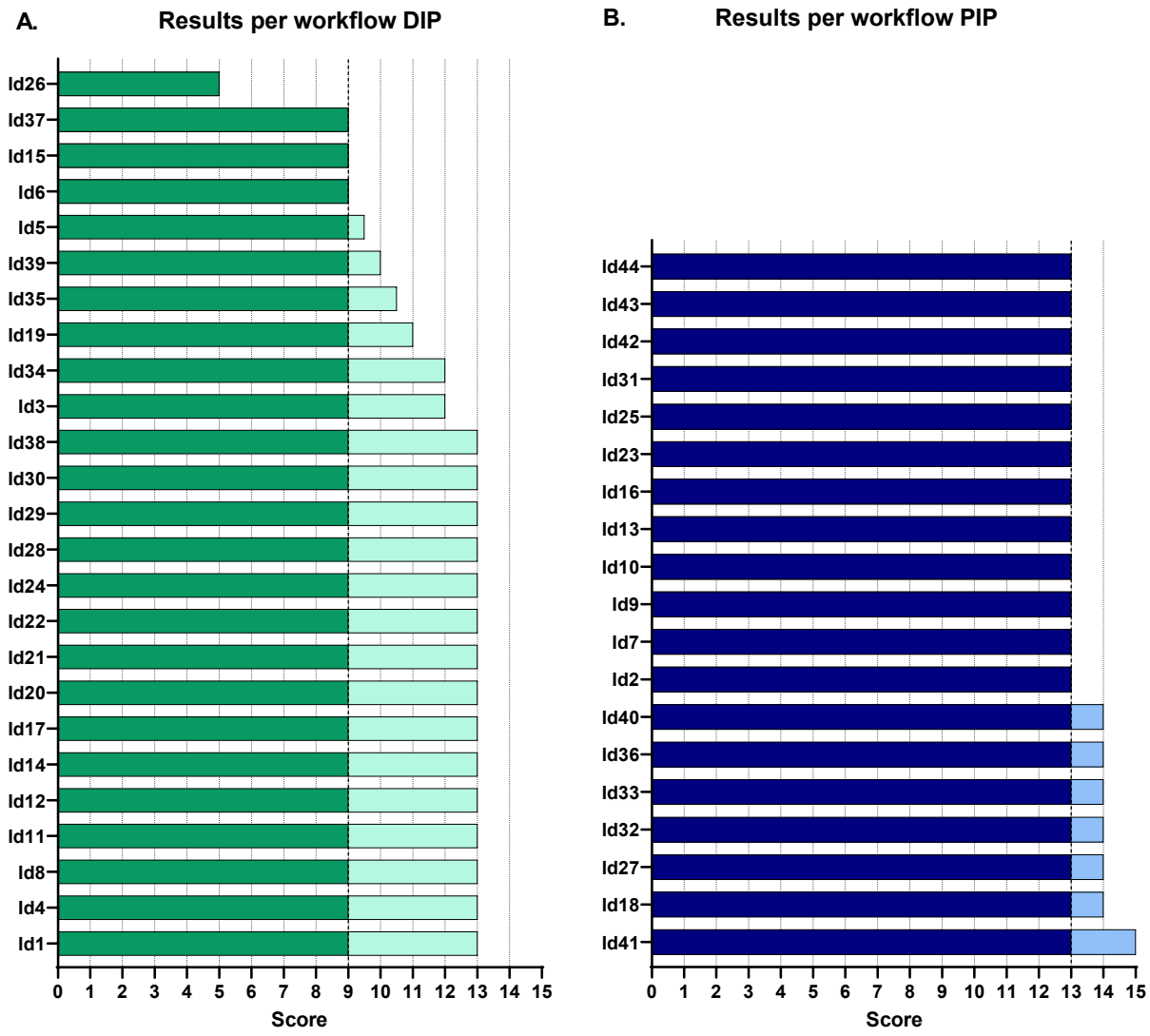


Figure 6. Overview of scoring for each workflow (Id) reported by sites, based on the detection of the core samples and educational samples present in the LEQA1 SARS-CoV-2 antigen panel. A distinction is made between the types of testing methods, either (A) dipping a swab in the sample (DIP; n=25) or (B) pipetting the sample (PIP; n=19). The panel contained 9 core- and 6 educational samples for DIP and 13 core- and 2 educational samples for PIP. The sites were given the option to evaluate samples with the following terms: Positive, Weak positive, Negative, or Inconclusive. The performance of each reported workflow was evaluated after which they were scored on a scale from 0 to 15. Workflows were given 1 point for each correct results and 0.5 points for an "Inconclusive" result. A workflow scoring 9 out of 15 points for DIP performs according to the national average. For PIP, a workflow scoring 13 out of 15 points performs according to the national average. A maximum score of 15 points could be achieved.

