National Institute for Public Health
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Ministry of Health, Welfare and Sport

External Quality Assessment of laboratories Performing SARS-CoV-2 Diagnostics for the Dutch Population, February 2021

Colophon

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AM coordinated the project. AM, GG and JS conceptualized the study, developed and produced the panels and reporting system. SvdB and LW performed reference PCR analysis. JS performed the analysis of the reported data and wrote the initial version of the report. AM critically reviewed the data analysis and report and JS together with AM wrote the final version.

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Contents

Version 3 20-05-2021: errata compared to version 2:
The number of copies of SARS-CoV-2 RNA was updated due to an error in calculations. This does not affect the quality of the PCR tests used for diagnostics. We know that the majority of them perform with the highest sensitivity possible from comparison to each other using dilution series of SARS-CoV-2. Changes made on page: 7, 8, 21, 22, 23, 24 and 32.

Version 2 7-4-2021: errata compared to version 1:
Page 4: Changed number (16>14) of laboratories scoring a grade of 6.5 and below.
Page 10: Changed number (10>16) of laboratories using two workflows scoring grade 8.
Page 36: Changed number (16>14) of laboratories scoring a grade of 6.5 and below.

Summary

Background

Since January 2020 many workflows for molecular diagnostics of SARS-CoV-2 were implemented and checked for performance using specificity and sensitivity panels distributed by the National Institute for Public Health and the Environment (RIVM). Although panels have been largely similar in load components for checking SARS-CoV-2 Nucleic Acid Amplification Tests (NAAT) performance, they were not exactly similar in constituents. Because of this heterogeneity in the past, the fact that patchy quality checks were implemented only when workflows change or laboratories were added to the network, and because 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') that the performance of the network of COVID-19 molecular diagnostic labs is checked as a whole regularly, a National External Quality Assessment (EQA) (Landelijk EQA; LEQA) program has been developed. This program consists of three rounds of LEQA. This report includes the second round of the LEQA program.

Objective

The goal of this LEQA round is to inventorize the quality of the Dutch SARS-CoV-2 diagnostics field, using a panel that consists of 10 simulated clinical specimens, containing heat inactivated SARS-CoV-2, including one variant of concern (VOC) B.1.1.7; 20B/501Y.V1, or other respiratory viruses or genetic material. Each of the laboratories was asked to conduct molecular detection of SARS-CoV-2 according to their workflows normally used for SARS-CoV-2 diagnostics.

Materials and Methods

In January 2021 the LEQA panel was produced and pre-tested at the RIVM. After obtaining the correct results per sample, all laboratories performing SARS-CoV-2 diagnostics in the Dutch network were contacted and notified of the distribution of the panel in the first week of February 2021. A number of workflows, especially the molecular point of care (mPOCT) ones, use expensive cartridges or pouches of which laboratories only receive a limited number every week. Therefore laboratories with limited resources that wanted to test their workflows were asked to indicate so. Then they were sent an email to limit testing for these workflows to samples 3, 5, 8 and 9. Laboratories were asked to report their results via an online form. Workflows were given a score of 8 for 100% correct results for the 8 core samples and reduced by 1 point per sample for an incorrect result and 0.5 points for a result "Indeterminate", "Equivocal" or "Inconclusive" for a core SARS-CoV-2 positive sample. When a workflow tested the reduced panel (containing samples 3, 5, 8 and 9), 2 points per sample for an incorrect result and 1 point for a result "Indeterminate", "Equivocal" or "Inconclusive" for a sample were subtracted from the maximum score.

Results

Out of 180 workflows reported, 148 scored a 100% correct score for all 8 core specimens (8 points) and thus met all criteria set for reliable SARS-CoV-2 diagnostics, 18 scored between 7-7.5, making it likely that only minor adjustments need to be made to meet all criteria and 14 workflows scored a 6.5 or lower, indicating that a lot of improvements still need to be made for these workflows to be reliable for SARS-CoV-2 diagnostics in clinical diagnostic settings and surveillance. For the SARS-CoV-2 negative core samples one false positive result was reported and two workflows gave indeterminate, equivocal or inconclusive results for non-SARS-CoV-2 containing samples. For the SARS-CoV-2-containing core samples, false negative results (n=23) and false indeterminate, equivocal or inconclusive results (n=12) were reported. Some workflows reported a negative result for SARS-CoV-2 presence in SARS-CoV-2-

containing samples due to cut-off values used in the assay; up to 1.5% of each SARS-CoV-2 positive sample (range: 0% – 1.5%) was reported as SARS-CoV-2 negative despite one (or more) of the target genes against SARS-CoV-2 in the assay giving a Ct value. The VOC was detected by 176/177 (99.4%) workflows. Despite the wide variety of kits, equipment and enzymes that are used in the implemented workflows, the influence on the quality of molecular diagnostics for SARS-CoV-2 was limited.
Compared to the results obtained during the first round of quality control in this program (LEQA1), no difference in overall quality of the SARS-CoV-2 diagnostic laboratory network was detected.

Conclusions
Overall the workflows used for SARS-CoV-2 diagnostics perform very well and laboratories using them provide a reliable network. A small number of workflows should be further optimized to achieve full potential. The Dutch SARS-CoV-2 diagnostics laboratory network performs on a very high level with the vast majority of workflows detecting the core SARS-CoV-2 containing specimens correctly, including VOC B.1.1.7; 20B/501Y.V1 that has become the major strain (up to 82%) in circulation by week 9/2021 in The Netherlands. The wide variety of kits, equipment and enzymes used in the Dutch SARS-CoV-2 diagnostic field do not affect adversely the quality of diagnostics. Instead, it allows for great flexibility during times of shortages in supplies and likely improves the capacity to detect possible future variants of SARS-CoV-2.

1. Introduction

Since January 2020 many workflows for molecular diagnostics of COVID-19 were implemented and checked for performance using specificity and sensitivity panels distributed by the National Institute for Public Health and the Environment (RIVM). Although panels have been largely similar in load components for checking SARS-CoV-2 Nucleic Acid Amplification Tests (NAAT) performance, they rapidly varied from SARS-CoV-1 RNA initially, to SARS-CoV-2 RNA, followed by SARS-CoV-2 whole heat inactivated virus particles, depending on when materials became available. Because of this heterogeneity in the past, the fact that patchy quality checks were implemented only when workflows changed or laboratories were added to the network, and because 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') that we check the performance of the network of COVID-19 molecular diagnostic labs as a whole regularly, a National External Quality Assessment (EQA) (Landelijk EQA; LEQA) program has been developed. This program consists of three rounds of EQA. In the first week of February 2021 the second round of EQA panels was distributed to all laboratories performing SARS-CoV-2 diagnostics on clinical samples derived from Dutch patients. This panel consisted of 10 simulated clinical specimens that contained either heat inactivated SARS-CoV-2, including one variant of concern (VOC) B.1.1.7; 20B/501Y.V1 that has become the major strain (up to 82%) in circulation by week 9/2021 in The Netherlands [1], or other respiratory viruses or genetic material. Each of the laboratories was asked to conduct molecular detection of SARS-CoV-2 on this panel 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

Except for the VOC containing sample the LEQA panel was produced at the RIVM in October 2020 and pretested at the RIVM and Erasmus MC. Both centers obtained similar results. In January 2021 aliquoted samples for LEQA2 were thawed and analysed again at RIVM. This confirmed that the samples were of unchanged quality. The sample containing the VOC was prepared and pretested at RIVM in January 2021. All 85 laboratories excluding the RIVM performing SARS-CoV-2 diagnostics in the Dutch network by January 2021 were contacted and notified of the distribution of the panel in the first week of February. All laboratories were asked to report their findings using an online form using Formdesk software (Wassenaar, The Netherlands) to allow for a more streamlined method of data collection. Laboratories had until the 21st of February to report their obtained results. After the 21st of February laboratories that had not reported their results yet were given one week grace time to report their results, after which the submission was closed on the 28th of February. A number of workflows, especially the molecular point of care (mPOCT) ones, use expensive cartridges or pouches of which laboratories only receive a limited number every week. Therefore laboratories with limited resources that wanted to test their workflows were asked to indicate so. Then they were sent an email to limit testing for these workflows to samples 3, 5, 8 and 9 (Table 1), covering three different concentrations of SARS-CoV-2 of which one was the VOC and a hCoV-NL63 containing sample.

2.2 Contents of LEQA2 panel

The LEQA2 panel consisted of 10 simulated clinical specimens (1ml) containing either whole infectious human respiratory seasonal viruses, genetic material of relevant viruses or heat-inactivated SARS-CoV-2 viruses. SARS-CoV-2 was isolated from clinical specimens on VERO E6 cells and heat-inactivated by heat treatment at 60 °C for two hours. The number of detectable copies of SARS-CoV-2 positive strand RNA in the stocks of SARS-CoV-2 was back-calculated from determination of the copy number after

extraction of RNA by digital SARS-CoV-2 E-gene and RdRP-gene PCR. For hCoV-19/Netherlands/NoordBrabant_10003/2020 at 1.28*10^10 and 1.73*10^10 copies of E-gene and RdRP-gene positive strand RNA/ml, respectively. And for hCoV-19/Netherlands/NH-RIVM-20432/2020 at 3.39*10^9 and 2.32*10^9 copies of E-gene and RdRP-gene positive strand RNA/ml, respectively. Because the viruses were not purified from the supernatant, the whole virus preparation contains in addition to genomic RNA, intermediate replication negative strand genomic RNA and subgenomic E-gene RNA that contribute to detection in routine one-step RT-qPCR for SARS-CoV-2 RNA. Virus dilutions were made in MEM with Hanks' salts. HEp2 cells were added to the dilution at a concentration of 10.000 cells per ml panel sample to simulate a clinical sample. The 10 samples included in the panel contained the following viruses: SARS-CoV-2 (hCoV-19/Netherlands/NoordBrabant_10003/2020) in various concentrations, SARS-CoV-2 B.1.1.7/20B/501Y.V1 VOC-202012/01 (UK) variant (hCoV-19/Netherlands/NH-RIVM-20432/2020), hCoV-NL63 (kindly provided by Lia van der Hoek, Amsterdam University Medical Hospital), hCoV-229E (ATCC), hCoV-OC43 (ATCC), SARS-CoV-1 (RNA) (kindly provided by Bart Haagmans, Erasmus MC) and a sample without any virus. Except for the A(H3N2) containing sample in LEQA1 [2] that was replaced by the VOC SARS-CoV-2 in LEQA2, all other samples in LEQA1 and 2 had the same content, but in different order. In Table 1 all samples are listed together with the expected target specific Ct values obtained at RIVM with routinely used diagnostic RT-qPCRs for the respective pathogens and the expected conclusion for SARS-CoV-2 detection in the samples. The digital copies of RdRP-gene and E-gene are also listed in Table 1 for the SARS-CoV-2 containing samples.

Table 1: Composition of LEQA2 together with the target specific expected Ct values[1] based on the in-house assay(s) of the RIVM.

Panel coding	Virus[2]	Number of copies E gene target/ml specimen, determined with dPCR[3]	Number of copies RdRP gene target/ml specimen, determined with dPCR[3]	Target specific Ct[4]	E-gene (Sarbeco) Ct	RdRP-gene (SARS-CoV-2) Ct	Conclusion SARS-CoV-2
LEQA2_CoV20-1[5]	SARS-CoV-1	-	-	NA	28.57 (4/4)	Neg	Negative[5]
LEQA2_CoV20-2	SARS-CoV-2 (d1)	1.28*10^5	1.73*10^5	NA	28.52 (4/4)	28.37 (4/4)	POSITIVE
ELQA2_CoV20-3	hCoV-NL63	-	-	28.10 (4/4)	Neg	Neg	Negative
LEQA2_CoV20-4	hCoV-229E	-	-	17.22 (4/4)	Neg	Neg	Negative
LEQA2_CoV20-5[6]	SARS-CoV-2 (d4)	1.28*10^2	1.73*10^2	NA	36.95 (2/4)	35.59 (2/4)	Weakly POSITIVE
LEQA2_CoV20-6	SARS-CoV-2 (d3)	1.28*10^3	1.73*10^3	NA	34.80 (4/4)	34.88 (4/4)	POSITIVE
LEQA2_CoV20-7	hCoV-OC43	-	-	27.77 (4/4)	Neg	Neg	Negative
LEQA2_CoV20-8	SARS-CoV-2 (d3)	1.28*10^3	1.73*10^3	NA	34.68 (4/4)	34.74 (4/4)	POSITIVE
LEQA2_CoV20-9[7]	SARS-CoV-2 VOC	3.39*10^5	2.32*10^5	NA	26.86 (4/4)	27.33 (4/4)	POSITIVE
LEQA2_CoV20-10	No virus	-	-	Neg	Neg	Neg	Negative

[1] The expected 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 4-fold; after the average Ct value between brackets () the number of times found positive is shown. 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] d1, d3 and d4 indicate that d3 is a 1:100 dilution of d1 and d4 is a 1:10 dilution of d3; SARS-CoV-2 is heat inactivated. SARS-CoV-1 is RNA stabilized with yeast tRNA.

[3] dPCR has been performed on + strand genomic RNA for RdRP-gene and E-gene; for E-gene, subgenomic messengers present are also detected. The one-step E-gene and RdRP-gene diagnostic RT-qPCR also detects - strand replicative form genomic RNA and the one-step E gene RT-qPCR in addition also detects subgenomic messengers, which probably increases the actual number of target templates for the diagnostic RT-qPCR in the sample after extraction.

[4] For hCoV-NL63 and hCoV-229E N-gene and hCoV-OC43 M-gene; n/a = not applicable.

[5] Educational specimen. Laboratories using only the Corman E-gene Sarbeco specific RT-PCR will epidemiologically rightly label this specimen as SARS-CoV-2 positive. The combination of low Ct with Sarbeco specific PCR and absence of positive signal with another SARS-CoV-2 target would prompt further research. One of the two targets positive with SARS-CoV-2 usually only occurs with very low viral load.

[6] Educational specimen: repeats of this specimen may have the E-gene and/or RdRP-gene negative; only 54.7% of reported workflows having tested this specimen reported this specimen positive for SARS-CoV-2

[7] SARS-CoV-2 variant B.1.1.7; 20B/501Y.V1 VOC-202012/01 (UK); TaqPath assay: S-dropout; TibMolbiol or other N501Y assay: 501Y variant positive. This is a core specimen.

2.3 Scoring the workflows

The performance of each reported workflow was evaluated after which they were scored on a scale from 0 to 8, with 8 being the best grade. This scoring system was implemented based on the detection of the core samples present in the panel. All samples except LEQA2_CoV20-1 and LEQA2_CoV20-5 (containing SARS-CoV-1 RNA and an educational load of SARS-CoV-2, respectively) were deemed core samples (samples with clinically relevant amounts of virus or no virus). The laboratories were given the option to evaluate samples with the following scores: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. Each workflow started with 8 points for all correct results for each of the 8 core samples. For each wrongly determined core sample (being positive for a sample containing no SARS-CoV-2 or vice versa) 1 point was deducted (out of 8). When a core sample was scored with an "Indeterminate", "Equivocal" or "Inconclusive" result, 0.5 point was deducted from the final mark of the workflow.

For some workflows (e.g. molecular point-of-care test (mPOCT) workflows) an option was given to test only a smaller subset of samples specimens in order to be able to make a limited statement about the sensitivity of detection of SARS-CoV-2 with the mPOCT used. These workflows only had to test LEQA2_CoV20-3, LEQA2_CoV20-5, LEQA2_CoV20-8 and LEQA2_CoV20-9. When notifying the Dutch SARS-CoV-2 diagnostics network, the laboratories were informed about the option to apply for a reduced (mPOCT) LEQA panel. Especially for these workflows the score "Not tested" was added as an option. The workflows testing the reduced panel were also graded according to a scale from 0 – 8 points. For each wrongly determined core sample (being positive for a sample containing no SARS-CoV-2 or vice versa) 2 points were deducted (out of 8). When a sample was scored with an "Indeterminate", "Equivocal" or "Inconclusive", 1 point was deducted from the final mark of the workflow.

When the entire panel was supposed to be tested using a workflow and a sample was given a score of "Not tested", 1 point was deducted from the final score for that workflow. This might have occurred when a laboratory used a second or more workflows for confirmation of some of the results in the first workflow used.

A workflow scoring 8 out of 8 passed all criteria set for SARS-CoV-2 diagnostics in terms of sensitivity and specificity deemed necessary for SARS-CoV-2 diagnostics in accordance with the set requirements for new workflows and laboratories. [3] Workflows scoring 7.5 or 7 out of 8 might still be valuable for SARS-CoV-2 diagnostics, but need adjustments in order to perform as desired. Adjustments depend on the type of result, e.g. an "Indeterminate", "Equivocal" or "Inconclusive" result for low viral load LEQA2_CoV20-6 or LEQA2_CoV20-8 samples is less severe than detection of SARS-CoV-2 targets in specimens which were SARS-CoV-2 negative (false positive). Any workflows scoring below 7 out of 8 points needs serious adjustments in order to be fit for SARS-CoV-2 diagnostics.

3. Results

3.1 Aggregated overview

Eighty-five laboratories were contacted with the announcement of panel distribution for this second EQA round. Seventy-nine (92.9%) of these laboratories reported their findings for 180 workflows. The workflow conclusions reported for each panel sample are summarized in Table 2 ('Not tested' result excluded). The panel scores obtained per laboratory and by number of workflows used are summarized in Table 3.

Table 2. Aggregated overview of workflow conclusions by LEQA panel sample.

Panel sample	Content	№ of workflows with test result reported (n=180)[1]	SARS-CoV-2 detection workflow conclusion			
			№ Positive	№ Indeterminate, Equivocal or Inconclusive	№ Negative	Errors
LEQA2_CoV20-1	SARS-CoV-1	161	70 (43.5%)	11 (6.8%)	80 (49.7%)	Not applicable, educational sample
LEQA2_CoV20-2	SARS-CoV-2 (d1)	160	160 (100%)	0	0	None
ELQA2_CoV20-3	hCoV-NL63	176	0	1 (0.6%)	175 (99.4%)	False indeterminate, equivocal or inconclusive (n=1)
LEQA2_CoV20-4	hCoV-229E	160	0	0	160 (100%)	None
LEQA2_CoV20-5	SARS-CoV-2 (d4)	179	98 (54.7%)	12 (6.7%)	69 (38.5%)	Not applicable, educational sample
LEQA2_CoV20-6	SARS-CoV-2 (d3)	162	147 (90.7%)	5 (3.1%)	10 (6.2%)	False indeterminate, equivocal or inconclusive (n=5), false negative (n=10)
LEQA2_CoV20-7	hCoV-OC43	160	0	0	160 (100%)	None
LEQA2_CoV20-8	SARS-CoV-2 (d3)	176	157 (89.2%)	7 (4.0%)	12 (6.8%)	False indeterminate, equivocal or inconclusive (n=7), false negative (n=12)
LEQA2_CoV20-9	SARS-CoV-2 VOC	177	176 (99.4%)	0	1 (0.6%)	False negative (n=1)
LEQA2_CoV20-10	No virus	160	1 (0.6%)	1 (0.6%)	158 (98.8%)	False positive (n=1), false indeterminate, equivocal or inconclusive (n=1)

[1] 15 of the 180 workflows tested were mPOCT testing samples 3, 5, 8 and 9 only. For 5 of 180 workflows laboratories made another limited selection of panel samples to test. Therefore the number of workflows with test result per sample is less than 180.

Table 3. Aggregated overview of scores for core specimens obtained by laboratories using various numbers of workflows.

№ of workflows per lab	№ of labs	№ of workflows per lab with indicated score (No of labs)		
		Score 8	Score 7 or 7.5	Score < 7
9	1	6 (n=1)	0	3 (n=1)
7	2	5-6 (n=2)	1 (n=2)	1 (n=1)
6	1	5 (n=1)	1 (n=1)	0
5	4	1-5 (n=4)	1-4 (n=2)	1 (n=1)
4	8	3-4 (n=8)	1 (n=2)	1 (n=1)
3	10	1-3 (n=10)	1 (n=3)	1 (n=2)
2	16	2 (n=16)	0	0
1	37	1 (n=26)	1 (n=5)	1 (n=6)

Despite not all workflows obtained fully correct results with the core specimens (Table 2), nearly all laboratories (73/79; 92.4%) used at least one workflow with which a score 7-8 was obtained (Table 3). All laboratories using two or more workflows had at least one workflow with which a score of 8 for fully correct results was obtained. Of the laboratories that used one workflow only 26/37 (70.3%) used a workflow with which a score of 8 was obtained**.** In the subsequent chapters a more detailed insight in the background of the results and the results themselves are presented.