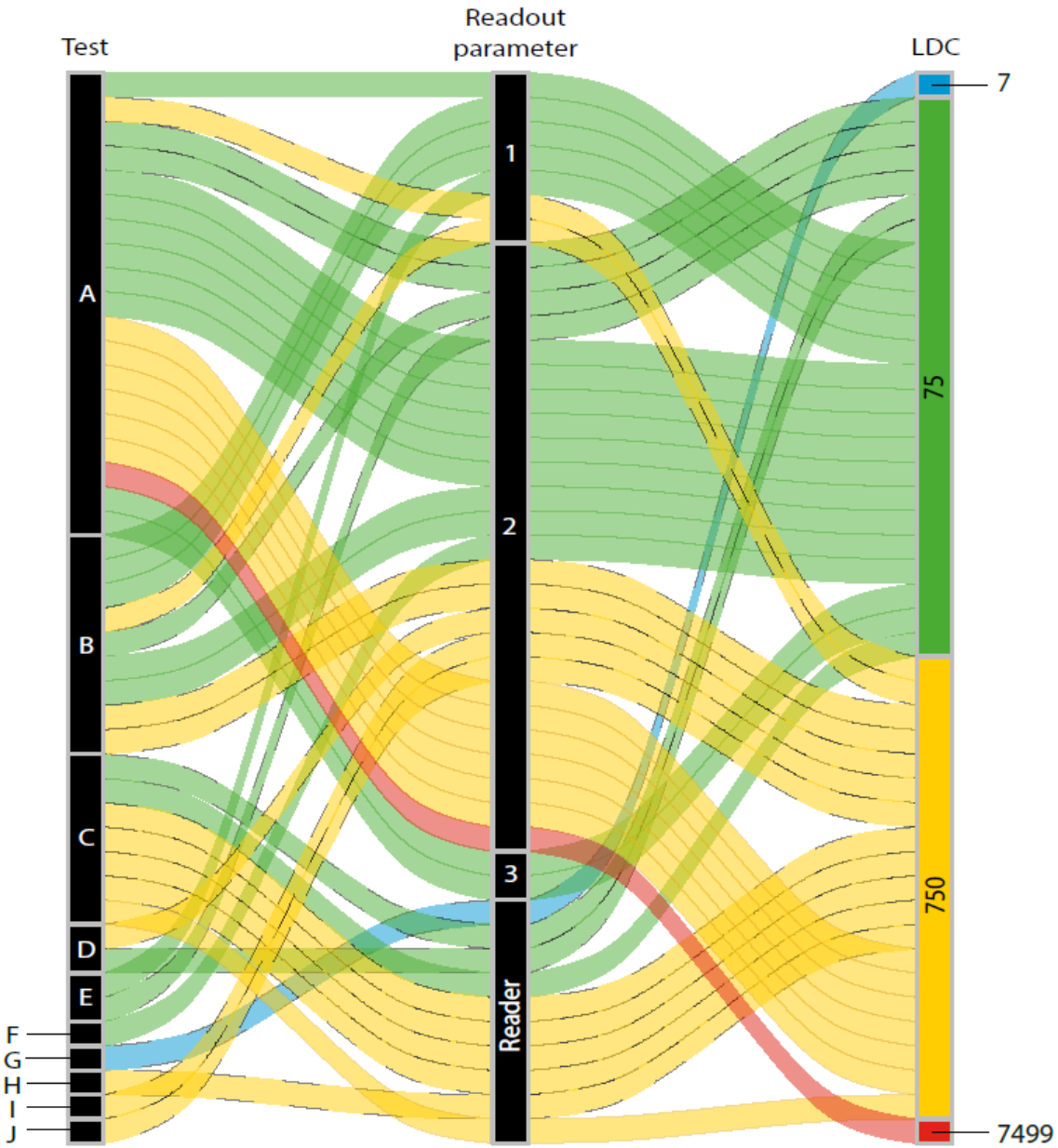


Figure 7. A flow diagram showing all workflows reported to have tested the LEQA SARS-CoV-2 antigen panel. The test brand, method of read-out and Lowest Detected Concentration (LDC; TCID50/ml) are connected for each workflow. Color of trails per workflow are based on the reported LDC of the SARS-CoV-2 B.11 19A (WT) dilution series. To correct for the lower sample volume used in the DIP method compared to PIP, the reported LDC was divided by 10 for the DIP workflows. The workflows using PIP are indicated with dashed lines.

A : Abbott - Panbio COVID-19-Ag rapid test;
B : Roche - SARS-CoV-2 Rapid Antigen Test;
C : Diasorin - Liaison SARS-CoV-2 Ag;
D : Becton Dickinson - BD Veritor COVID test;
E : Biozek - Covid-19 antigen rapid test cassette;
F : Siemens Healthineers - Clinitest Rapid COVID-19 Antigen test;
G : Siemens Healthineers - Atellica IM CoV2Ag;
H : SD Biosensor - Standard F-Covid-19 Ag;
I : GR Biotech - Rapid Test Swab (Shenzhen Lvshiyuan Biotechnology Co., Ltd.);
J : GR Biotech - Saliva test (Shenzhen Lvshiyuan Biotechnology Co., Ltd.)

1 : Test results are visually interpreted by 1 technician;
2 : Test results are visually interpreted independently by 2 technicians;
3 : Test results are visually interpreted independently by 3 technicians;
Reader: test results are interpreted by a reader

4. Discussion and conclusion

Overall the SARS-CoV-2 antigen test workflows (lateral flow assay-based RATs and Chemiluminescence Immunoassay-based high-throughput assays) used for SARS-CoV-2 diagnostics in the Netherlands perform well. Out of the 44 workflows reported, 43 scored a 100% correct score for the core samples of the LEQA1 SARS-CoV-2 antigen panel. Overall, all reported test brands are able to correctly identify SARS-CoV-2 in the core samples. Furthermore, there is no difference in sensitivity (based on the dilution series of the four SARS-CoV-2 strains) of the tests between the SARS-CoV-2 variants, including the VOC.

A significant amount of workflows - 80% (20/25) workflows using DIP and 36.8% (7/19) workflows using PIP - were also able to correctly identify educational samples of the panel (containing SARS-CoV-2 concentrations around the LOD of the respective tests). The results of the LEQA1 SARS-CoV-2 antigen could not identify a single factor explaining the variation in reported LDC between workflows, since variation in LDC occurs between workflows using the same test brand and similar read-out methods (number of independent visual interpretations or reader). The variation in reported LDC between the workflows using visual interpretation of colloidal gold-based lateral flow assays indicate that probably training of personnel to interpret results is important for consistent assessment of samples containing relatively low concentrations of SARS-CoV-2 virus.

It should be noted that this LEQA1 SARS-CoV-2 antigen panel focused on the analytical sensitivity of the SARS-CoV-2 antigen test workflows. The performance of the workflows on specificity, thus false-positive results due to cross-reactivity with other viruses or contaminants in clinical samples, was not specifically assessed in this LEQA. The only negative sample in the panel contained no virus and was interpreted correctly negative by 100% of the workflows. The absence of cross-reactivity with multiple other viruses has been assessed and validated by the manufacturers of these tests. Although the LEQA1 antigen panel samples consists of simulated clinical samples, actual clinical samples might contain more contaminants that may cause a very weak signal in the tests. Correctly discriminating between SARS-CoV-2-specific weak signals or aspecific weak signals is challenging for workflows. This aspect can only be addressed with (large) real-live clinical validation studies performed at the testing sites.

Overall we can conclude that the SARS-CoV-2 antigen test workflows used for SARS-CoV-2 diagnostics for the Dutch population perform well and that the currently used SARS-CoV-2 antigen tests are a reliable diagnostic tool.

5. References

1. RIVM. Varianten van het coronavirus SARS-CoV-2. 2021 [cited 2021 31-3-2021]; Available from: https://www.rivm.nl/coronavirus-covid-19/virus/varianten.

6. Participating testing sites

All participating testing sites and the responsible laboratories are listed below. We would like to thank colleagues from these sites for their participation in the SARS-CoV-2 antigen test LEQA.