3.1 Used volumes, equipment, kits and reagents

Because the sensitivity of a workflow is partly defined by the sample equivalent input volume in the RT-qPCR/other NAAT, a subset of questions revolved around the volumes used for testing of clinical samples for each specific workflow: volume specimen in nucleic acid extraction; elution volume; volume RNA/total NA in RT-qPCR reaction or other NAAT; end volume of RT-qPCR reaction or other NAAT. Figure 1 shows each of the volumes used for RNA isolation and RT-qPCR or other NAAT for all workflows for which results were reported. For those workflows for which extraction input, elution and RT-qPCR/other NAAT input volumes were reported the sample equivalent input volume in RT-qPCR/other NAAT reaction was calculated and plotted (Figure 1).

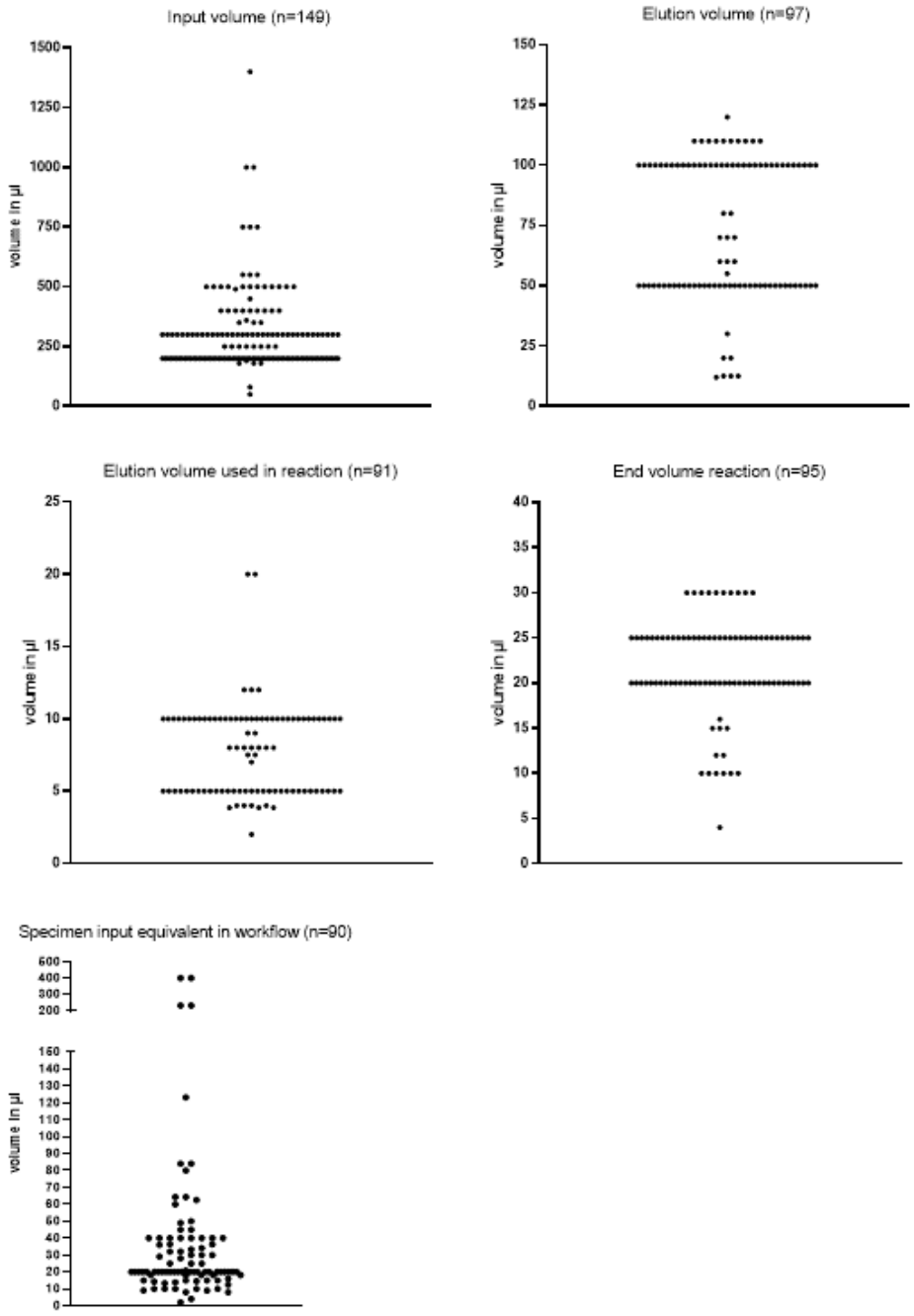


Figure 1: Volumes used in the RT-PCRs or other NAATs described for the workflows reported. Most commonly used volumes: 200 µl input volume; 50 µl elution volume; 10 µl elution input volume in reaction; 20 µl end volume of reaction; 20 µl specimen input equivalent.

Other factors that may determine the performance of the workflows are the used kits, equipment and/or separate enzymes used for extraction and amplification implemented in SARS-CoV-2 diagnostics for the Dutch population. Therefore for each workflow these details were inventoried. Figure 2 shows the kits used for RNA/total NA isolation, Figure 3 shows the RNA isolation equipment, Figure 4 shows the kits used for the RT-PCR or other NAAT reaction, Figure 5 shows the separate enzymes used for the in-house RT-PCR or other NAAT reaction and Figure 6 shows the equipment used for the RT-PCR or other NAAT reaction. In several occasions the kit used for extraction and for RT-qPCR or other NAAT has the same name because these are all-in-one kits.

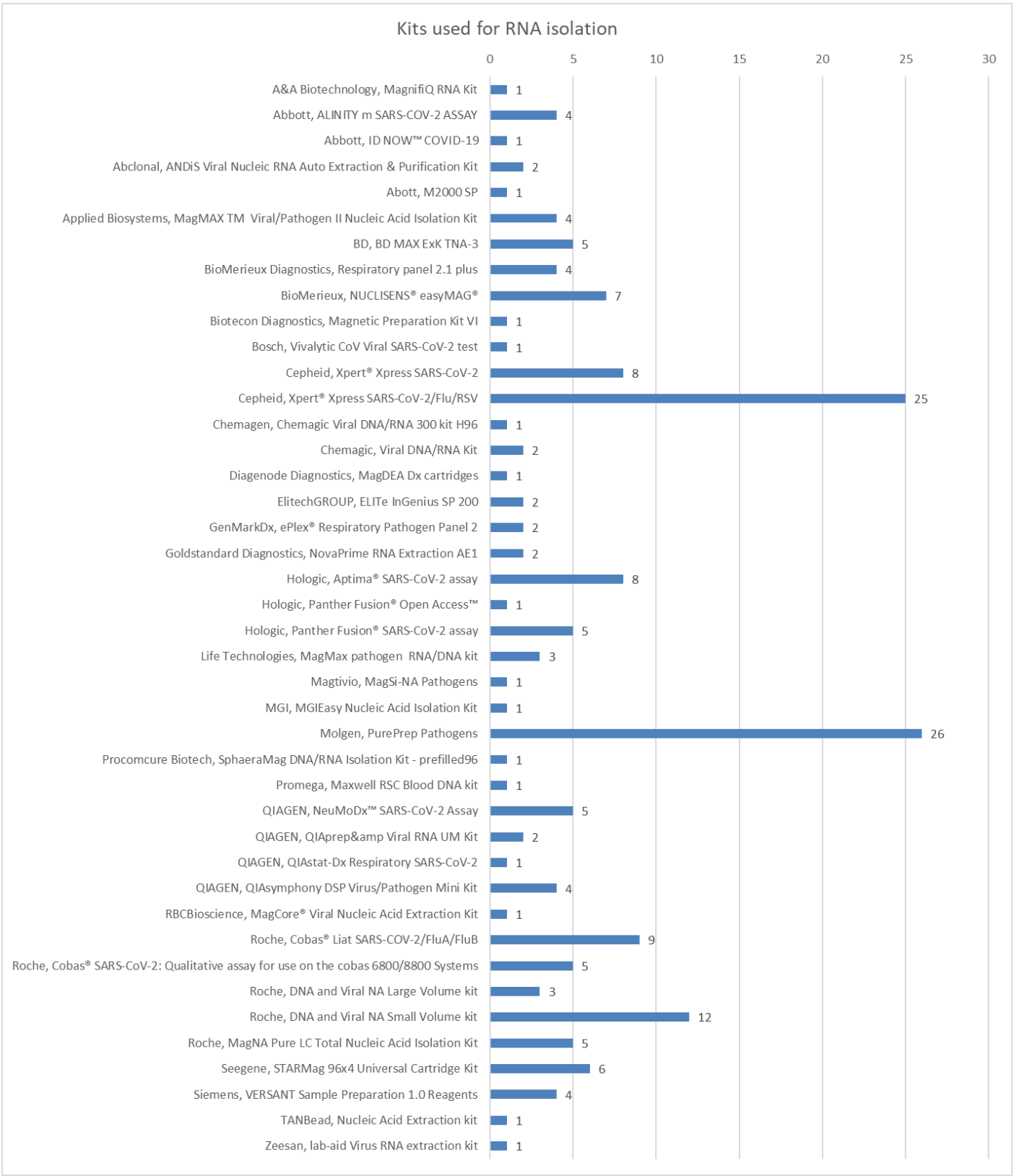


Figure 2: The RNA isolation kits used by workflows testing for SARS-CoV-2 together with the number of workflows per kit (n=180)

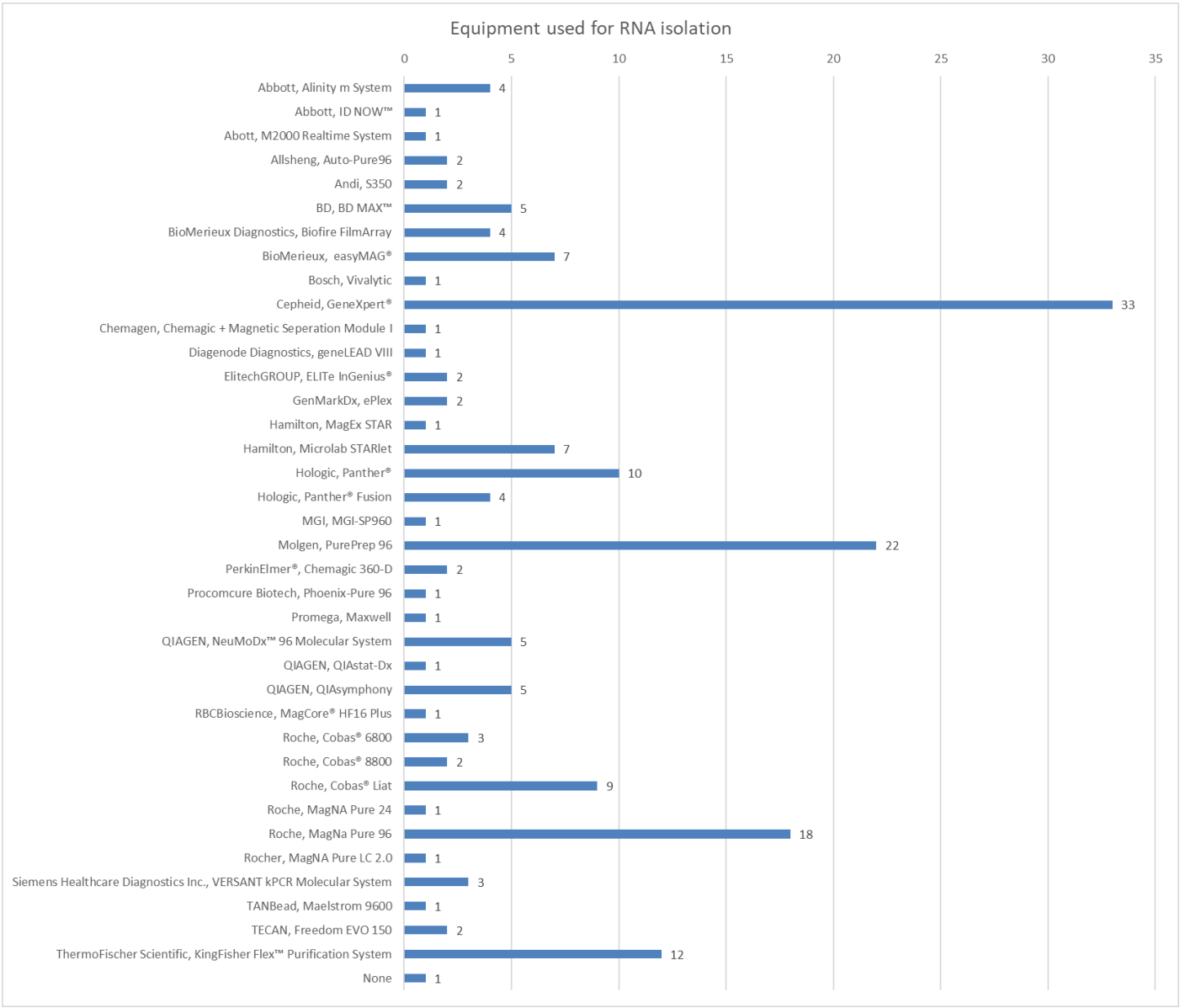


Figure 3: The RNA isolation equipment used by workflows testing for SARS-CoV-2 together with the number of workflows per machine (n=180)

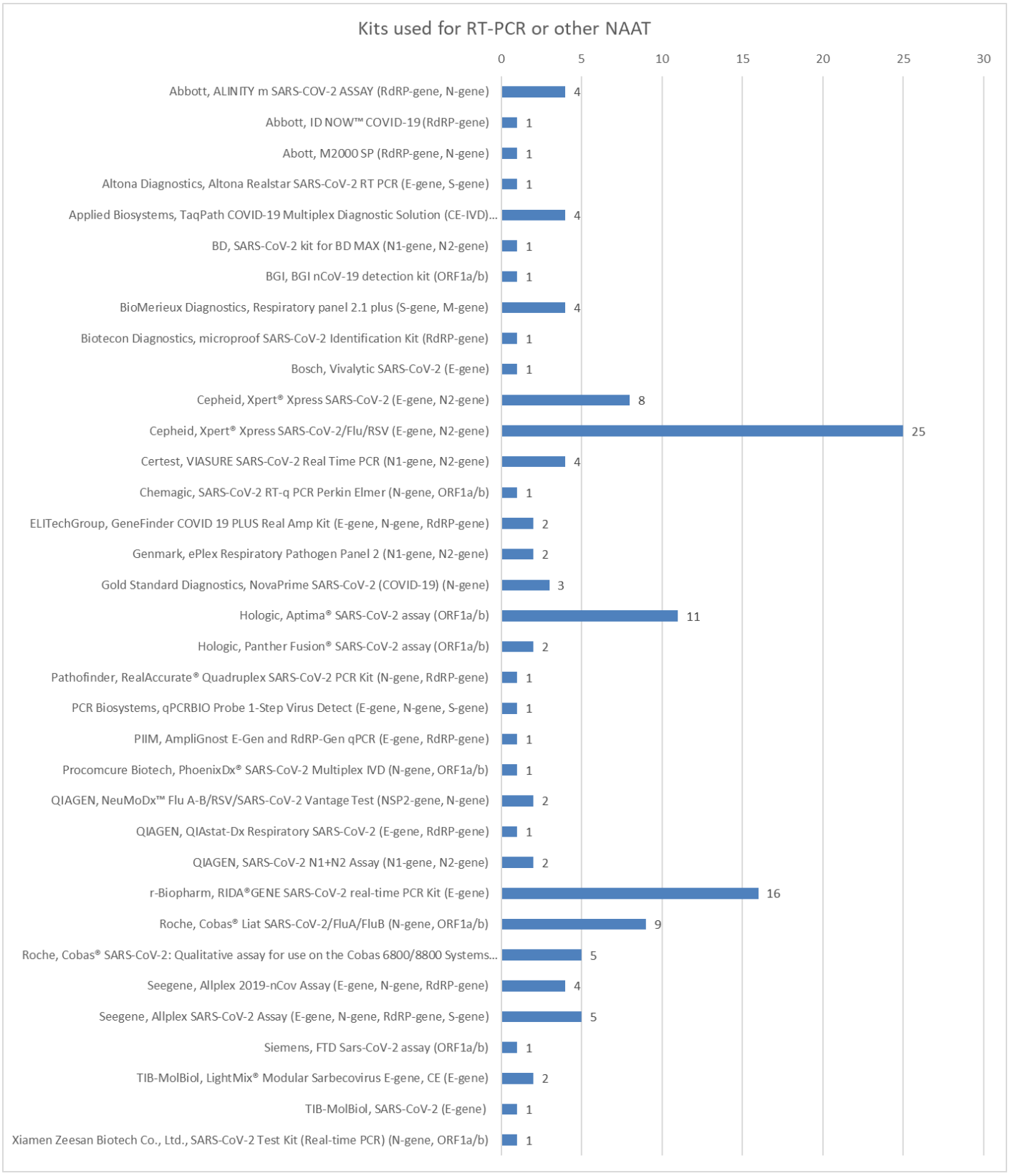


Figure 4: The RT-qPCR or other NAAT kits used by workflows testing for SARS-CoV-2 together with the number of workflows per kit (n=130). Not all workflows use 'ready to use' kits for their RT-qPCR or other NAAT, so the total N is not equal to the amount of workflows tested. For each kit the used target genes are listed. Workflows using separate enzymes and primers and probe are listed in Figure 5.

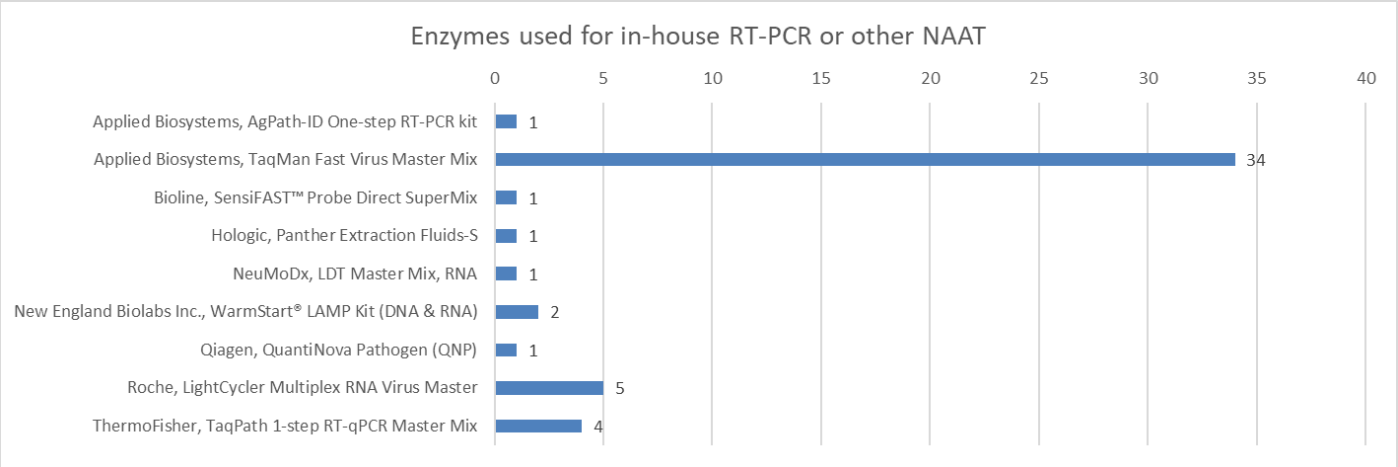


Figure 5: The enzymes used for performing RT-PCR or other NAAT by workflows testing for SARS-CoV-2 together with the number of workflows per enzyme (n=50). Not all workflows use separate enzymes for their RT-PCR or other NAAT, so the total N is not equal to the amount of workflows tested. In total 41/50 of the above mentioned workflows use 1 target gene to test for SARS-CoV-2 presence (E-gene Sarbeco specific (n=33); N2-gene (n=1); N-gene (n=2); ORF1a/b (n=2); RdRP-gene (n=3)). 9/50 workflows use 2 target genes to test for SARS-CoV-2 presence (E-gene Sarbeco specific + N1-gene (n=4); E-gene Sarbeco specific + N-gene (n=1); E-gene Sarbeco specific + RdRP-gene (n=2); E-gene SARS-CoV-2 specific + N-gene (n=1); E-gene SARS-CoV-2 specific + RdRP-gene (n=1)). Workflows using complete 'ready to use' kits for SARS-CoV-2 detection are listed in Figure 4.

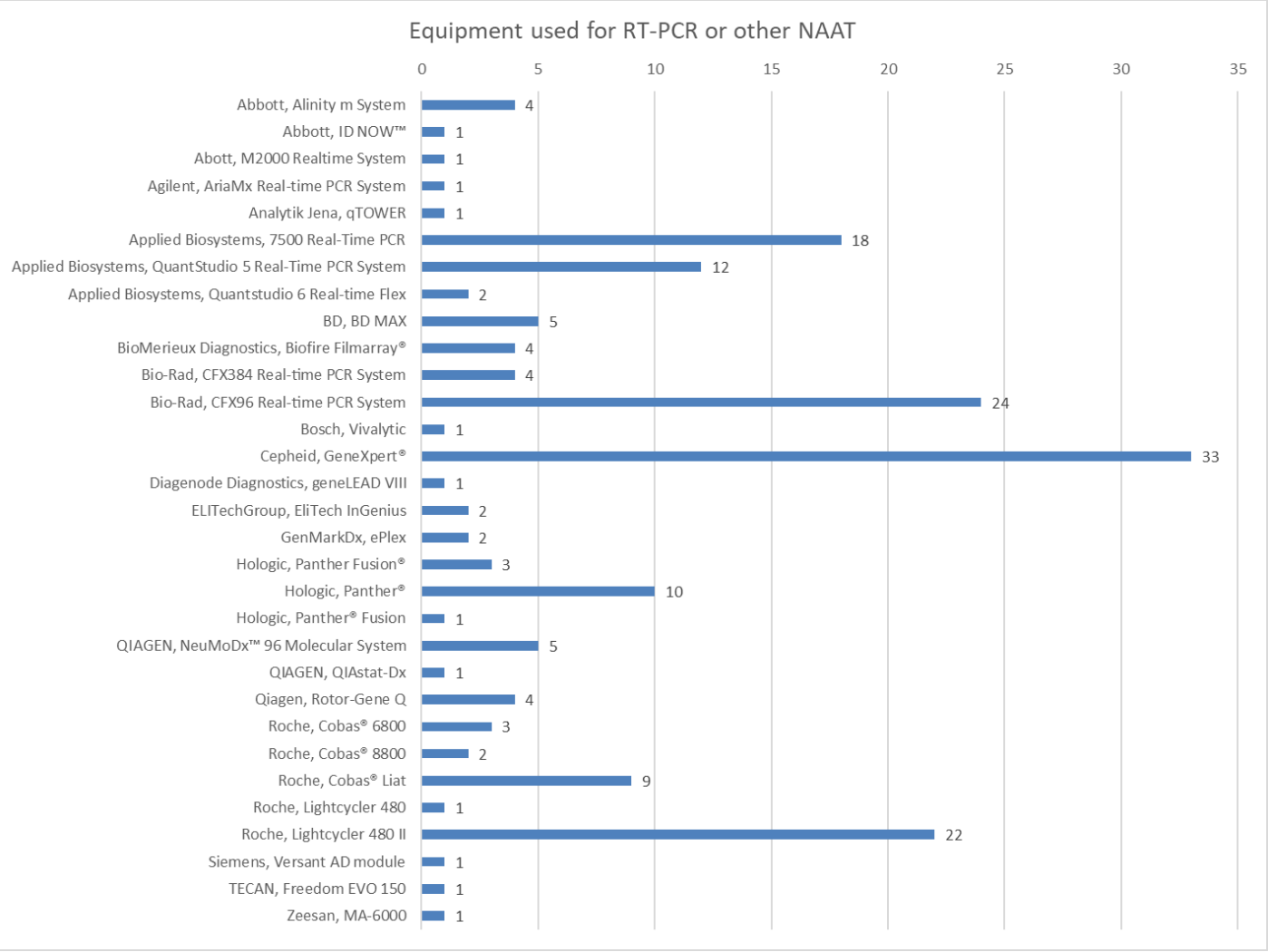


Figure 6: The RT-PCR or other NAAT equipment used by workflows testing for SARS-CoV-2 together with the number of workflows per machine (n=180)

3.2 Target genes used for RT-PCR or other NAAT

As the sensitivity of a workflow may also depend on the used gene or genes, for all workflows the target genes used were inventoried. Some workflows used up to 4 target genes. From the 180 workflows a total of 87 workflows used 1 target gene, 78 workflows used 2 target genes, 11 workflows used 3 target genes and 4 workflows used 4 target genes. In Figure 7, the target genes used in the order reported for each workflow are shown. In Figure 8 the combinations of target genes used in the workflows is displayed. Combinations of genes used by number of workflows are listed in Table 4. Some workflows using more than one gene do not generate separate result for each independent gene but rather a composite conclusion.

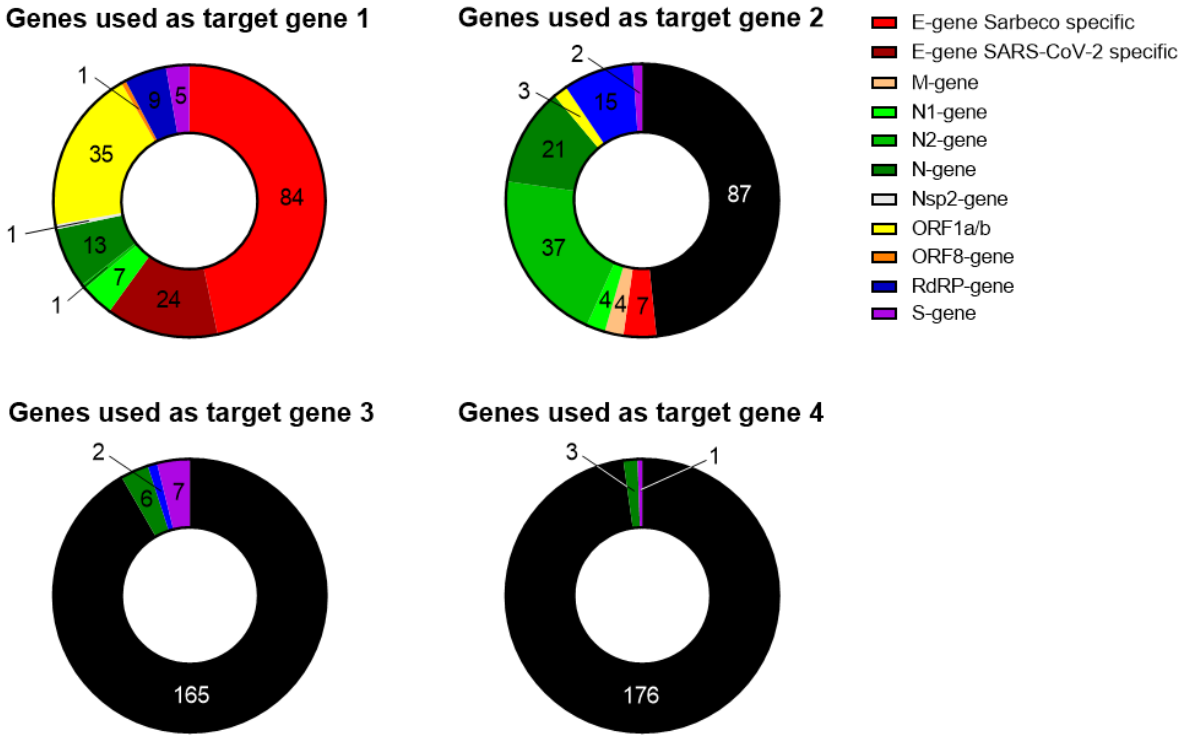


Figure 7: Target genes used in the workflows as reported in the questionnaire (n=180). Color coding genes is shown in the legend. Black color indicates workflows that do not contain a 2nd, 3rd or 4th gene target.

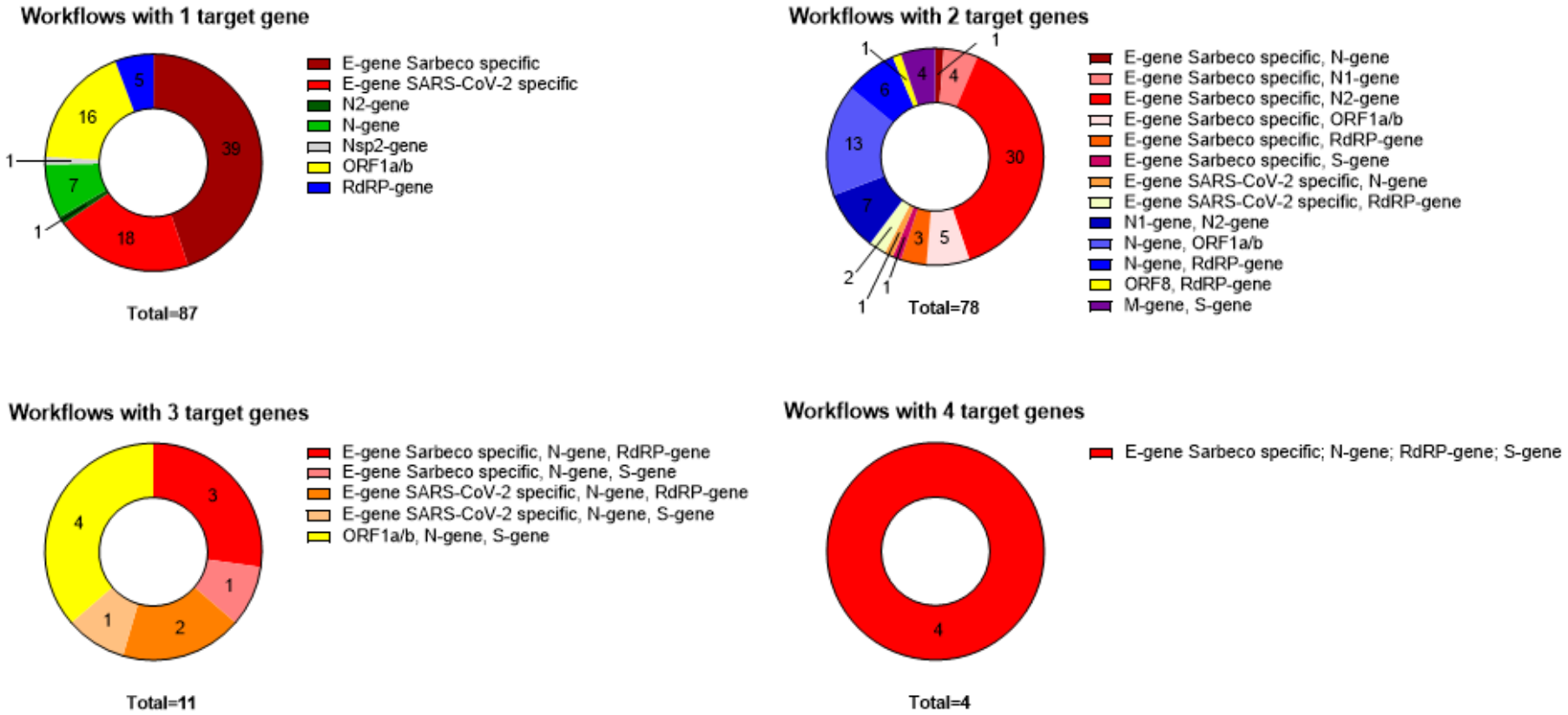


Figure 8: Combinations of target genes used in the workflows as reported in the questionnaire (n=180). Color coding genes is shown in the legend.

Table 4. Overview of number and type of target genes used per reported workflow.

№ target genes in workflow	Target gene(s)	№ workflows
1	E-gene Sarbeco specific	39
	E-gene SARS-CoV-2 specific	18
	N2-gene	1
	N-gene	7
	Nsp2-gene	1
	ORF1a/b	16
	RdRP-gene	5
2	E-gene Sarbeco specific; N1-gene	1
	E-gene Sarbeco specific; N2-gene	4
	E-gene Sarbeco specific; N-gene	30
	E-gene Sarbeco specific; ORF1a/b	5
	E-gene Sarbeco specific; RdRP-gene	3
	E-gene Sarbeco specific; S-gene	1
	E-gene SARS-CoV-2 specific; N-gene	1
	E-gene SARS-CoV-2 specific; RdRP-gene	2
	N1-gene; N2-gene	7
	N-gene; ORF1a/b	13
	N-gene; RdRP-gene	6
	ORF8; RdRP-gene	1
	M-gene; S-gene	4
3	E-gene Sarbeco specific; N-gene; RdRP-gene	3
	E-gene Sarbeco specific; N-gene; S-gene	1
	E-gene SARS-CoV-2 specific; N-gene; RdRP-gene	2
	E-gene SARS-CoV-2 specific; N-gene; RdRP-gene	1
	ORF1a/b; N-gene; S-gene	4
4	E-gene Sarbeco specific; N-gene; RdRP-gene; S-gene	4

3.3 Performance of the workflows

All laboratories participating in testing of the LEQA were asked to score each of the 10 samples contained in the panel for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. As said before, for some workflows only a smaller subset of samples needed to be tested. These workflows had to test only LEQA2_CoV20-3, LEQA2_CoV20-5, LEQA2_CoV20-8 and LEQA2_CoV20-9. Figure 9 – Figure 18 show the obtained results for LEQA2_CoV20-1 up to LEQA2_CoV20-10 for all target genes tested, in the order how the genes have been reported and are displayed in Figure 7 and a summary plot for the total workflow results. Some workflows using more than one gene do not generate separate result for each independent gene but rather show a composite conclusion. Due to this, some results obtained of multiple target genes are combined into and shown as one target gene in Figure 9 – Figure 18. The sample containing B.1.1.7/20B/501Y.V1 VOC-202012/01 (UK) variant SARS-CoV-2 was detected by 176/177 (99.4%) workflows, indicating primary diagnostic tests perform well detecting this variant (Table 2, Figure 17). A re-test of the panel was performed on the one workflow for which the B.1.1.7/20B/501Y.V1 VOC-202012/01 (UK) variant SARS-CoV-2 containing specimen (specimen

number 9) was reported negative for SARS-CoV-2. In this second test, the workflow managed to detect the SARS-CoV-2 VOC. During the initial test, this workflow found LEQA2 specimen number 10, containing no virus, positive for SARS-CoV-2 presence, whereas it remained negative during the second test. It is likely that specimen number 9 and 10 were switched during the first round of testing the LEQA2 panel. When including the results obtained during the re-test of this workflow, 100% of the workflows included in this quality round could detect B.1.1.7/20B/501Y.V1 VOC-202012/01 (UK) variant SARS-CoV-2. No results on performance of variant specific assays were obtained.

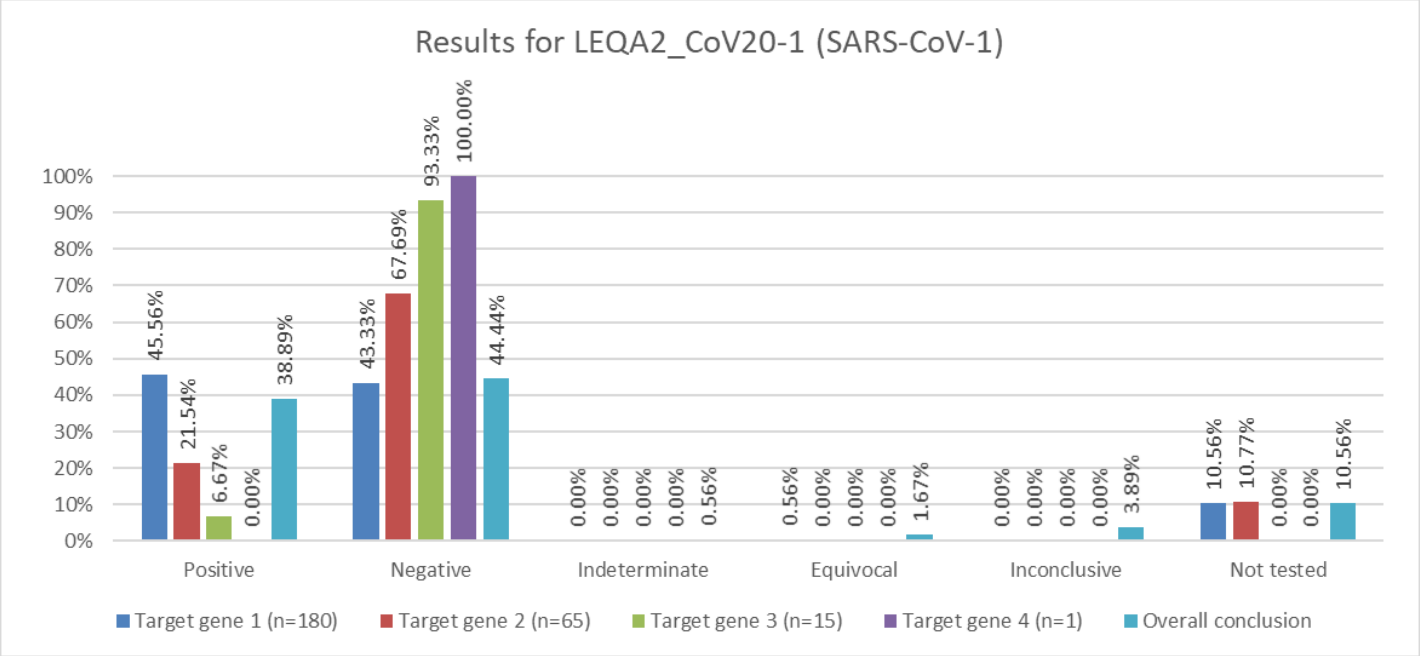


Figure 9: Results obtained for LEQA2_CoV20-1 containing SARS-CoV-1 combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. This sample was not deemed a core sample in the panel. Overall, of 161 workflows that reported an overall conclusion 80/161 (49.7%) did correctly not detect SARS-CoV-2 in this sample. 11/161 (6.8%) workflows reported an indeterminate, equivocal or inconclusive result and 70/161 (43.5%) reported a positive result, highly likely because in the current epidemiological situation no other Sarbeco virus than SARS-CoV-2 would be expected.