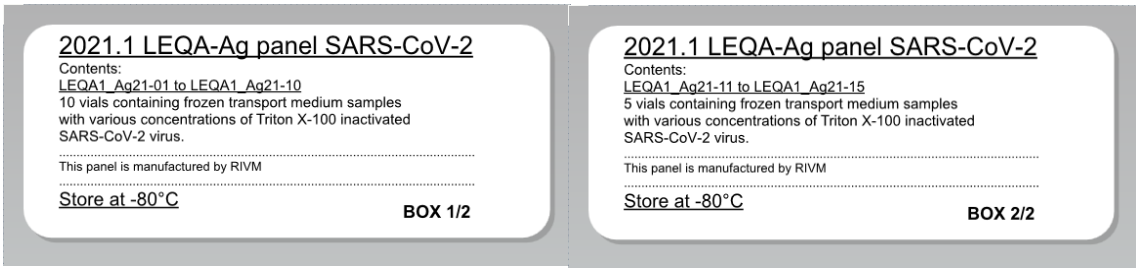
Admiraal de Ruyter Ziekenhuis, Goes
Amphia ziekenhuis, Breda
Bravis ziekenhuis, Roosendaal
Cansius Wilhelmina ziekenhuis, Nijmegen
Centraal Bacteriologisch en Serologisch Laboratorium, Hilversum
Certe, Groningen
COMICRO, Hoorn
Diakonessenhuis, Utrecht
Elisabeth Tweesteden Ziekenhuis, Tilburg
Erasmus MC, Rotterdam
Gelderse Vallei Ziekenhuis, Ede
Gelre ziekenhuizen, Apeldoorn
GGD Drenthe
GGD IJsselland - IJsselhallen, Zwolle
GGD West Brabant test straat, Bergen op Zoom
GGD West Brabant test straat, Breda
GGD WTC Heliconweg, Leeuwarden
GGD XL AHOY, Rotterdam
GGD XL Rotterdam The hague Airport, Rotterdam
GGD XL-teststraat, Utrecht
Isala, Zwolle
Izore, Leeuwarden
Jeroen Bosch Ziekenhuis, 's-Hertogenbosch
Laboratorium Microbiologie Twente en Achterhoek, Hengelo
Langeland ziekenhuis, Zoetermeer
Medisch Centrum Leeuwarden, Leeuwarden
Microvida, Breda
Nij smellinghe, Drachten
Ommelander Ziekenhuis Groningen, Scheemda
Reinier de Graaf Gasthuis, Delft
Reinier Haga Medisch Diagnostisch Centrum, Delft
Sportgeneeskunde Friesland, Heerenveen
Stichting PAMM, Veldhoven
Streeklaboratorium v.d. Volksgezondheid Kennemerland, Haarlem
Treant Zorggroep Scheper ziekenhuis, Emmen
UMC Utrecht
Zuyderland MC, Heerlen

Gebruiksaanwijzing
Landelijk External Quality Assessment (LEQA) panel voor SARS-CoV-2 antigeen sneltesten

Bewaar het panel bij -80 ° C of lager tot het moment van testen

Inhoud

Blind LEQA-panel: 15 gesimuleerde klinische monsters (1 ml) die Triton X-100 geïnactiveerde SARS-CoV-2-virussen ofwel geen virus in viraal transportmedium GLY bevatten.



Deze monsters kunnen op 2 manieren behandeld worden. De monsters moeten binnen 4 uur na het ontdooien gebruikt worden!

1) ***Behandelen door middel van pipetteren.*** Ontdooi de monsters op kamer temperatuur, waarna de inhoud goed mengen door te vortexen of over lengte van het epje te zwenken. Volg de instructies die meegeleverd zijn met de antigeen sneltest met als enige verschil dat (i.p.v. een swabs) 350 µl van het monster bij de antigeen sneltest buffer wordt gepipetteerd. (circa 1:1 menging). Dit geldt ook voor high-throughput systemen.
2) ***Behandelen door middel van een swab.*** Ontdooi de monsters op kamer temperatuur, waarna de inhoud goed mengen door te vortexen of over lengte van het epje te zwenken. Dip de swab (alleen het uiteinde waar de vezels zitten) in het monster. Roteer de swab gedurende 10 seconden in het monster. Volg vanaf hier de instructies die meegeleverd zijn met de antigeen sneltest. Dit geldt ook voor high-throughput systemen.

Verzoek aan de laboratoria en testlocaties
Gebruik voor de detectie van SARS-CoV-2 in het LEQA-panel de antigeen sneltest die u hebt geïmplementeerd voor detectie van SARS-CoV-2 in klinische monsters. U bent vrij om uit de behandelingen van de monsters te kiezen zoals die hierboven zijn beschreven.