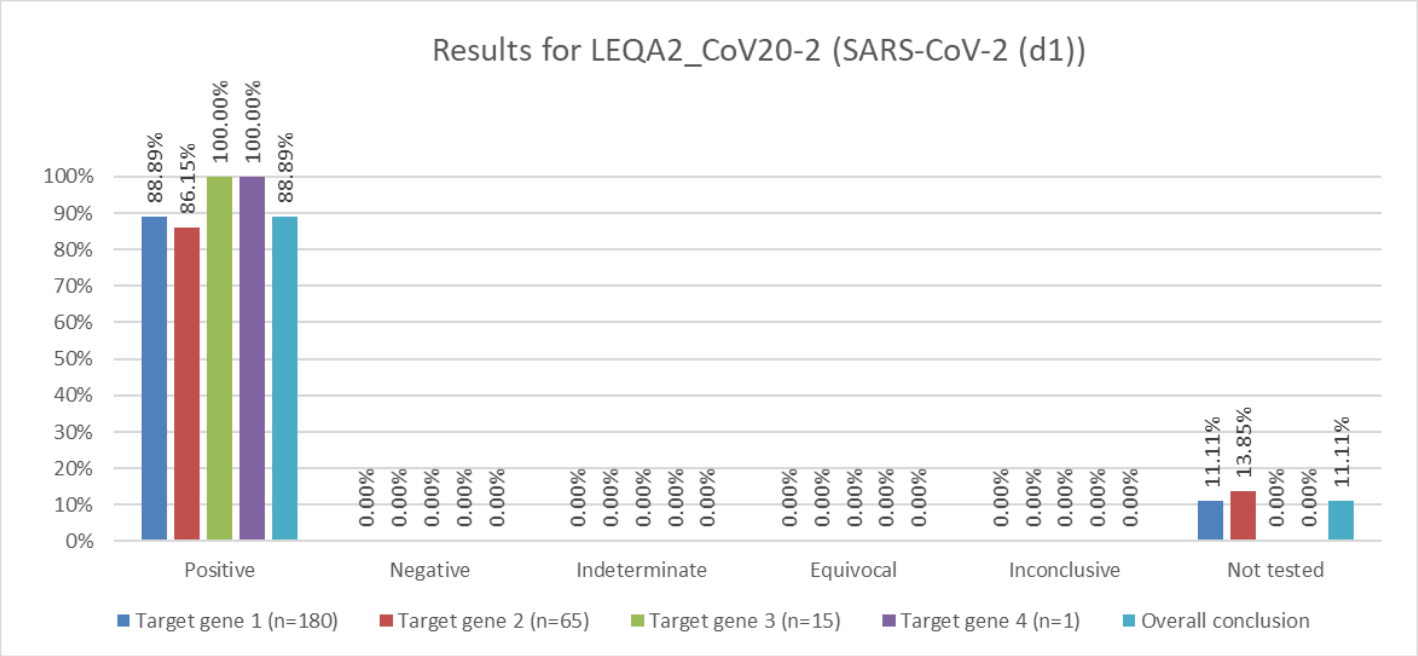


*Figure 10: Results obtained for LEQA2_CoV20-2 containing SARS-CoV-2 (with 1.28*10^5 copies E target/ml sample (determined with dPCR) and 1.73*10^5 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. Overall, all 160 workflows that reported an overall conclusion did correctly detect SARS-CoV-2 in this sample.*

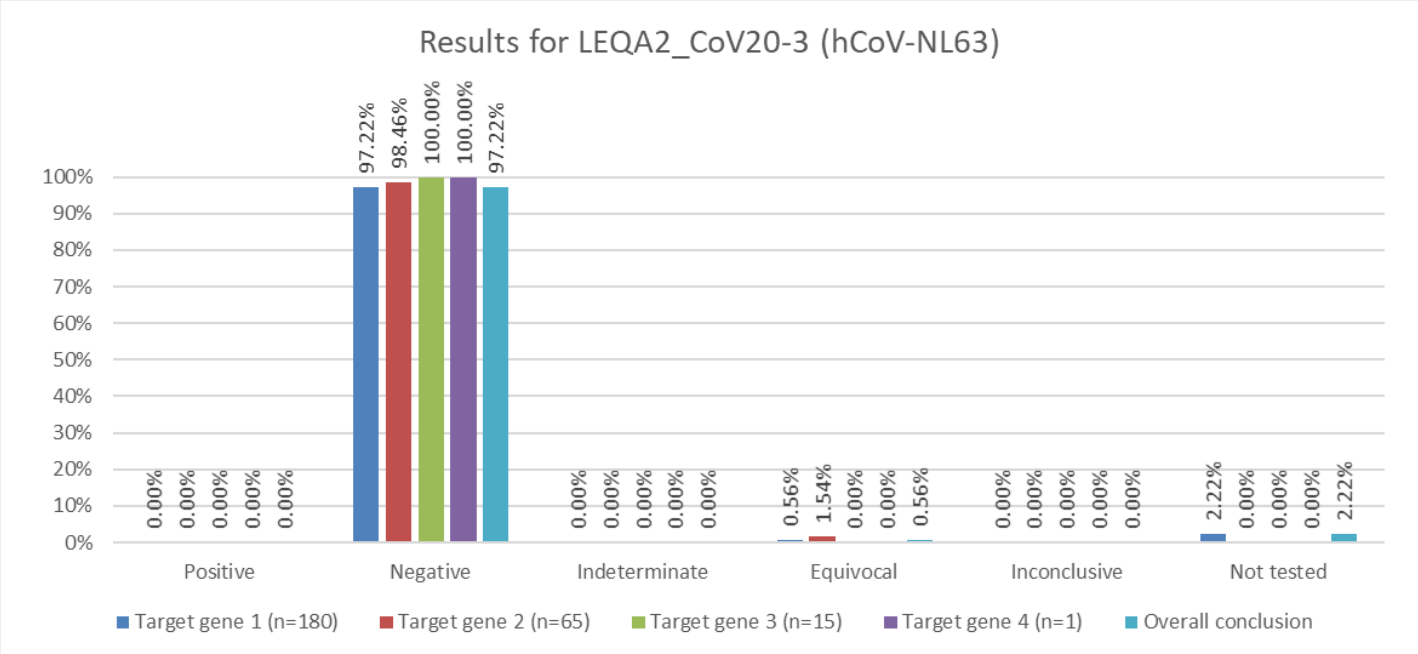


Figure 11: Results obtained for LEQA2_CoV20-3 containing hCoV-NL63 combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. This sample was not deemed a core sample in the panel. Overall, of all 176 workflows that reported an overall conclusion 175/176 (99.4%) identified this sample as SARS-CoV-2 negative and 1/176 (0.4%) with an indeterminate, equivocal or inconclusive result.

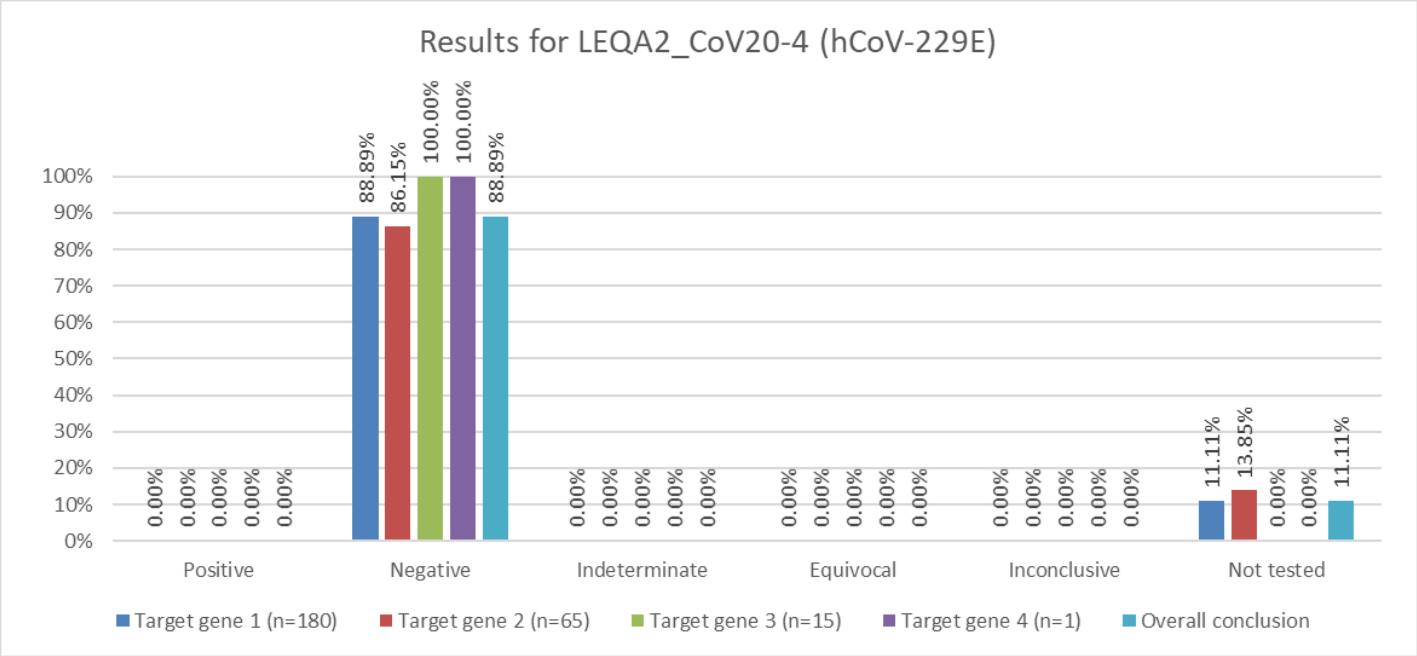


Figure 12: Results obtained for LEQA2_CoV20-4 containing hCoV-229E combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. Overall, of all 160 workflows that reported an overall conclusion 160/160 (100%) workflows correctly did not detect SARS-CoV-2 in this sample.

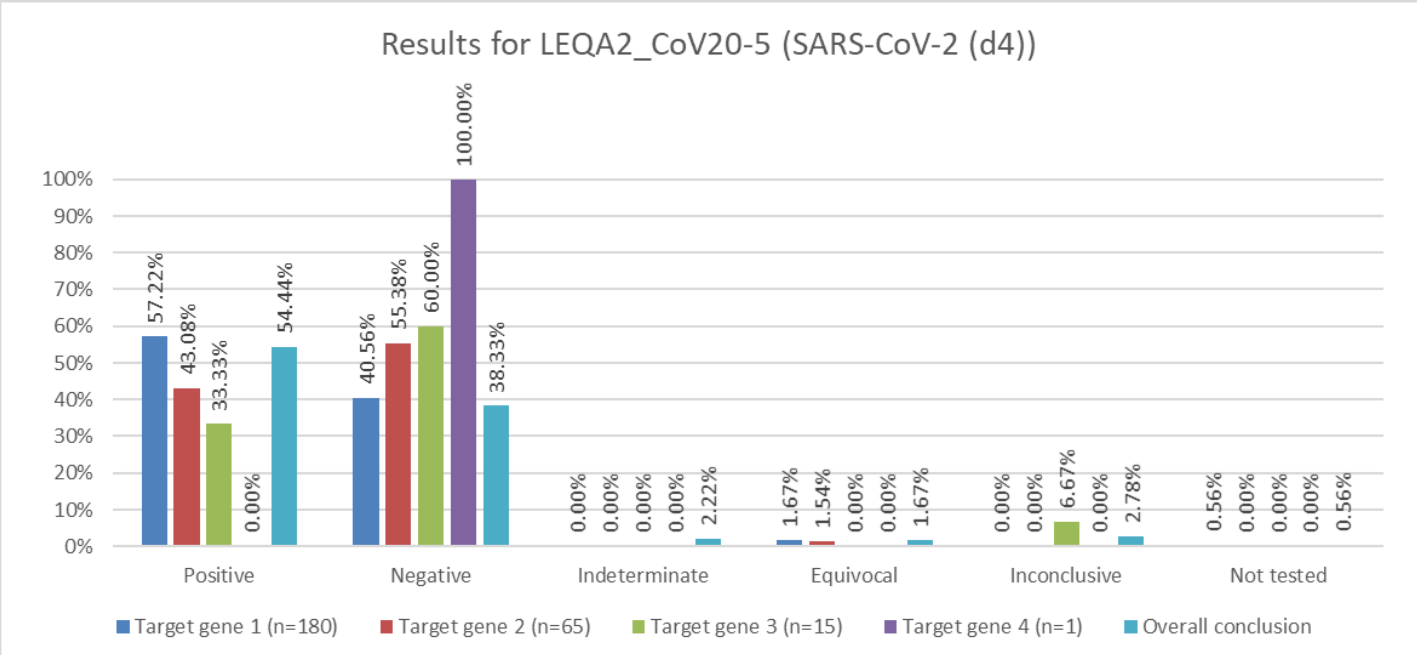


*Figure 13: Results obtained for LEQA2_CoV20-5 containing SARS-CoV-2 (with 1.28*10^2 copies E target/ml sample (determined with dPCR) and 1.73*10^2 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. This sample was not deemed a core sample in the panel. Overall, of all 179 workflows that reported an overall conclusion 98/179 (54.7%) correctly identified this sample as SARS-CoV-2 positive and 12/179 (6.7%) workflows gave an indeterminate, equivocal or inconclusive overall conclusion. 69/179 (38.5%) workflows reported incorrectly a negative result.*

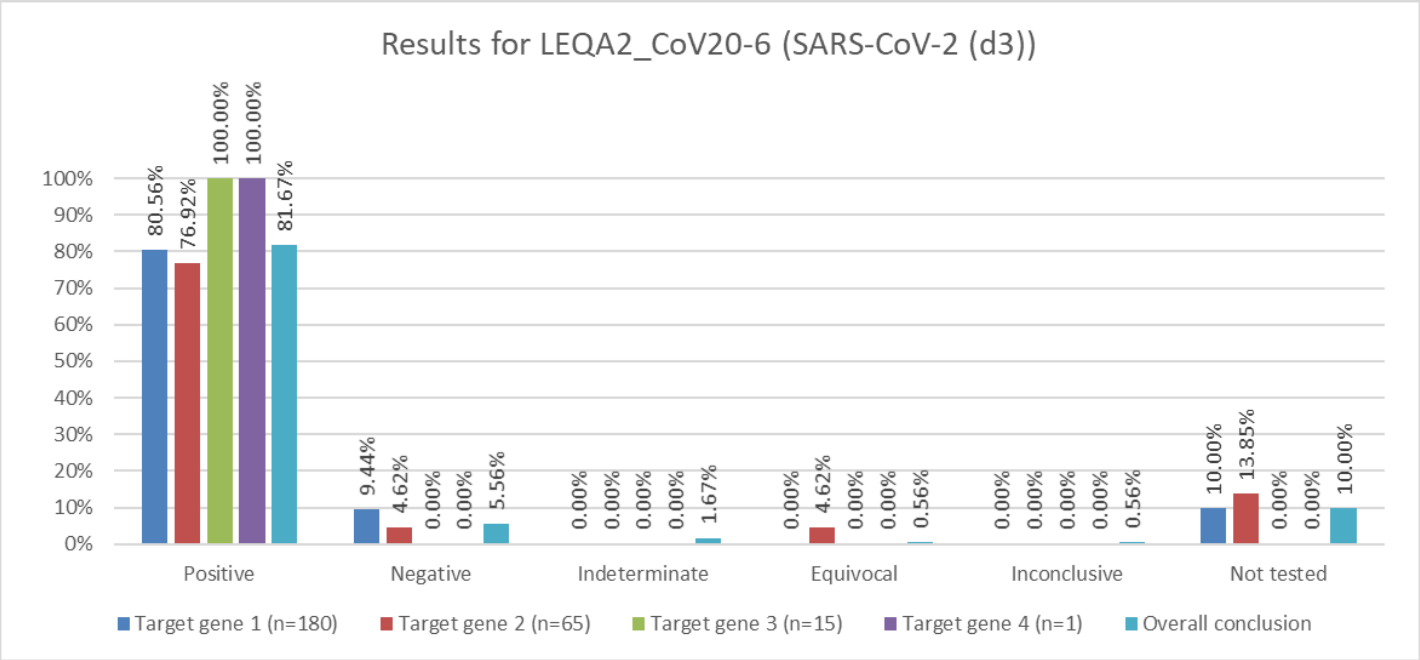


*Figure 14: Results obtained for LEQA2_CoV20-6 containing SARS-CoV-2 (with 1.28*10^3 copies E target/ml sample (determined with dPCR) and 1.73*10^3 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. Overall, of all 162 workflows that reported an overall conclusion 147/162 (90.7%) correctly identified this sample as SARS-CoV-2 positive and 5/162 (3.1%) workflows gave an indeterminate, equivocal or inconclusive overall conclusion. 10/162 (6.2%) workflows reported incorrectly a negative result.*

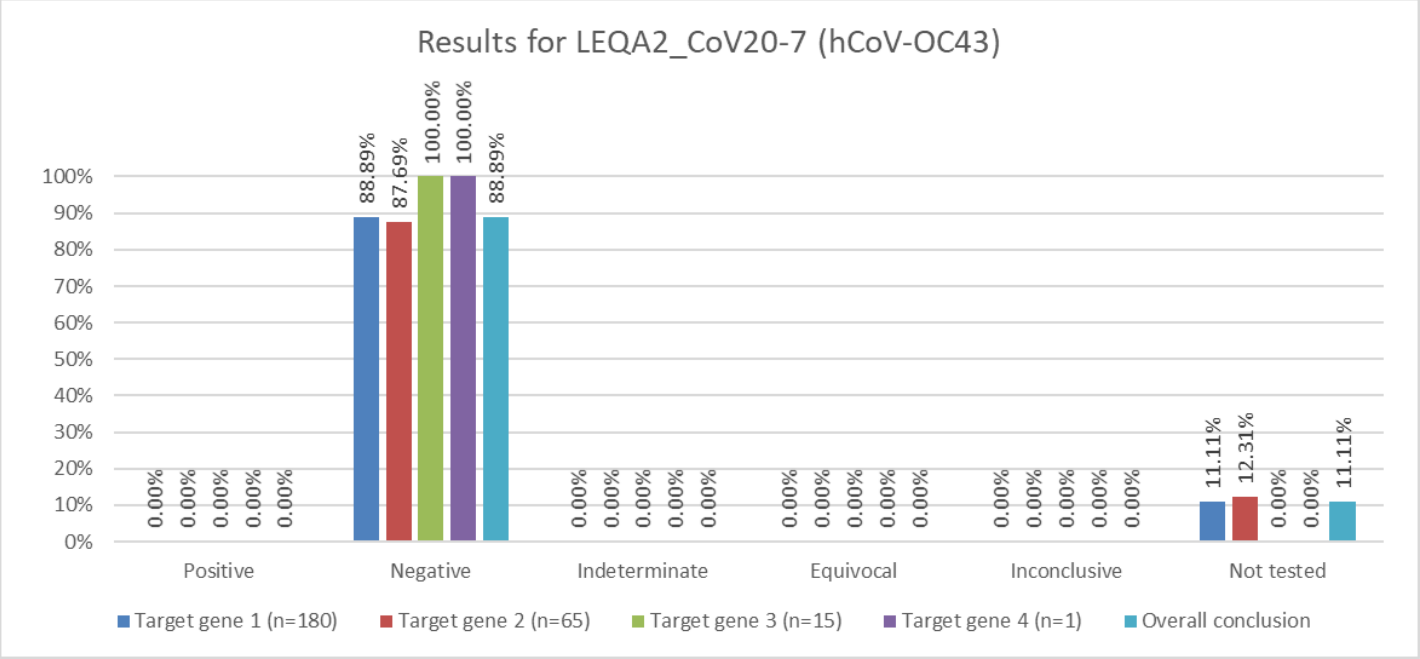


Figure 15: Results obtained for LEQA2_CoV20-7 containing hCoV-OC43 combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. Overall, of all 160 workflows that reported an overall conclusion 160/160 (100%) workflows correctly did not detect SARS-CoV-2 in this sample.