Rapportage van de resultaten
Wij verzoeken u vriendelijk om de resultaten van de panels, inclusief informatie over de gebruikte antigeen sneltest en overige condities ter evaluatie aan ons te rapporteren, uiterlijk 26 maart 2021. Rapporteer uw bevindingen zo snel mogelijk na ontvangst van de panels via de online module die beschikbaar is via deze link: https://www.formdesk.nl/rivm/reporting_form_LEQA1_antigen_test.
We verzoeken u om foto's te maken van de antigeen sneltest resultaten en deze in het bijgeleverde PowerPoint document te plaatsen: 'Enter name location and antigen test_LEQA1_Antigen_test_results.ppt' bijvoorbeeld: 'GGD_XL-teststraat_Utrecht_Roche_LEQA1_Antigen_test_results.ppt'. Dit document kan geüpload worden in formdesk. We verwachten afzonderlijk voor elk type antigen sneltest en elke methode monsterbehandeling die u gebruikt resultaten te ontvangen. Let op: bij het invullen van het online formulier kunnen vragen worden toegevoegd of verwijderd, afhankelijk van uw eerdere antwoorden. Het volledige formulier bestaat uit een grotere set vragen, waarvan u slechts een kleine deelverzameling hoeft te beantwoorden, afgestemd op uw specifieke antigeen sneltest.

Feedback over resultaten
U ontvangt de decodering van het panel met verwachte resultaten zo spoedig mogelijk na afsluiting van de oefening op 26 maart 2021. Een rapport met de geanonimiseerde resultaten van alle deelnemende laboratoria en testlocaties volgt nadat alle gegevens zijn verwerkt. Indien mogelijk, zullen de resultaten gebruikt worden om een publicatie over onze bevindingen te schrijven.

Vragen
Heeft u vragen, neem dan contact met ons op via COVID-19.testen@rivm.nl. Voor vragen die direct beantwoord moeten worden, kunt u Wanda Han bellen op + 31- (0)6 3111 5605

Verwachte resultaten LEQA1 Antigeen panel

Het landelijke External Quality Assessment panel voor SARS-CoV-2 antigeen sneltesten bestaat uit 4 verschillende SARS-CoV-2 stammen, namelijk: hCoV-19/Netherlands/NoordBrabant_10003/2020 (B.11, 19A), hCoV-19/Netherlands/GE-RIVM-20300/2020 (B.1.177, 20AEU1), hCoV-19/Netherlands/NH-RIVM-20432/2020 (B.1.1.7, 20B/501Y.V1) en hCoV-19/Netherlands/NB-RIVM-20274/2020 (B.1.5, 20A). Deze stammen zijn geïnactiveerd door middel van 2 uur incubatie met Triton X-100 op kamertemperatuur. De kwaliteitscontrole is als volgt uitgevoerd op het RIVM: 6 analisten hebben 6 verschillende SARS-CoV-2 sneltesten (Roche SARS-CoV-2 Rapid Antigen Test/SD Biosensor Standard Q COVID-19 Ag, Abbott Panbio COVID-19 Ag RAPID TEST DEVICE, Quidel Sofia2 SARS- Antigen FIA, Healgen Coronavirus Ag Rapid Test Cassette, BD Veritor System For Rapid Detection of SARS-CoV-2 en SD Biosensor Standard F COVID-19 Ag FIA) in 3-voud ingezet met het LEQA panel. Vervolgens heeft 1 analist de uitslag van de SARS-CoV-2 sneltesten beoordeeld en direct opvolgend heeft de eindverantwoordelijke van de kwaliteitscontrole als extra controle de uitslag van de SARS-CoV-2 sneltesten onafhankelijk beoordeeld. De Diasorin LIAISON® SARS-CoV-2 Ag assay is extern uitgevoerd met het panel, we willen het laboratorium van Elisabeth Tweesteden Ziekenhuis bedanken voor het leveren van deze resultaten.

Tabel 1. LEQA1 Antigeen panel samenstelling en resultaten bij **het pipetteren van 350 µl panelmonster** in Roche SARS-CoV-2 Rapid Antigen Test/SD Biosensor Standard Q COVID-19 Ag, Abbott Panbio COVID-19 Ag RAPID TEST DEVICE, Quidel Sofia2 SARS- Antigen FIA, Healgen Coronavirus Ag Rapid Test Cassette, BD Veritor System For Rapid Detection of SARS-CoV-2, SD Biosensor Standard F COVID-19 Ag FIA en Diasorin LIAISON® SARS-CoV-2 Ag assay. Bij de colloidal-goud-gebaseerde antigeen testen, zijn de resultaten tevens beoordeeld op de intensiteit van de teststreep (weergegeven in de 1e kolommen onder elke test). Aan een intensere kleuring van de teststreep is een sterker signaal gekoppeld. Alle Sars-CoV-2 antigeen sneltesten zijn in 3-voud per panelmonster uitgevoerd; naast de signaalsterkte van de teststreep staat tussen () het aantal keren positief (weergegeven in de 2e kolommen onder elke test). Voor SD Biosensor Standard F COVID-19 Ag FIA en Diasorin LIAISON® SARS-CoV-2 Ag assay is kwalitatief resultaat positief of negatief weergegeven, het aantal keren positief van getest tussen () en de range aan gemeten fluorescentiewaarden.

Panel codering	SARS-Cov-2 Stam	Concentratie TCID50/ml	qRT-PCR Ct-waarde[1]			Roche		Abbott		Quidel[2]		Healgen		BD		SD-Biosensor F[2,3]			LIAISON[2,4]		
			E-gen	RdRp-gen	N1-gen																
LEQA1_Ag21-01	B.1.177 20A.EU1	1334	31.4	23.2	25.6	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	46.77-52.41	+	(3/3)	9869-10088
LEQA1_Ag21-02	B.11; 19A; wildtype	7499	29.1	21.1	23.7	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	30.02-37.33	+	(3/3)	11128-12791
LEQA1_Ag21-03	B.1.1.7 20B/501Y.V1	23714	30.7	24.4	26.0	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	57.51-58.58	+	(3/3)	57365-69663
LEQA1_Ag21-04[5]	B.11; 19A; wildtype	75	35.3	28.1	30.4	+-	(3/3)	+-	(2/3)	+	(3/3)	+-	(3/3)	+-	(3/3)	-	(0/3)	0.22-0.40	-	(0/2)	157-161
LEQA1_Ag21-05	B.1.1.7 20B/501Y.V1	237	36.1	30.8	32.7	+	(3/3)	+-	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	3.89-4.58	+	(3/3)	528-583
LEQA1_Ag21-06	B.1.177 20A.EU1	13335	28.1	19.9	22.5	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	57.94-58.30	+	(3/3)	88940-91785
LEQA1_Ag21-07	B.1.5 20A	2371	32.1	23.9	26.9	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	52.31-53.60	+	(3/3)	5520-9847
LEQA1_Ag21-08	B.1.177 20A.EU1	133	33.7	26.7	29.1	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	5.59-10.27	+	(3/3)	1043-1091
LEQA1_Ag21-09	Negatief					-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	0.12-0.28	-	(0/3)	<22
LEQA1_Ag21-10	B.1.1.7 20B/501Y.V1	2371	34.3	27.7	29.1	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	53.37-59.97	+	(3/3)	4570-4730
LEQA1_Ag21-11	B.11; 19A; wildtype	7		31.2	33.6	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	0.15-0.33	-	(0/3)	27-40
LEQA1_Ag21-12	B.1.5 20A	23714	28.9	20.6	23.6	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	75.01-75.31	+	(3/3)	90632-93767
LEQA1_Ag21-13	B.11; 19A; wildtype	750	32.4	24.7	27.2	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	4.77-5.30	+	(3/3)	1407-1452
LEQA1_Ag21-14	B.1.5 20A	237	35.4	27.4	30.1	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	10.20-11.56	+	(3/3)	1081-1127
LEQA1_Ag21-15	B.11; 19A; wildtype	74989	26.1	18.1	20.9	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	73.38-75.13	+	(3/3)	>100000

Voetnoot volgende pagina

Bij colloidal-goud-gebaseerde antigeen testen voor visuele inspectie: ++ = Sterk positief, de intensiteit van de teststreep is sterker dan controle streep; + = Positief, de intensiteit van de teststreep is gelijkwaardig als controle streep; +- = Zwak positief, de intensiteit van de teststreep is lichter dan controle streep; - = Negatief, er is geen teststreep aanwezig.