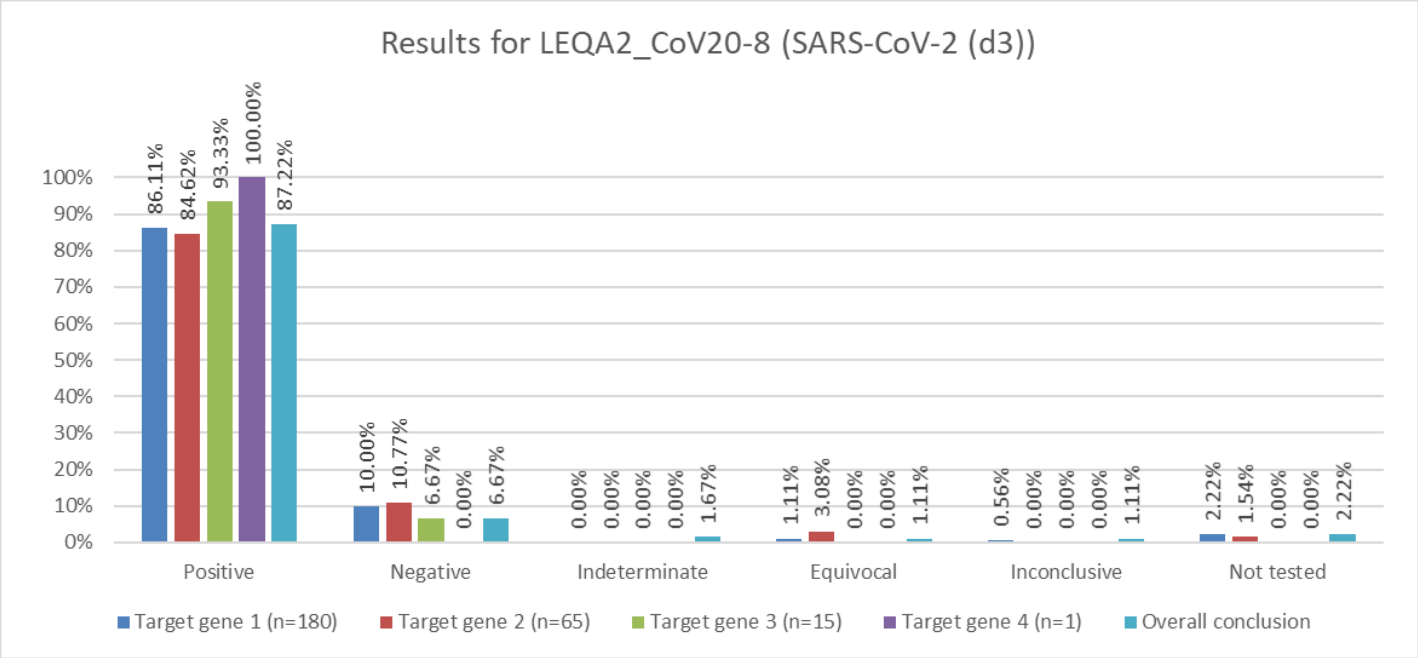


*Figure 16: Results obtained for LEQA2_CoV20-8 containing SARS-CoV-2 (with 1.28*10^3 copies E target/ml sample (determined with dPCR) and 1.73*10^3 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. Overall, of all 176 workflows that reported an overall conclusion 157/176 (89.2%) correctly identified this sample as SARS-CoV-2 positive and 7/162 (4.0%) workflows gave an indeterminate, equivocal or inconclusive overall conclusion. 12/176 (6.8%) workflows reported incorrectly a negative result.*

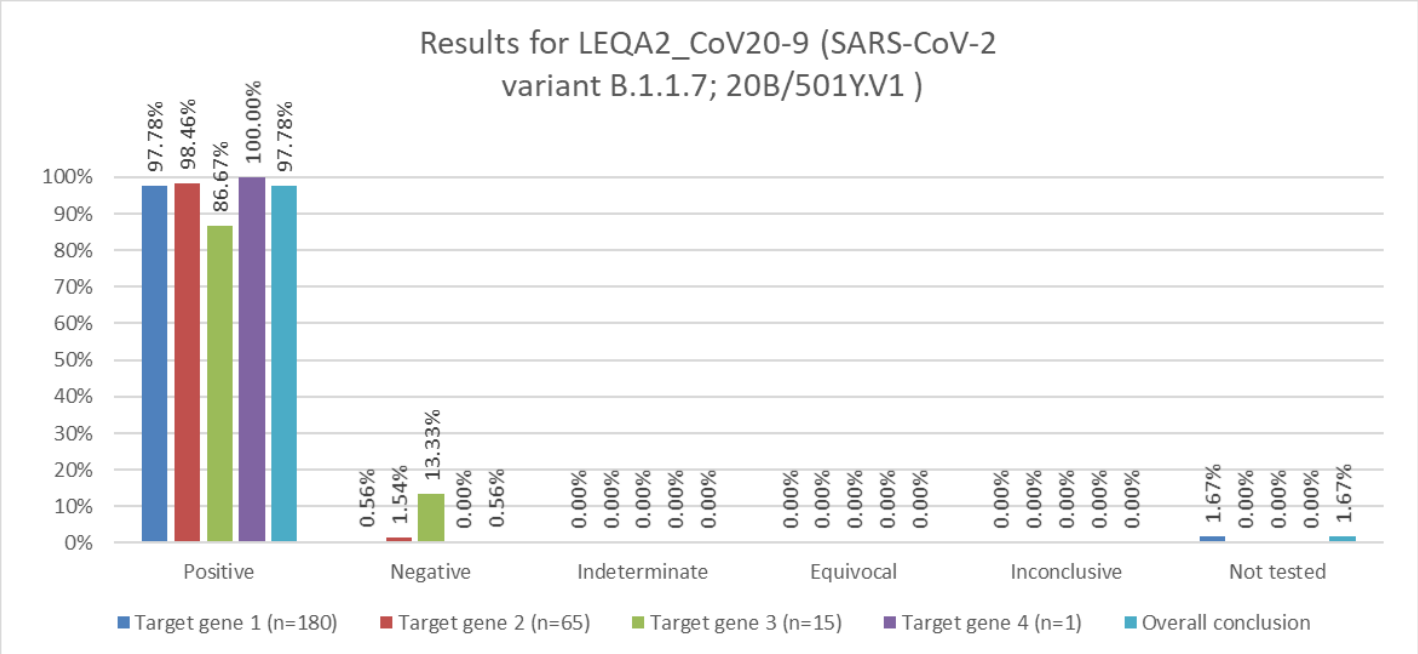


*Figure 17: Results obtained for LEQA2_CoV20-09 containing SARS-CoV-2 (SARS-CoV-2 variant B.1.1.7; 20B/501Y.V1) (with 3.39*10^5 copies E target/ml sample (determined with dPCR) and 2.32*10^5 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. Overall, of all 177 workflows that reported an overall conclusion 176/177 (99.4%) correctly identified this sample as SARS-CoV-2 positive and 1/177 (0.6%) workflows reported incorrectly a negative result.*

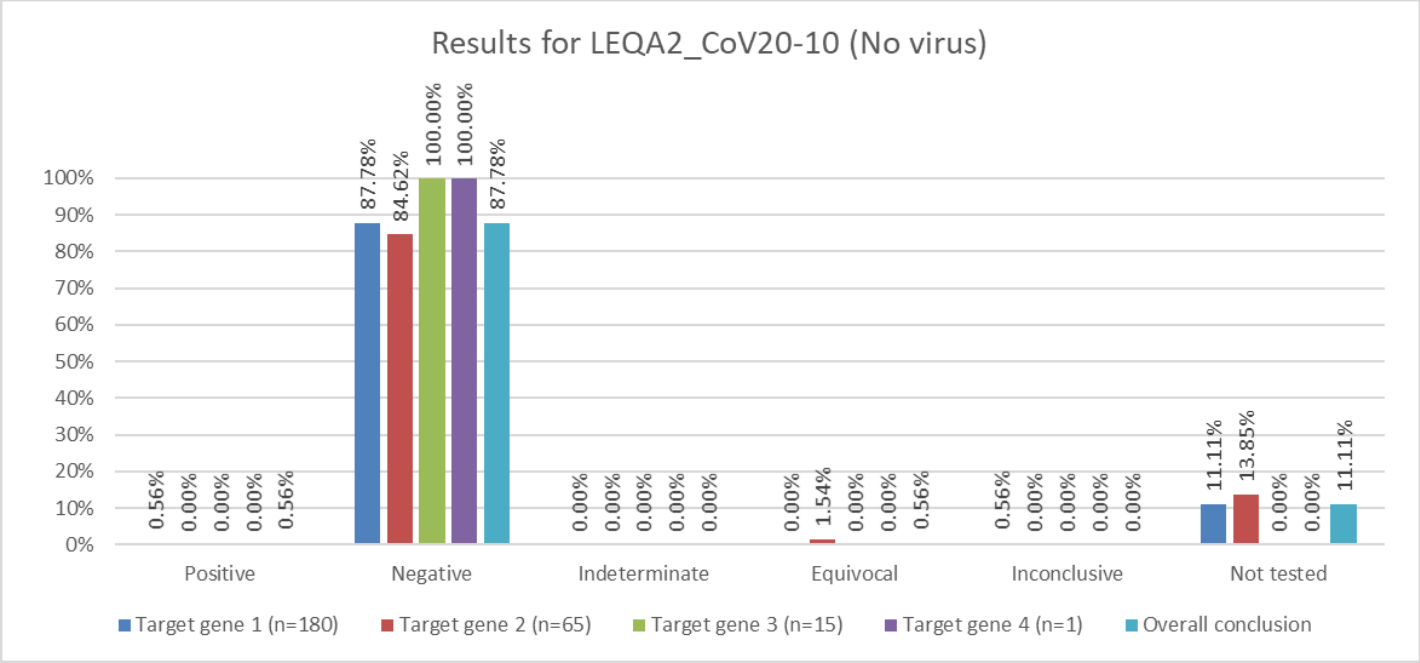


Figure 18: Results obtained for LEQA2_CoV20-10 containing no virus (negative control) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested. For each of the target genes used (which were processed in the order in which they were reported) the percentage of results reported per scoring category are shown. Overall, of 160 workflows that reported an overall conclusion 176/177 (99.4%) identified this sample as SARS-CoV-2 negative and 1/176 (0.4%) with an indeterminate, equivocal or inconclusive result. 1/177 (0.6%) workflows reported incorrectly a positive result.

As described before, all workflows were graded using a point system from 0 (being the lowest grade) up to 8 (highest grade). 148 workflows were given an '8', five workflows scored a '7.5', 13 workflows scored a '7', one workflow scored a '6.5', eight workflows scored a "6", one workflow scored a '5', one workflow scored a '3' and three workflows scored a '1'. It should be noted that all workflows scoring less than 6 points did not test the full panel or all samples of the reduced panel, thus these workflows lost a lot of points due to samples being scored 'Not tested'. Figure 19 shows all grades given to the reported workflows.

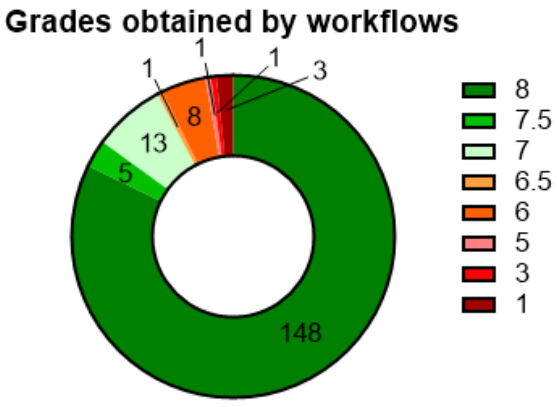


Figure 19: All grades obtained by the reported workflows out of the maximum of 8 points (n=180).

An overview containing the results obtained per target gene per panel sample for workflows reporting Ct values is shown in Figure 20. In this figure for each of the target genes used (shown in the order in which they were reported) the Ct values are shown for each of the tested samples. Also shown are the number of tested samples using each of the target genes, the percentage of results showing a Ct < 50, the number of reported negative results and the percentage of reported negative results (likely due to implemented cut-off values) with Ct < 50 .

The obtained scores per workflow are also coupled to the extraction kit or method used, the PCR or other NAAT test performed and the number of target genes used in order to assess the effect of different techniques on the performance of workflows. An overview of these factors on the grade is shown in Figure 21. Unfortunately the specimen input equivalent volume in the PCR could only be

calculated for 90/180 workflows and therefore this factor is not included in Figure 21. For the 90 workflows for which it was calculated the specimen equivalent volume was median 20 µl (range 2 µl – 400 µl). For the 148 workflows for which specimen input volume in extraction was reported the median volume was 250 µl (range 50 µl – 1400 µl). The median reaction volume reported for 95 workflows was 20 µl (range 4 µl – 30 µl).

In total one laboratory reported data for 9 workflows, two laboratories reported data for 7 workflows, one laboratory reported data for 6 workflows, four laboratories reported data for 5 workflows, eight laboratories reported data for 4 workflows, ten laboratories reported data for 3 workflows, sixteen laboratories reported data for 2 workflows and thirty-seven laboratories reported data for 1 workflow. There are six laboratories which only have scores of < 7 for all reported workflows. The obtained scores per workflow are sorted (anonymously) per laboratory and shown in Figure 22. Of the 79 laboratories, 68 laboratories reported at least one workflow with fully correct results. When excluding all mPOCT assays from the analysis, one laboratory reported data for 7 workflows, one laboratory reported data for 6 workflows, one laboratory reported data for 5 workflows, two laboratories reported data for 4 workflows, nine laboratories reported data for 3 workflows, eighteen laboratories reported data for 2 workflows, forty-three laboratories reported data for 1 workflow and four laboratories did not report any other workflows than mPOCT assays. Four laboratories only have scores of < 7 for all reported workflows. This is shown in Figure 23. It should be noted that all workflows (also including mPOCT assays) scoring less than 6 points did not test the full panel or all samples of the reduced panel, thus these workflows lost a lot of points due to samples being scored “Not tested”. Therefore for these laboratories and workflows a true performance estimate cannot be given.

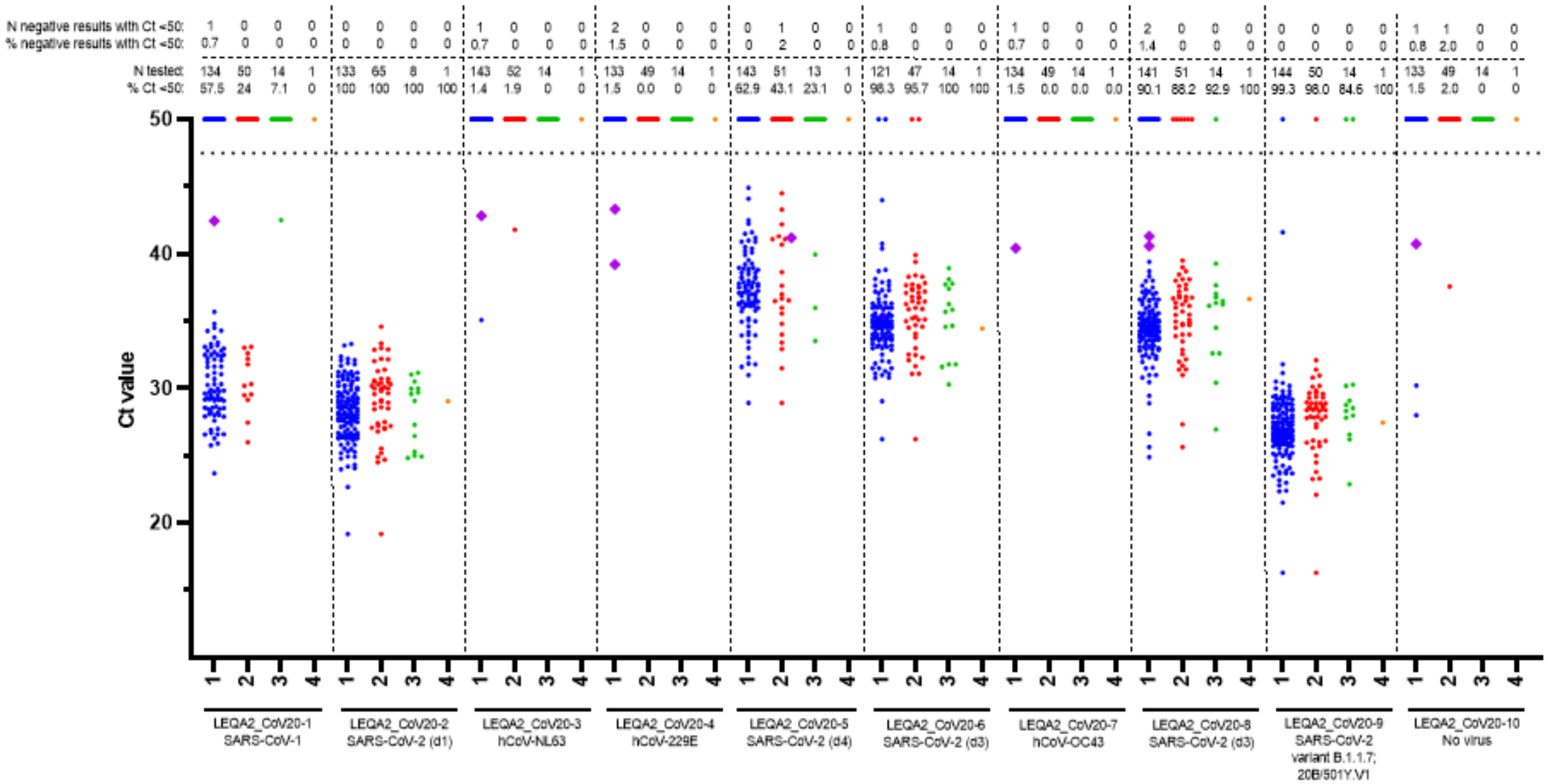
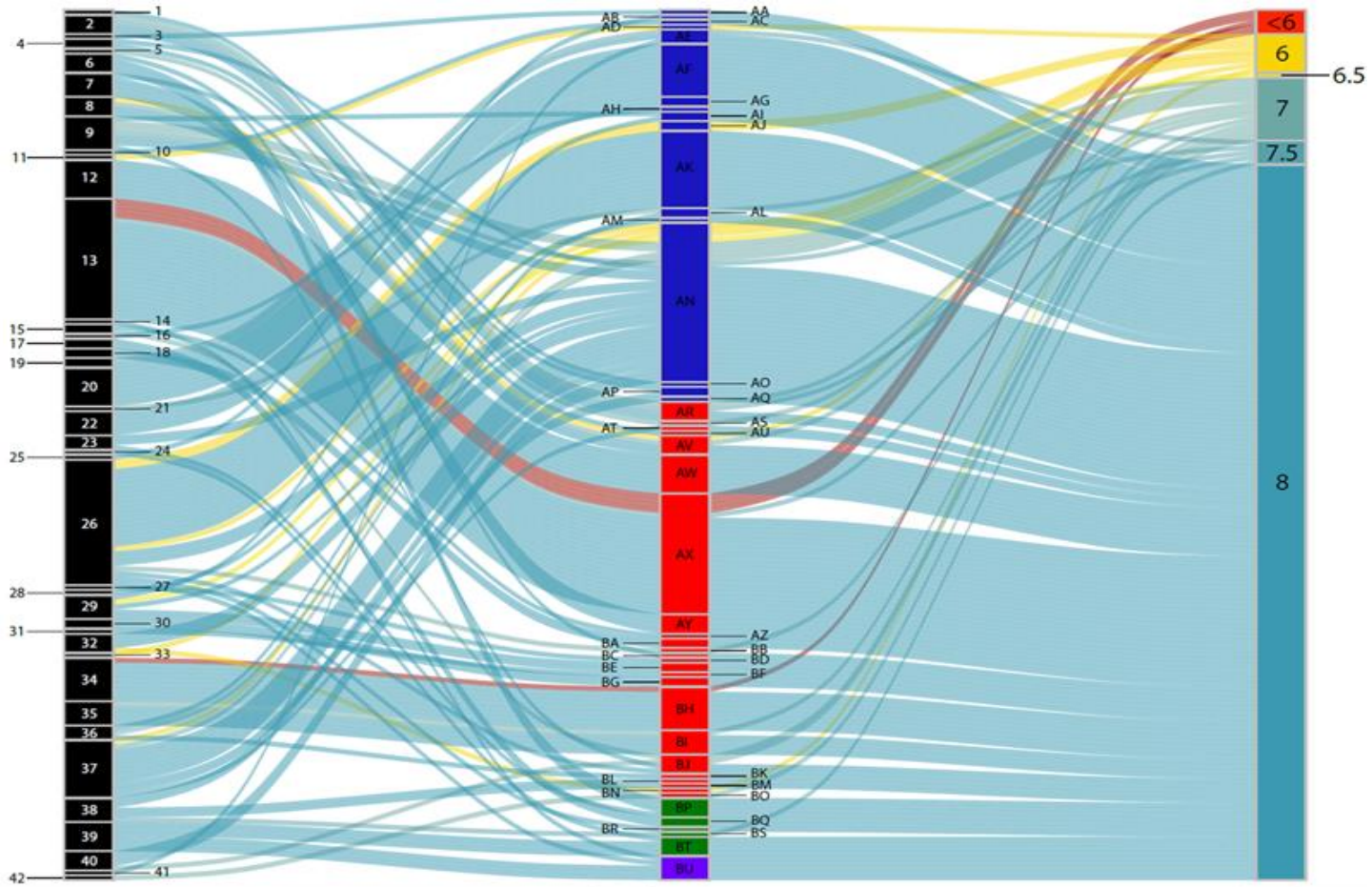


Figure 20: Results obtained per target gene per panel sample for workflows reporting Ct values. The numbers on the X-axis indicate which target gene (in order in which they were reported) is used for the detection of each sample. Underneath these numbers the contents of the sample are shown. All negative values for which no Ct value was given by the reporters have been given an artificial Ct value of 50. Not all negative results have a Ct value of 50. Some results with Ct < 50 are deemed negative by laboratories, likely due to Ct cutoff values used in the interpretation of an obtained result. Above the graph the number of tests (N) and the percentage of Ct values below 50 is shown per sample per target gene. Above the graph the number (N) and the percentage of tests with a negative results reported with a Ct value below 50 is shown as well per sample per target gene. Samples deemed negative with a Ct value below 50 are indicated with a purple diamond inside the graph.



RNA isolation kit	
A&A Biotechnology, MagnifiQ RNA Kit	1
Abbott, ALINITY m SARS-COV-2 ASSAY	2
Abbott, ID NOW™ COVID-19	3
Abclonal, ANDiS Viral Nucleic RNA Auto Extraction & Purification Kit	4
Abott, M2000 SP	5
Applied Biosystems, MagMAX TM Viral/Pathogen II Nucleic Acid Isolation Kit	6
BD, BD MAX ExK TNA-3	7
BioMerieux Diagnostics, Respiratory panel 2.1 plus	8
BioMerieux, NUCLiSENS® easyMAG®	9
Biotecon Diagnostics, Magnetic Preparation Kit VI	10
Bosch, Vivalytic CoV Viral SARS-CoV-2 test	11
Cepheid, Xpert® Xpress SARS-CoV-2	12
Cepheid, Xpert® Xpress SARS-CoV-2/Flu/RSV	13
Chemagen, Chemagic Viral DNA/RNA 300 kit H96	14
Chemagic, Viral DNA/RNA Kit	15
Diagenode Diagnostics, MagDEA Dx cartridges	16
ElitechGROUP, ELITe InGenius SP 200	17
GenMarkDx, ePlex® Respiratory Pathogen Panel 2	18
Goldstandard Diagnostics, NovaPrime RNA Extraction AE1	19
Hologic, Aptima® SARS-CoV-2 assay	20
Hologic, Panther Fusion® Open Access™	21
Hologic, Panther Fusion® SARS-CoV-2 assay	22
Life Technologies, MagMax pathogen RNA/DNA kit	23
Magtivio, MagSi-NA Pathogens	24
MGI, MGIEasy Nucleic Acid Isolation Kit	25
Molgen, PurePrep Pathogens	26
Procomcure Biotech, SphaeraMag DNA/RNA Isolation Kit - prefilled96	27
Promega, Maxwell RSC Blood DNA kit	28
QIAGEN, NeuMoDx™ SARS-CoV-2 Assay	29
QIAGEN, QIAprep& Viral RNA UM Kit	30
QIAGEN, QIAstat-Dx Respiratory SARS-CoV-2	31
QIAGEN, QIAsymphony DSP Virus/Pathogen Mini Kit	32
RBCBioscience, MagCore® Viral Nucleic Acid Extraction Kit	33
Roche, Cobas® Liat SARS-COV-2/FluA/FluB	34
Roche, Cobas® SARS-CoV-2: Qualitative assay for use on the cobas 6800/8800 Systems	35
Roche, DNA and Viral NA Large Volume kit	36
Roche, DNA and Viral NA Small Volume kit	37
Roche, MagNA Pure LC Total Nucleic Acid Isolation Kit	38
Seegene, STARMag 96x4 Universal Cartridge Kit	39
Siemens, VERSANT Sample Preparation 1.0 Reagents	40
TANBead, Nucleic Acid Extraction kit	41
Zeesan, lab-aid Virus RNA extraction kit	42

PCR tests	
Tests with 1 target gene	
Abbott, ID NOW™ COVID-19	AA
BGI, BGI nCoV-19 detection kit	AB
Biotecon Diagnostics, microproof SARS-CoV-2 Identification Kit	AC
Bosch, Vivalytic SARS-CoV-2	AD
Gold Standard Diagnostics, NovaPrime SARS-CoV-2 (COVID-19)	AE
Hologic, Aptima® SARS-CoV-2 assay	AF
Hologic, Panther Fusion® SARS-CoV-2 assay	AG
N2-gene, Lu et al. 2020	AH
N-gene, Corman et al. 2020	AI
ORF1a/b (LAMP assay)	AJ
r-Biopharm, RIDA®GENE SARS-CoV-2 real-time PCR Kit	AK
RdRp-gene, Corman et al. 2020	AL
RdRP-gene, Corman et al. 2020 (adapted)	AM
Sarbeco E-gene, Corman et al. 2020	AN
Siemens, FTD Sars-CoV-2 assay	AO
TIB-MolBiol, LightMix® Modular Sarbecovirus E-gene, CE	AP
TIB-MolBiol, SARS-CoV-2	AQ
Tests with 2 target genes	
Abbott, ALINITY m SARS-COV-2 ASSAY	AR
Abott, M2000 SP	AS
Altona Diagnostics, Altona Realstar SARS-CoV-2 RT PCR	AT
BD, SARS-CoV-2 kit for BD MAX	AU
BioMerieux Diagnostics, Respiratory panel 2.1 plus	AV
Cepheid, Xpert® Xpress SARS-CoV-2	AW
Cepheid, Xpert® Xpress SARS-CoV-2/Flu/RSV	AX
Certest, VIASURE SARS-CoV-2 Real Time PCR	AY
Chemagic, SARS-CoV-2 RT-q PCR Perkin Elmer	AZ
Genmark, ePlex Respiratory Pathogen Panel 2	BA
Pathofinder, RealAccurate® Quadruplex SARS-CoV-2 PCR Kit	BB
PIIM, AmpliGnost E-Gen and RdRP-Gen qPCR	BC
Procomcure Biotech, PhoenixDx® SARS-CoV-2 Multiplex IVD	BD
QIAGEN, NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Test	BE
QIAGEN, QIAstat-Dx Respiratory SARS-CoV-2	BF
QIAGEN, SARS-CoV-2 N1+N2 Assay	BG
Roche, Cobas® Liat SARS-CoV-2/FluA/FluB	BH
Roche, Cobas® SARS-CoV-2: Qualitative assay for use on the Cobas 6800/8800 Systems	BI
Sarbeco E-gene, Corman et al. 2020; N1-gene, Lu et al. 2020	BJ
Sarbeco E-gene, Corman et al. 2020; N-gene, Lu et al. 2020	BK
Sarbeco E-gene, Corman et al. 2020; RdRP-gene, Corman et al. 2020	BL
Sarbeco E-gene, Corman et al. 2020; RdRP-gene, Corman et al. 2020 (adapted)	BM
Sarbeco E-gene, Corman et al. 2020; RdRP-gene, Lu et al. 2020	BN
Xiamen Zeesan Biotech Co., Ltd., SARS-CoV-2 Test Kit (Real-time PCR)	BO
Tests with 3 target genes	
Applied Biosystems, TaqPath COVID-19 Multiplex Diagnostic Solution (CE-IVD)	BP
ELITechGroup, GeneFinder COVID 19 PLUS Real Amp Kit	BQ
PCR Biosystems, qPCRBIO Probe 1-Step Virus Detect	BR
Sarbeco E-gene, Corman et al. 2020; N1-gene, Lu et al. 2020; N2-gene, Lu et al. 2020	BS
Seegene, Allplex 2019-nCov Assay	BT
Tests with 4 target genes	
Seegene, Allplex SARS-CoV-2 Assay	BU

Figure 21: A flow diagram showing all workflows reported to have tested the LEQA panel with extraction method, PCR test, the number of target genes used and the final score achieved by each workflow. In the alluvial plot PCR tests using 1 target gene are depicted in blue, PCR tests using 2 target genes are shown in red, PCR tests using 3 target genes are shown in green and PCR tests using 4 target genes are shown in purple. For the target gene combinations used per kit, see Figure 5. Color of trails per workflow are based on the grade obtained for LEQA2 All workflows receiving grades below 6 are grouped in <6.

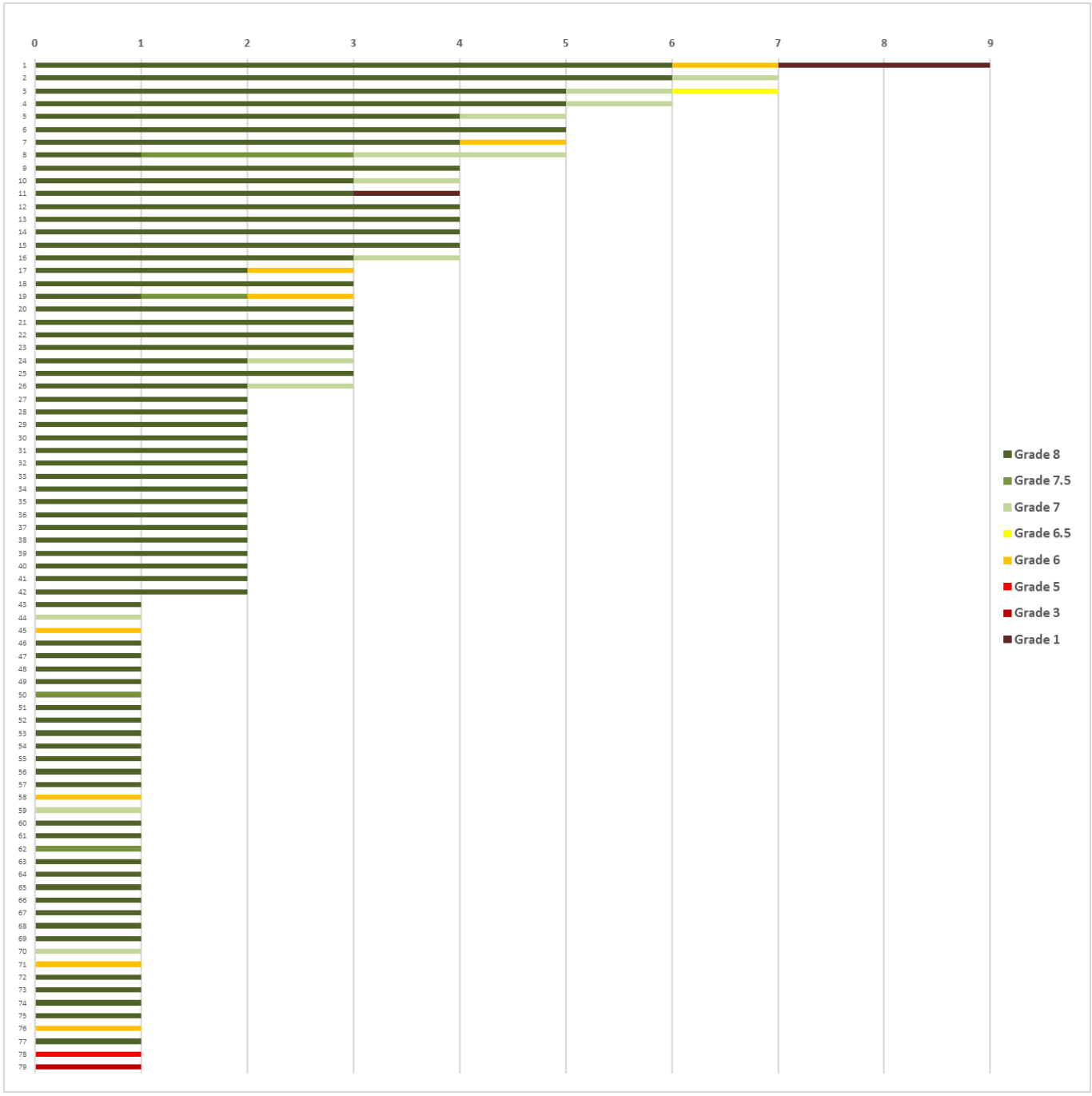


Figure 22: Grades obtained per workflow per laboratory (anonymized). For each of the laboratories the amount of reported workflows is shown on the X-axis together with their accompanying grades. In total 79 laboratories sent in data of their workflows. There are six laboratories which only have scores of < 7 for all reported workflows. It should be noted that all workflows scoring less than 6 points did not test the full panel or all samples of the reduced panel, thus these workflows lost a lot of points due to samples being scored "Not tested". The same numbering is maintained as in Figure 23, where all workflows excluding mPOCT assays are listed.

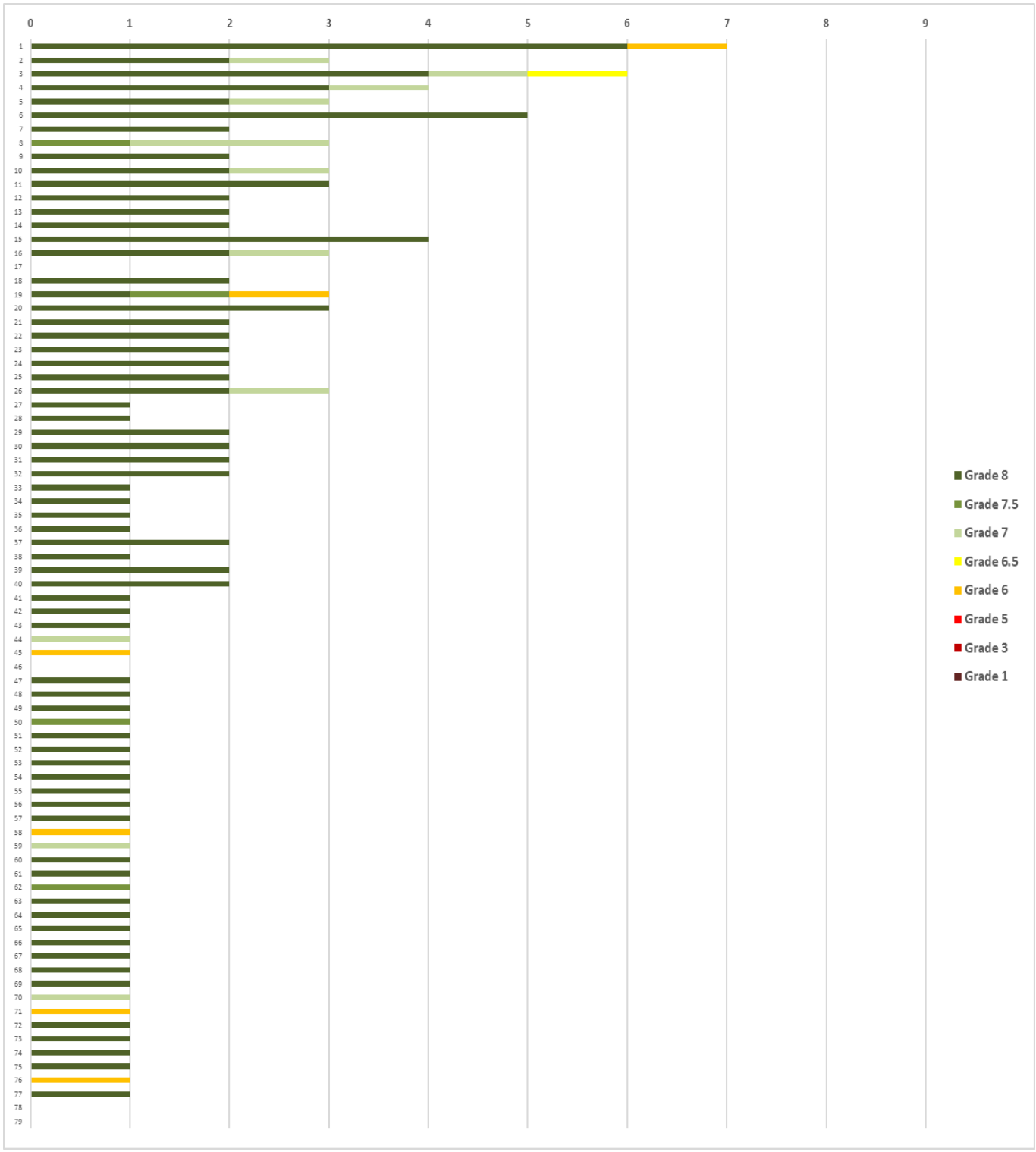


Figure 23: Grades obtained per workflow per laboratory excluding all mPOCT assays (anonymized). For each of the laboratories the amount of reported workflows is shown on the X-axis together with their accompanying grades. In total 79 laboratories sent in data of their workflows. There are four laboratories which only have scores of < 7 for all reported workflows. Four laboratories do not report any other workflows than mPOCT assays. It should be noted that all workflows scoring less than 6 points did not test the full panel or all samples of the reduced panel, thus these workflows lost a lot of points due to samples being scored "Not tested". The same numbering is maintained as in Figure 22, where all workflows including mPOCT assays are listed.

3.4 Comparing results LEQA2 to LEQA1

Whereas 65 laboratories reported data for 164 workflows for LEQA1, 79 laboratories reported data for 180 workflows for LEQA2. This is an increase of 21.5% of reporting laboratories and an increase of 9.8% of reported workflows. The grades obtained by the workflows for both LEQA1 and LEQA2 are listed in Table 5. It should be noted that 7/8 core samples were exactly the same in both panels. The single different sample was LEQA1 influenza virus A(H3N2) replaced by LEQA2 B.1.1.7/20B/501Y.V1 VOC-202012/01 (UK) variant SARS-CoV-2. Because the concentration of the variant virus in the sample in LEQA2 was high the number of workflows detecting this variant was expected to be 100%, similar to expected 100% of workflows not detecting A(H3N2) in panel LEQA1. Therefore, grades can directly be compared between LEQA1 and LEQA2.

	Grade obtained						
	8	7.5	7	6.5	6	<6	Total workflows tested
LEQA1	132 (80.5%)	7 (4.3%)	11 (6.7%)	0	8 (4.9%)	6 (3.7%)	164
LEQA2	148 (82.2%)	5 (2.7%)	13 (7.2%)	1 (0.1%)	8 (4.4%)	5 (2.7%)	180

Table 5. Overview of grades obtained by the workflows having tested LEQA1 and LEQA2. All workflows receiving grades below 6 are grouped in <6

The shift in grades obtained by the workflows tested between LEQA1 and LEQA2 are shown in Table 6. Not all workflows were tested for both LEQA1 and LEQA2. These workflows are labelled 'Not Tested' or 'NT' abbreviated. This data is also visualized in Figure 24.