[1] De Ct waarden zijn een gemiddelde van de 3-voud bepaling door middel van real-time RT-PCR met Fast-Virus Mastermix na extractie van 200 µl op MagNApure 96 met total nucleic acid kit small volume, elutie in 50 µl en 5 µl extract per RT-PCR reactie.

[2] Deze SARS-CoV-2 antigeen sneltesten worden afgelezen m.b.v. een apparaat die fluoroscentie afleest (resultaten weergegeven in 3^{e} kolommen onder de test). Quidel met Sofia2; SD-Biosensor met F2400; LIAISON met LIAISON® XL

[3] De threshold van de SD Biosensor Standard F COVID-19 Ag FIA is een COI waarde van 1.0.

[4] De threshold van de Diasorin LIAISON® SARS-CoV-2 Ag assay is 200 RFU.

[5] Bij herhalingen van dit monster kan de SARS-CoV-2 antigeen sneltest negatief zijn. Voorlopige indicatie: educatief monster. Na binnenkomst van resultaten van alle laboratoria kan een definitieve status toegekend worden.

Tabel 2. LEQA1 Antigeen panel samenstelling en resultaten bij **het dippen van een swab in het panelmonster** waarna de swab volgens gebruiksaanwijzing gebruikt wordt in Roche SARS-CoV-2 Rapid Antigen Test/SD Biosensor Standard Q COVID-19 Ag, Abbott Panbio COVID-19 Ag RAPID TEST DEVICE, Quidel Sofia2 SARS- Antigen FIA, Healgen Coronavirus Ag Rapid Test Cassette, BD Veritor System For Rapid Detection of SARS-CoV-2, SD Biosensor Standard F COVID-19 Ag FIA en Diasorin LIAISON® SARS-CoV-2 Ag assay. Bij de colloidal-goud-gebaseerde antigeen testen, zijn de resultaten tevens beoordeeld op de intensiteit van de teststreep (weergegeven in de 1e kolommen onder elke test). Aan een intensere kleuring van de teststreep is een sterker signaal gekoppeld. Alle Sars-CoV-2 antigeen sneltesten zijn in 3-voud per panelmonster uitgevoerd; naast de signaalsterkte van de teststreep staat tussen () het aantal keren positief (weergegeven in de 2e kolommen onder elke test). Voor SD Biosensor Standard F COVID-19 Ag FIA en Diasorin LIAISON® SARS-CoV-2 Ag assay is kwalitatief resultaat positief of negatief weergegeven, het aantal keren positief van getest tussen () en de range aan gemeten fluorescentiewaarden.

Panel codering	SARS-Cov-2 Stam	Concentratie TCID50/ml	qRT-PCR Ct-waarde[1]			Roche		Abbott		Quidel[2]		Healgen		BD		SD-Biosensor F[2,3]			LIAISON[2,4]		
			E-gen	RdRp-gen	N1-gen																
LEQA1_Ag21-01	B.1.177 20A.EU1	1334	31.4	23.2	25.6	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	20.64-26.06	+	(3/3)	2155-2394
LEQA1_Ag21-02	B.11; 19A; wildtype	7499	29.1	21.1	23.7	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	6.34-7.19	+	(3/3)	2588-3150
LEQA1_Ag21-03	B.1.1.7 20B/501Y.V1	23714	30.7	24.4	26.0	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	55.99-57.65	+	(3/3)	13910-17918
LEQA1_Ag21-04	B.11; 19A; wildtype	75	35.3	28.1	30.4	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	0.11-0.13	-	(0/3)	44-51
LEQA1_Ag21-05	B.1.1.7 20B/501Y.V1	237	36.1	30.8	32.7	+-	(3/3)	+-	(3/3)	+	(1/3)	+-	(3/3)	+-	(3/3)	-	(0/3)	0.73-0.87	-	(0/3)	158-194
LEQA1_Ag21-06	B.1.177 20A.EU1	13335	28.1	19.9	22.5	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	57.66-58.03	+	(3/3)	16343-19638
LEQA1_Ag21-07	B.1.5 20A	2371	32.1	23.9	26.9	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	21.55-26.94	+	(3/3)	2034-2632
LEQA1_Ag21-08	B.1.177 20A.EU1	133	33.7	26.7	29.1	+-	(3/3)	+-	(3/3)	+	(2/3)	+-	(3/3)	+-	(3/3)	-	(0/3)	1.24-2.25	+	(3/3)	241-307
LEQA1_Ag21-09	Negatief					-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	0.04-0.16	-	(0/3)	<22
LEQA1_Ag21-10	B.1.1.7 20B/501Y.V1	2371	34.3	27.7	29.1	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	+	(3/3)	11.06-18.73	+	(3/3)	1138-1590
LEQA1_Ag21-11	B.11; 19A; wildtype	7		31.2	33.6	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	-	(0/3)	0.09-0.42	-	(0/3)	22-25
LEQA1_Ag21-12	B.1.5 20A	23714	28.9	20.6	23.6	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	73.48-74.37	+	(3/3)	21226-24026
LEQA1_Ag21-13[5]	B.11; 19A; wildtype	750	32.4	24.7	27.2	+-	(3/3)	+-	(3/3)	+	(2/3)	+-	(3/3)	+-	(3/3)	-	(0/3)	0.52-0.75	+	(2/2)	323-356
LEQA1_Ag21-14	B.1.5 20A	237	35.4	27.4	30.1	+-	(3/3)	+-	(3/3)	+	(2/3)	+-	(3/3)	+-	(3/3)	+	(3/3)	1.22-1.58	+	(3/3)	243-314
LEQA1_Ag21-15	B.11; 19A; wildtype	74989	26.1	18.1	20.9	++	(3/3)	++	(3/3)	+	(3/3)	++	(3/3)	++	(3/3)	+	(3/3)	70.86-72.19	+	(3/3)	25339-33854