Table 6. Overview of grades obtained by workflows that tested LEQA1 and how they scored testing LEQA2. Green cells indicate workflows performing better in LEQA2 than in LEQA1. Red cells indicate workflows performing worse in LEQA2 than in LEQA1. White cells indicate workflows performing equally in LEQA 2 and LEQA1. All workflows receiving grades below 6 are grouped in <6

		Grade obtained for LEQA 1							
		<6	6	6.5	7	7.5	8	NT[1]	Subtotal
Grade obtained for LEQA2	<6	1						4	5
	6						4	4	8
	6.5					1			1
	7				2	2	5	4	13
	7.5						2	3	5
	8	1	3		4	3	71	66	148
	NT[2]	4	5		5	1	50		
	Subtotal	6	8	0	11	7	132		180 / 164

[1] Short for 'Not Tested'. These workflows were not used to test LEQA1, but were used to test LEQA2

[2] Short for 'Not Tested'. These workflows were not used to test LEQA2, but were used to test LEQA1

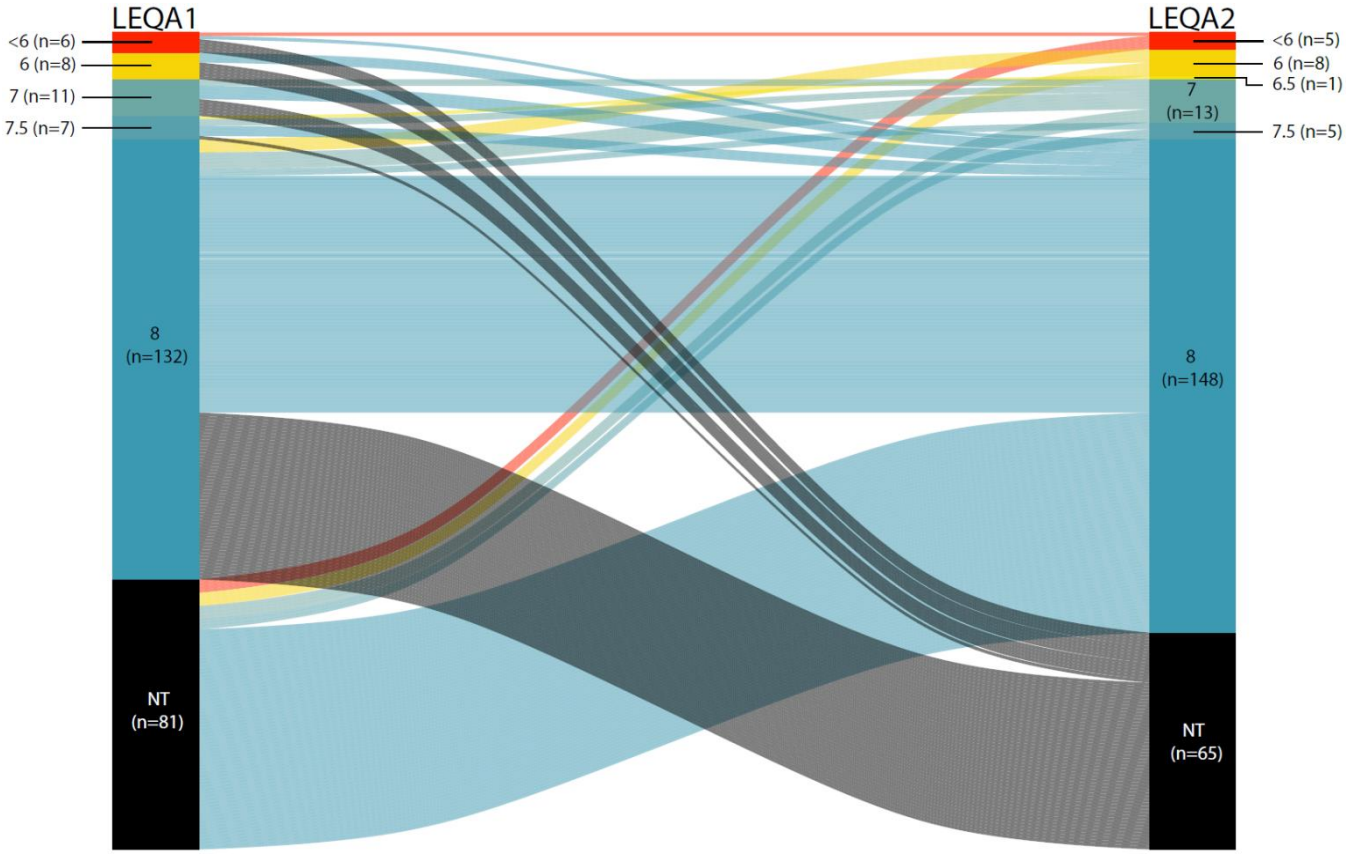


Figure 24: Overview of grades obtained by workflows that tested LEQA1 and how they scored testing LEQA2. 'NT' stands 'Not Tested'. These workflows were not used to test LEQA1, but have tested LEQA2 or vice versa. Color of trails per workflow are based on the grade obtained for LEQA2. All workflows receiving grades below 6 are grouped in <6

In total 65 workflows were not used to test LEQA2 but did test LEQA1. Twenty-seven of these workflows were mPOCT assays (41.5%), 22 workflows used 'ready to use' kits for their RT-qPCR (33.8%) and 16 workflows were in-house assays (24.%). Of the 81 newly implemented workflows in LEQA2 (which were not tested in LEQA1), 31 were mPOCT assays (38.3%), 30 workflows used 'ready to use' kits for their RT-qPCR (37.0%), 2 workflows were Loop-mediated isothermal amplification (LAMP) assays (2.5%) and 18 workflows were in-house assays (22.2%).

In total 78/162 (48.2%) of the workflows tested in LEQA1 could detect the educational load of SARS-CoV-2 (with 128 and 173 copies E gene and RdRP gene target/ml sample, respectively; dilution d4, see Table 1) whereas 98/179 (54.7%) of the workflows tested in LEQA2 could detect SARS-CoV-2 in this sample. For LEQA1 both LEQA1_CoV20-5 and LEQA1_CoV20-7 contained SARS-CoV-2 at a concentration of 1,280 and 1,730 copies E gene and RdRP gene target/ml specimen, respectively (dilution d3, see Table 1). LEQA1_CoV20-5 was measured positive for SARS-CoV-2 by a total of 131/148 (88.5%) of the workflows. For LEQA1_CoV20-7 139/158 (88.0%) of the workflows tested these samples positive for SARS-CoV-2. In LEQA2 identical samples with the same SARS-CoV-2 load but with different labelling were put in the panel: LEQA2_CoV20-6 and LEQA_CoV20-8 respectively. LEQA2_CoV20-6 was scored positive for SARS-CoV-2 by 147/162 (90.7%) workflows, an increase with 2.7% compared to LEQA1_CoV20-5. LEQA2_CoV20-8 was scored positive by 157/176 (89.2%) workflows, an increase with 1.2% compared to LEQA1_CoV20-7. Another set of specimens with the same SARS-CoV-2 loads in both LEQA panels were LEQA1_CoV20-1 and LEQA2_CoV20-2. These samples contained 1.28*10^5 copies E gene and 1.73*10^5 copies RdRP gene target/ml specimen, respectively (dilution d1, see Table 1). LEQA1_CoV20-1 was scored positive for SARS-CoV-2 by 161/162 (99.4%) workflows, whereas

LEQA2_CoV20-2 was scored positive by 160/160 (100%) workflows, an increase by 0.6%. So, at the highest SARS-CoV-2 concentration (d1), the amount of positive results increased from 99.4% to 100%. The percentage of positive results for both specimens with the intermediate SARS-CoV-2 load (d3) increased from 88.0%/88.5% to 89.2%/90.7%. Lastly the specimens with the lowest SARS-CoV-2 load (d4) of both panels show an increase in positive results from 48.2% to 54.7%. Taken together, for each of the samples with different SARS-CoV-2 concentrations, the workflows included in LEQA2 showed an increase in the percent of positive results compared to those included in LEQA1. This is an indication that more-sensitive workflows have been implemented since LEQA1 or that existing workflows have been made more sensitive.

In total, 99 workflows used to test LEQA1 were also used to test LEQA2 (Table 6). A detailed description of a comparison of the performance with these workflows is given below.

In total, 74/99 workflows did not show a change in grade between LEQA2 and LEQA1. One workflow obtained a grade below 6/8, two workflows obtained a grade of 7/8 and 71 workflows scored a grade of 8/8 for both LEQA rounds.

Of all workflows scoring a lower grade for LEQA2 than for LEQA1 (n=14/99), 11 (78.6%) previously scored a grade of 8/8 and 3 (21.4%) scored a grade of 7.5/8 for LEQA1. For LEQA2 the obtained grades were 6/8 by four (28.6%) workflows; 6.5/8 by one (7.1%) workflow; 7/8 by seven (50%) workflows; 7.5/8 by two (14.3%) workflows. For LEQA2 6 (42.9%) of the workflows reported one false negative result for one of the lowest concentration SARS-CoV-2 containing core specimens (dilution d3, see Table 1); 2 (14.3%) of the workflows reported a false negative result for both lowest concentration SARS-CoV-2 containing core specimens (dilution d3, see Table 1); 1 (7.1%) of the workflows reported a false indeterminate/inconclusive/equivocal result for one of lowest concentration SARS-CoV-2 containing core specimens (dilution d3, see Table 1); 2 (14.3%) of the workflows reported a false indeterminate/inconclusive/equivocal result for both lowest concentration SARS-CoV-2 containing core specimens (dilution d3, see Table 1); 1 (7.1%) of the workflows reported one false indeterminate/inconclusive/equivocal and one false negative result for the lowest concentration SARS-CoV-2 containing core specimens (dilution d3, see Table 1); 1 (7.1%) of the workflows reported a false negative result for SARS-CoV-2 variant B.1.1.7; 20B/501Y.V1 VOC-202012/01 (UK) and a false positive result for no virus control; 1 (7.1%) of the workflows reported a false indeterminate/inconclusive/equivocal result for the no virus control. Most of the incorrect results have been obtained for the SARS-CoV-2 dilution d3 of the core samples (10-fold higher concentration than the educational load sample), suggesting that the concerned workflows lost some sensitivity between both LEQA rounds. This data is summarized in Table 7.

Of all workflows scoring a higher grade for LEQA2 than for LEQA1 (n=11/99), 3 (27.3%) workflows previously scored a 7.5/8; 4 (36.4%) workflows scored a 7/8; 3 (27.3%) workflows scored a 6/8; 1 workflow (9.1%) scored a 4/8. When testing LEQA2, all these workflows scored an 8/8. So, all core samples were determined correctly using all the concerned workflows. For LEQA1 3 (27.3%) workflows reported a false indeterminate/inconclusive/equivocal result for the no virus control; 2 (18.2%) of the workflows reported one false negative result for the lowest concentration SARS-CoV-2 containing core specimens (dilution d3, see Table 1); 2 (18.2%) workflows reported of the workflows reported a false indeterminate/inconclusive/equivocal result for both lowest concentration SARS-CoV-2 containing core specimens (dilution d3, see Table 1); 2 (18.2%) workflows reported false negative results for both lowest concentration SARS-CoV-2 containing core specimens (dilution d3, see Table 1); 1 (9.1%) workflow only tested part of the LEQA1 panel and did not test the reduced mPOCT panel. Most of the improvements in LEQA2 compared to LEQA1 have been obtained for the SARS-CoV-2 dilution d3 of the core samples (10-fold higher concentration than the educational load sample), suggesting that the

concerned workflows gained some sensitivity between both LEQA rounds. These results are summarized in Table 8.

Table 7. Overview of the reasons workflows obtained lower grades for LEQA2 than for LEQA1. For LEQA1 the previously obtained grades are also shown and how they were obtained.

Lower \ Higher		**LEQA1**	
		1x false indeterminate/inconclusive/equivocal for SARS-CoV-2 dilution d3 (grade 7.5/8)	No errors (grade 8/8)
LEQA2	1x false indeterminate/inconclusive/equivocal for no virus control (grade 7.5/8)	na[1]	n=1
	1x false indeterminate/inconclusive/equivocal for SARS-CoV-2 dilution d3 (grade 7.5/8)	na	n=1
	1x false negative for SARS-CoV-2 dilution d3 (grade 7/8)	na	n=5
	2x false indeterminate/inconclusive/equivocal for SARS-CoV-2 dilution d3 (grade 7.5/8)	n=2	na
	1x false negative for SARS-CoV-2 dilution d3 *AND* 1x false indeterminate/inconclusive/equivocal for SARS-CoV-2 dilution d3 (grade 6.5/8)	n=1	na
	1x false negative for SARS-CoV-2 dilution d3 (mPOCT panel; grade 6/8)	na	n=1
	2x false negative for SARS-CoV-2 dilution d3 (grade 6/8)	na	n=2[3]
	1x false negative for SARS-CoV-2 variant B.1.1.7; 20B/501Y.V1 VOC-202012/01 (UK) *AND* 1x false positive for no virus control (grade 6/8)[2]	na	n=1

[1] na = not applicable.

[2] These samples were likely switched during testing. Upon retesting by the involved laboratory after closure of the reporting period and receiving the expected results, this workflow correctly determined all core samples of LEQA2.

[3] One of these workflows did find the educational SARS-CoV-2 containing specimen (dilution d4; see Table 1) positive for SARS-CoV-2. This might be an indication that the workflow does have a high sensitivity concerning SARS-CoV-2 detection.

Table 8. Overview of the reasons workflows obtained higher grades for LEQA2 than for LEQA1. For LEQA1 the previously obtained grades are also shown and how they were obtained.

Higher / Lower		**LEQA2**
		No errors (grade 8/8)
LEQA1	1x false indeterminate/inconclusive/equivocal for SARS-CoV-2 dilution d3 (grade 7.5/8)	n=3
	1x false negative for SARS-CoV-2 dilution d3 (grade 7/8)	n=2
	2x false indeterminate/inconclusive/equivocal for SARS-CoV-2 dilution d3 (grade 7/8)	n=2
	2x false negative for SARS-CoV-2 dilution d3 (grade 6/8)	n=3
	1x Testing only part of LEQA panel, but not mPOCT panel (grade 4/8)	n=1

On a laboratory level, when testing LEQA1, two laboratories had workflows with grades below 7/8 only. One of these laboratories (laboratory 61 in Figures 22 and 23) has since improved their workflow and no longer scores a grade below 7 with the implemented workflow. The other laboratory (laboratory 58 in Figures 22 and 23) unfortunately still scores a grade below 7/8. When testing LEQA2, unfortunately two new labs have received grades of below 7/8 only, whereas they did not in LEQA1 (laboratories 45 and 71 in Figures 22 and 23). The workflow of laboratory 71 (in Figure 22 and 23) scored a grade below 7/8 due to missing two core specimen containing SARS-CoV-2. This same workflow however was able to detect SARS-CoV-2 in the educational specimen (dilution d4, see Table 1). This might indicate that this workflow does have a high sensitivity for SARS-CoV-2 detection. The reason why the 10-fold higher concentration has been missed remains unclear.
Also three laboratories who did not participate in LEQA1 have received grades below 7/8 for LEQA2 (laboratories 76, 78 and 79 in Figures 22 and 23). Two of these latter laboratories did not test the full LEQA2 panel nor the reduced mPOCT panel, leading to a lower grade. And therefore the performance of these laboratories can in fact not be evaluated using these results.

4. Discussion and conclusion

Out of 180 workflows reported, 148 scored a 100% correct score for all 8 core specimens (8 points) and thus met all criteria set for reliable SARS-CoV-2 diagnostics, 18 scored between 7-7.5, making it likely that only minor adjustments need to be made to meet all criteria and 14 workflows scored a 6.5 or lower, indicating that a lot of improvements still need to be made for these workflows to be reliable for SARS-CoV-2 diagnostics in clinical diagnostic settings and surveillance. All workflows scoring grades below 6 (n=6) seemed to have obtained a low score due to testing only a small subset of the core samples while not testing the reduced panel of 4 core samples that has been recommended for mPOCT testing. Due to this, a lot of samples received a score of "Not tested" and thus decreased the overall grade of the test. When the workflows on which only the full panel or reduced mPOCT panel were tested are taken into account (n=174), none of the workflows submitted received a grade below 6.

When considering all workflows each laboratory has access to and have reported LEQA2 results for, six laboratories have workflows which do not have a grade of 7 or above. Although it is not necessary to use a workflow scoring a 7 or 7.5 out of 8 for SARS-CoV-2 diagnostics, due to slight reduced sensitivity to detect SARS-CoV-2, during the current period with a prevalence of COVID-19 around 10% or higher, it is likely that only minor adaptions of the concerned workflows are needed to perform within the desired criteria detecting all core samples correctly.

Only four laboratories have access to solely mPOCT based assays, limiting their maximal daily throughput of clinical specimens.

Throughout the reported workflows lots of different target genes and combinations of them are reported, but the E-gene as target gene as either a Sarbeco specific or SARS-CoV-2 specific target is most prevalent (115 out of 180 workflows). There was no significant difference in performance between workflows using different single target genes or combinations of target genes.

False indeterminate/inconclusive/equivocal results have been obtained for samples containing hCoV-NL63 (1/176; 0.6%) and no virus control (1/160; 0.6%). Otherwise no such results have been reported for non SARS-CoV-2 containing specimen. This shows that the implemented workflows show a high specificity to SARS-CoV-2 and the RT-PCRs or other NAATs rarely cross-react to other human coronaviruses or other non SARS-CoV-2 containing samples. One false positive result for LEQA2_CoV20-10 that does not contain virus has been reported by the laboratories. This was likely due to accidental swapping of specimens LEQA2_CoV20-9 and -10 by the technician. Additionally, many workflows reported the SARS-CoV-1 containing sample (LEQA2_CoV20-1) positive for SARS-CoV-2 due to using Sarbeco-specific RT-PCR. Considering the fact that SARS-CoV-1 is not circulating, this does not pose a problem for the accuracy of SARS-CoV-2 diagnostics performed in clinical and surveillance settings. However, it is striking that for a quite some workflows SARS-CoV-2 specific E-gene primers/probe are reported whilst it still detects SARS-CoV-1 (21.74%; Supplemental Figure 1). It appears that for these workflows most likely the specificity of E-gene primers/probe is wrongly labelled as SARS-CoV-2 specific rather than Sarbeco specific. Or have a strong cross-reaction to SARS-CoV-1, which is less likely.

Whereas very little false positive results have been reported for the core samples included in the panel, a number of workflows reported false negative results for some of the core samples containing SARS-CoV-2. Apparently sensitivity is a bigger issue than specificity for the workflows used for SARS-CoV-2 diagnostics.

Despite some workflows generated a Ct value using SARS-CoV-2 RT-PCR, the sample was deemed SARS-CoV-2 negative, possibly related to a used cut off value or other criterium, e.g. shape of the amplification curve. Up to 1.5% of each SARS-CoV-2 positive sample (range: 0% – 1.5%) was reported as SARS-CoV-2 negative despite one (or more) of the target genes against SARS-CoV-2 in the assay

giving a Ct value. For all LEQA samples tested with a workflow providing Ct values as an output, a broad range of Ct values for the individual SARS-CoV-2 containing samples has been reported. The biggest range of reported Ct values was found for LEQA_CoV20-9. The average Ct value was 26.89 (SD 2.40; data not shown) where the lowest Ct value reported was 16.29 and the highest value was 41.60 (making a 25.31 difference in Ct value), indicating a wide spread of Ct values reported for the same sample, generated by the large variety of workflows used. Despite this wide range of Ct values for the same sample by different workflows, this did not affect the sensitivity of the workflows significantly. This finding indicates clearly that comparing Ct values between workflows and laboratories should not be done without calibration using a standard.

A wide array of varying in-house and kit-based SARS-CoV-2 workflows have been reported, that has been changed and even broadened compared to LEQA1 [2]. Compared to the 2009 influenza pandemic, the Dutch clinical diagnostic field for respiratory diagnostics shows a divergent pattern in use of kits, reagents and equipment. [4] A more divergent use of kits, reagents and equipment can be quite useful in a laboratory network as a shortage of any of these can be compensated by switching to different equipment or when certain workflows are less capable of detecting new strains of SARS-CoV-2. This is highly relevant with the rising level of infections with new variants of SARS-CoV-2. Therefore it is encouraging that in the current LEQA2 variant of concern B.1.1.7; 20B/501Y.V1 that has become the major strain (up to 82%) in circulation by week 9/2021 in The Netherlands [1] was detected by 176/177 (99.4%) workflows that tested this sample.

Comparing this second round of quality assessment with the first round, 14 additional laboratories reported their findings. The total number of reported workflows increased with 15 compared to the earlier assessment. From the workflows initially scoring a grade <6 for LEQA1, 4/6 (66.7%) were not retested with LEQA2. Similarly, from 5/8 (62.5%) workflows initially scoring a 6, 5/11 (45.5%) workflows initially scoring a 7, 1/7 (14.3%) workflows initially scoring a 7.5 and 50/132 (37.9%) workflows initially scoring an 8 were not retested with LEQA2. The relative amount of LEQA1 workflows not re-tested in LEQA2 is bigger for the poorer scoring workflows (<7) compared to the better scoring workflows (7-8) in LEQA1. This data seems to indicate that laboratories use these quality assessments as a way to improve their performance in SARS-CoV-2 diagnostics. The absolute amount of workflows obtaining a score of <6 only decreased by 1 (from 6 to 5) from LEQA1 to LEQA2. This is due to an influx of four new workflows compared to LEQA1 with which not the full LEQA2 panel nor the reduced mPOCT panel was tested, leading to a lower grade. Two of these new workflows belong to laboratories that did not test the LEQA1 panel. Nevertheless, there seems to be a trend showing a disuse of lesser performing workflows. However, this data might be biased due to not all workflows being used by a laboratory have been tested and reported in this second round by all laboratories.

Overall we can conclude that the workflows used for SARS-CoV-2 diagnostics for the Dutch population perform very well. There are some workflows which need some work in order for them to perform as desired, but all in all the Dutch SARS-CoV-2 diagnostics laboratory network appears to perform on a very high level.

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.

2. J. Sluimer et al., "External Quality Assessment of laboratories Performing SARS-CoV-2 Diagnostics for the Dutch Population, November 2020", RIVM report. Available from: https://www.rivm.nl/sites/default/files/2021-02/EQA%2520of%2520Laboratories%2520Performing%2520SARS-CoV-2%2520Diagnostics%2520for%2520the%2520Dutch%2520Population%2520November-2020.pdf

3. RIVM. Aanvullende informatie diagnostiek COVID-19. 2021 [cited 2021 26-3-2021]; 24: Available from: https://lci.rivm.nl/covid-19/bijlage/aanvullend.