Voetnoot volgende pagina

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[1] De Ct waarden zijn een gemiddelde van de 3-voud bepaling door middel van real-time RT-PCR met Fast-Virus Mastermix na extractie van 200 µl op MagNApure 96 met total nucleic acid kit small volume, elutie in 50 µl en 5 µl extract per RT-PCR reactie.

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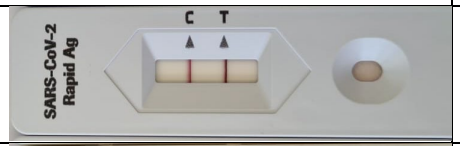
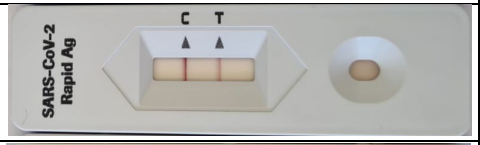
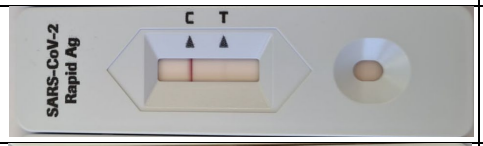
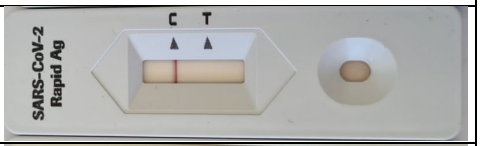
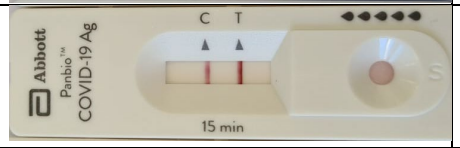
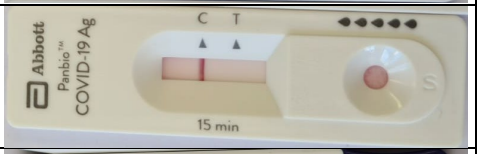
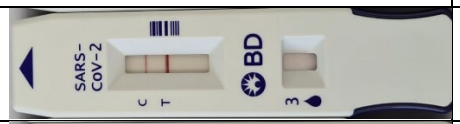
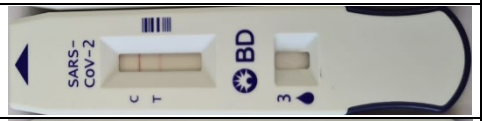
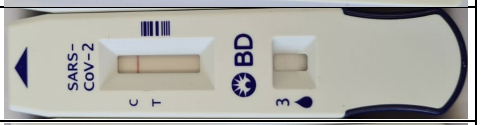
Tabel 3. Visuele beoordeling van 4 SARS-CoV-2 antigeen sneltest. De resultaten en beoordeling die word weergegeven zijn van de volgende panelmonsters; ++ = LEQA1_Ag21-06, + = LEQA1_Ag21-14, LEQA1_Ag21-04 en - = LEQA1_Ag21-09

Type antigeen sneltest	++	+	+-	-
Roche				
Abbott				
BD				
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