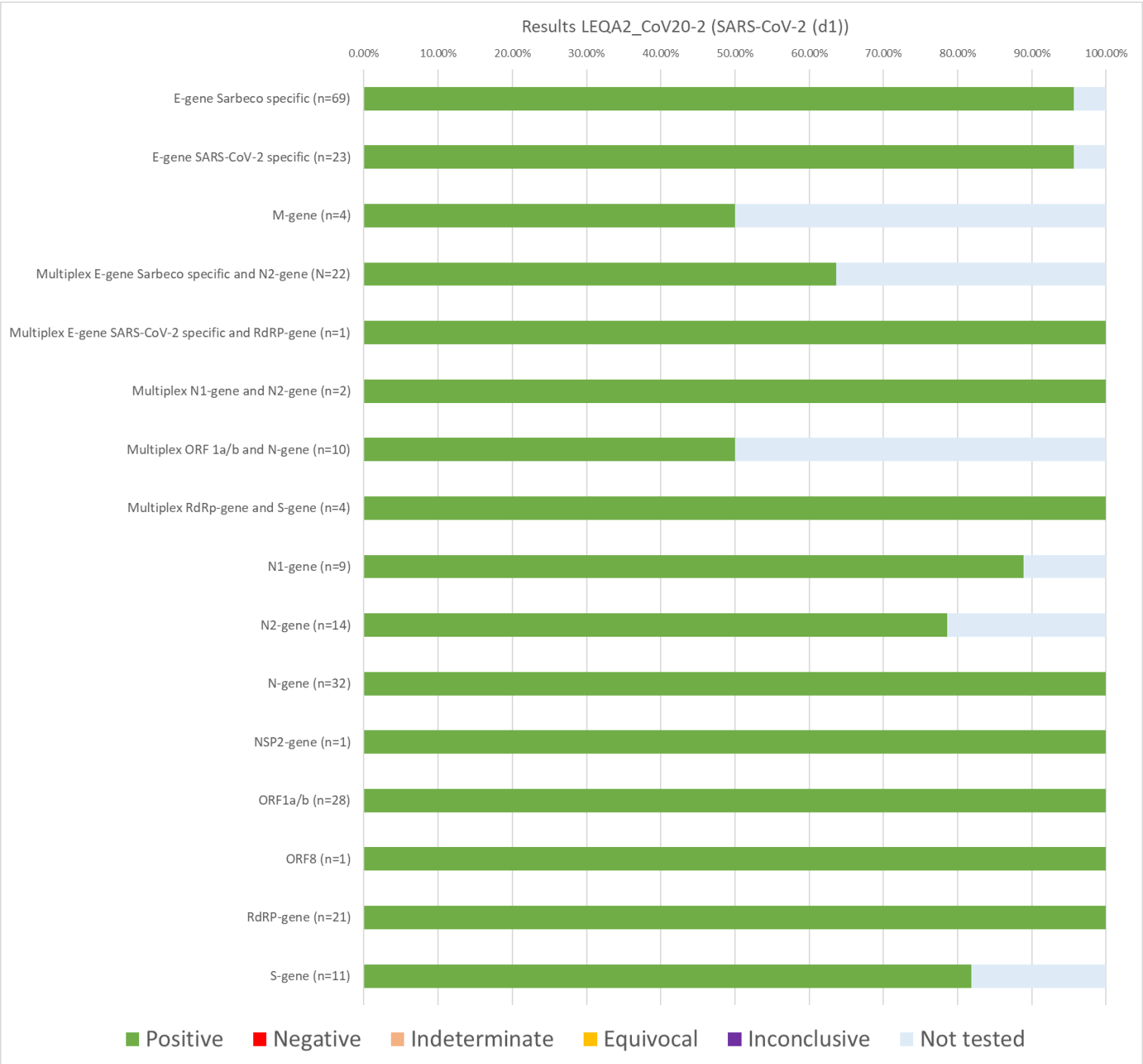
4. A. Meijer et al., "Preparing the outbreak assistance laboratory network in the Netherlands for the detection of the influenza virus A(H1N1) variant", J. Clin. Virol., vol. 45, no. 3, pp. 179–184, 2009.

6. Supplemental material

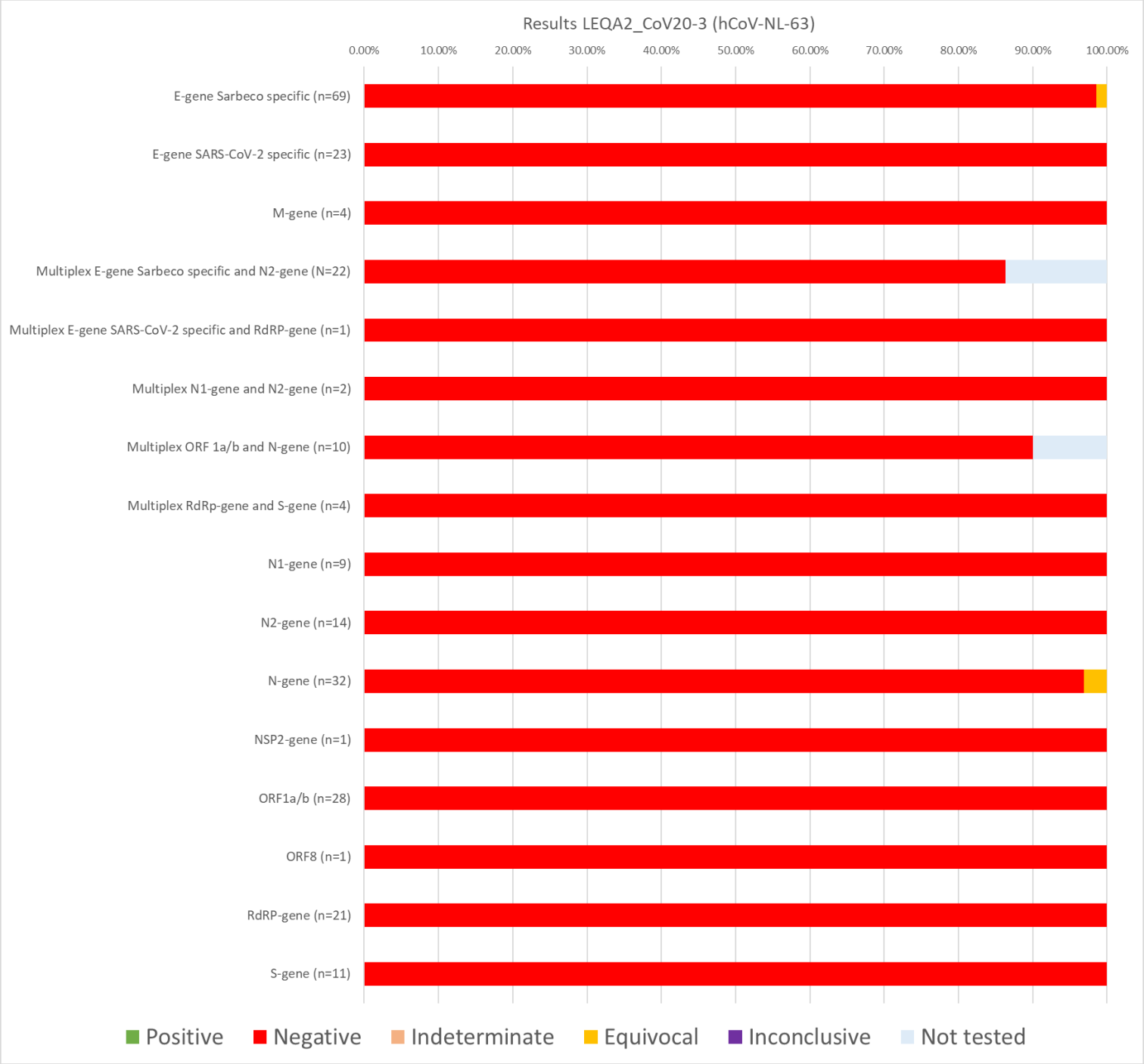
6.1 Results obtained per target gene per sample

Here all results (any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive or Not tested) obtained per target gene are shown in percentages per panel sample number. Some workflows using more than one gene do not generate separate result for each independent gene but rather a composite conclusion. In Supplemental Figure 1-10 these are shown together as one target gene.

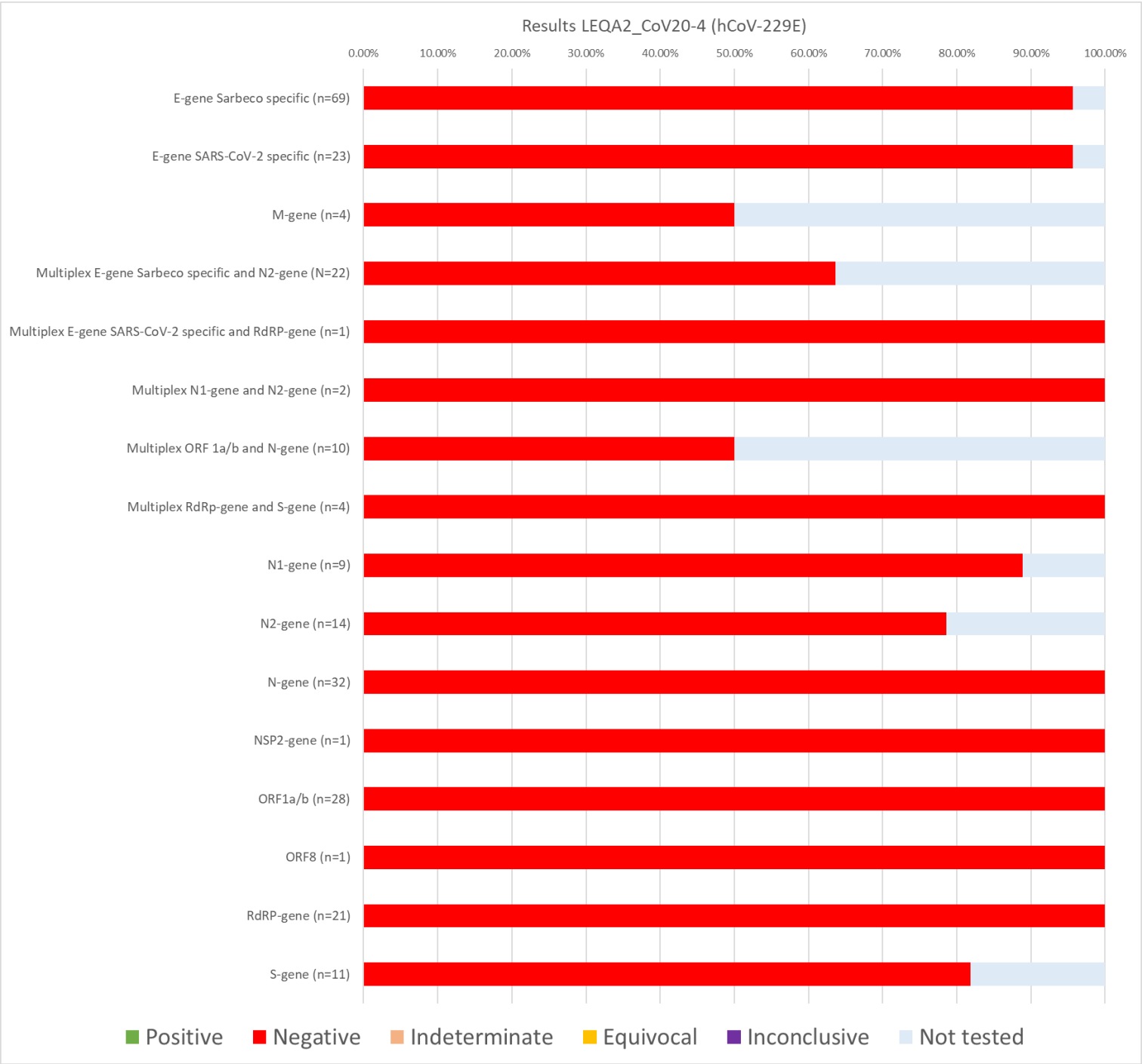
Supplemental Figure 1: The percentages of the various scores obtained for LEQA2_CoV20-1 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



Supplemental Figure 2: The percentages of the various scores obtained for LEQA2_CoV20-2 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample

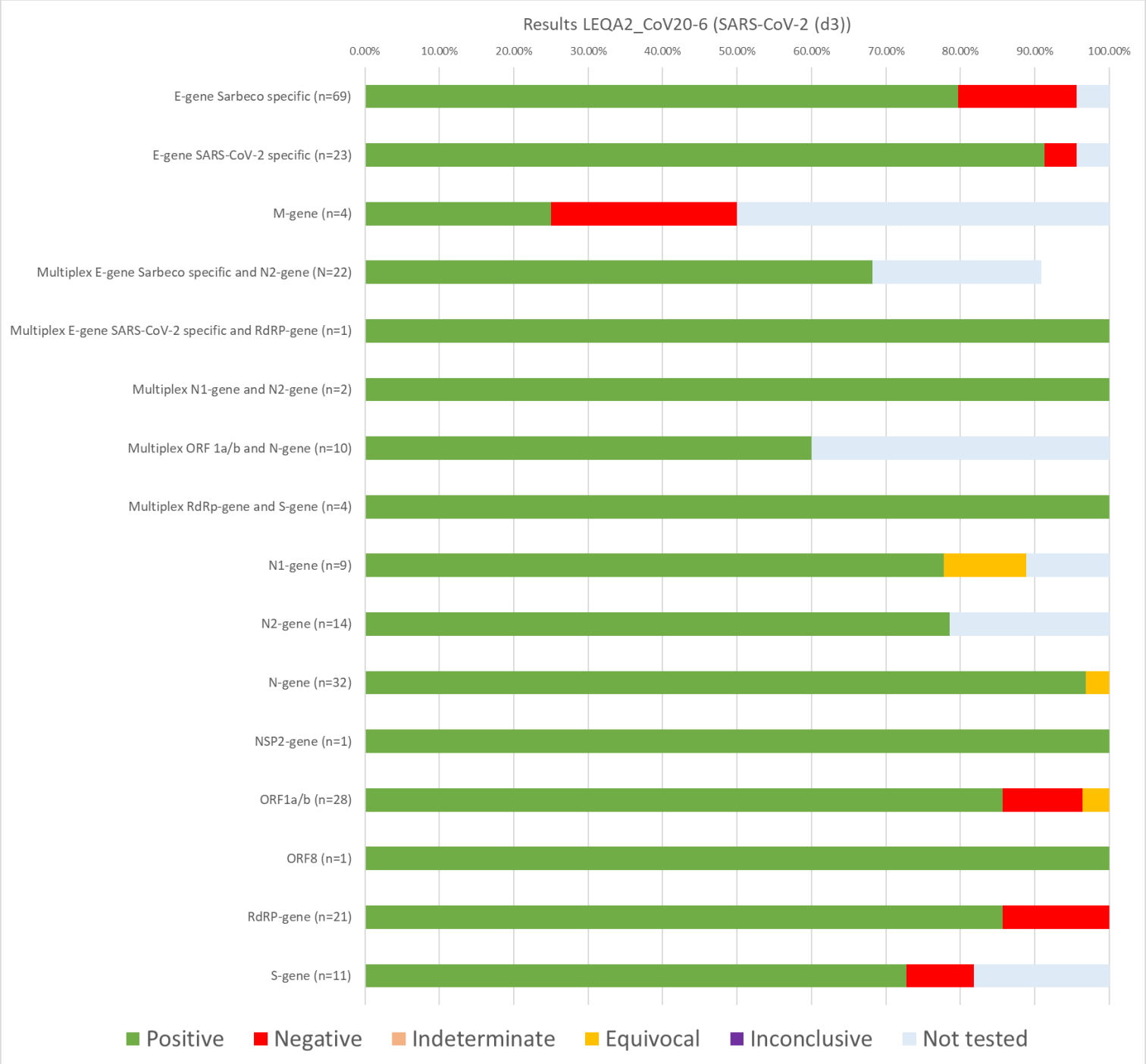


Supplemental Figure 3: The percentages of the various scores obtained for LEQA2_CoV20-3 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample

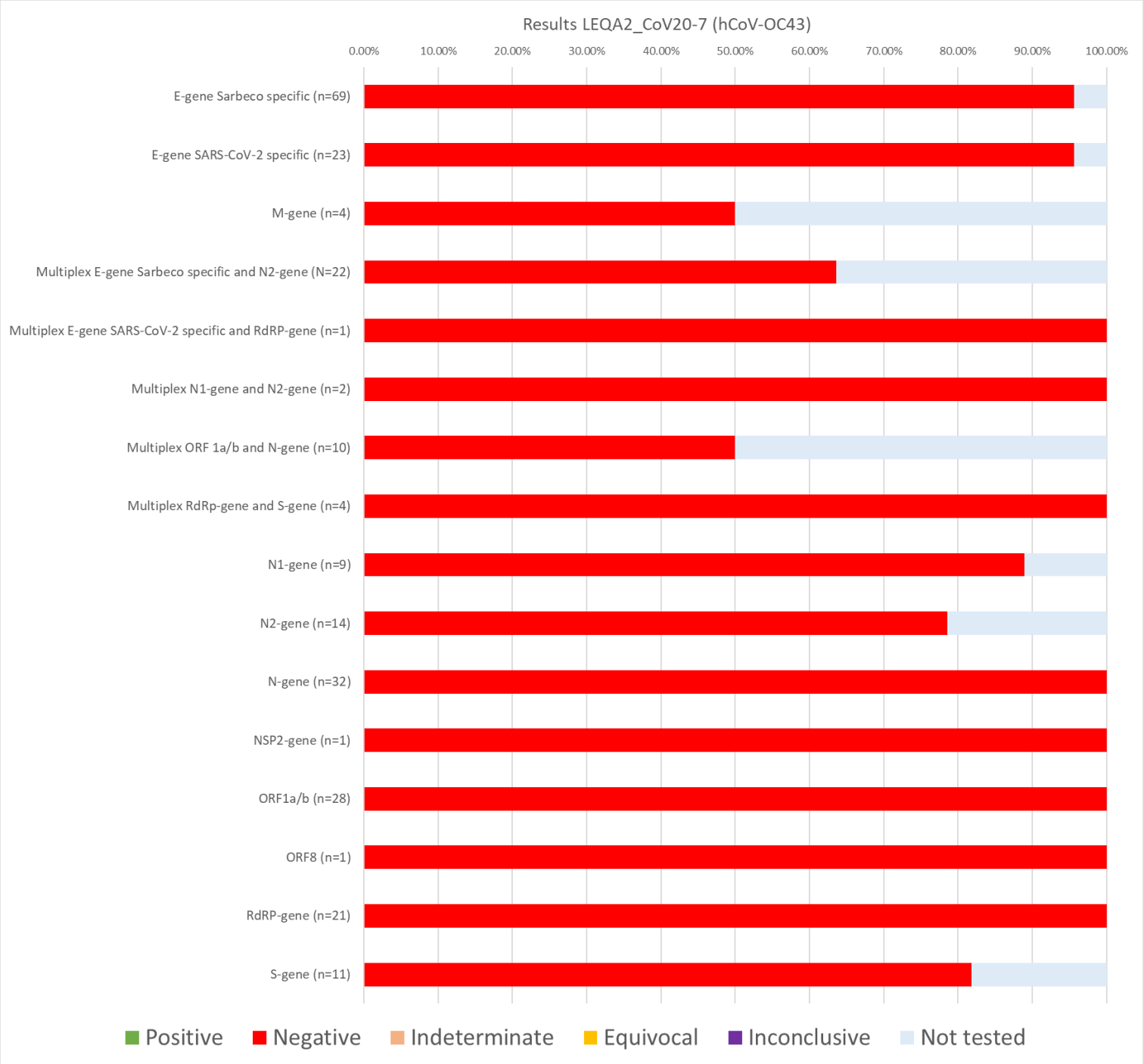


Supplemental Figure 4: The percentages of the various scores obtained for LEQA2_CoV20-4 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample. This sample was not deemed a core sample from the LEQA panel.

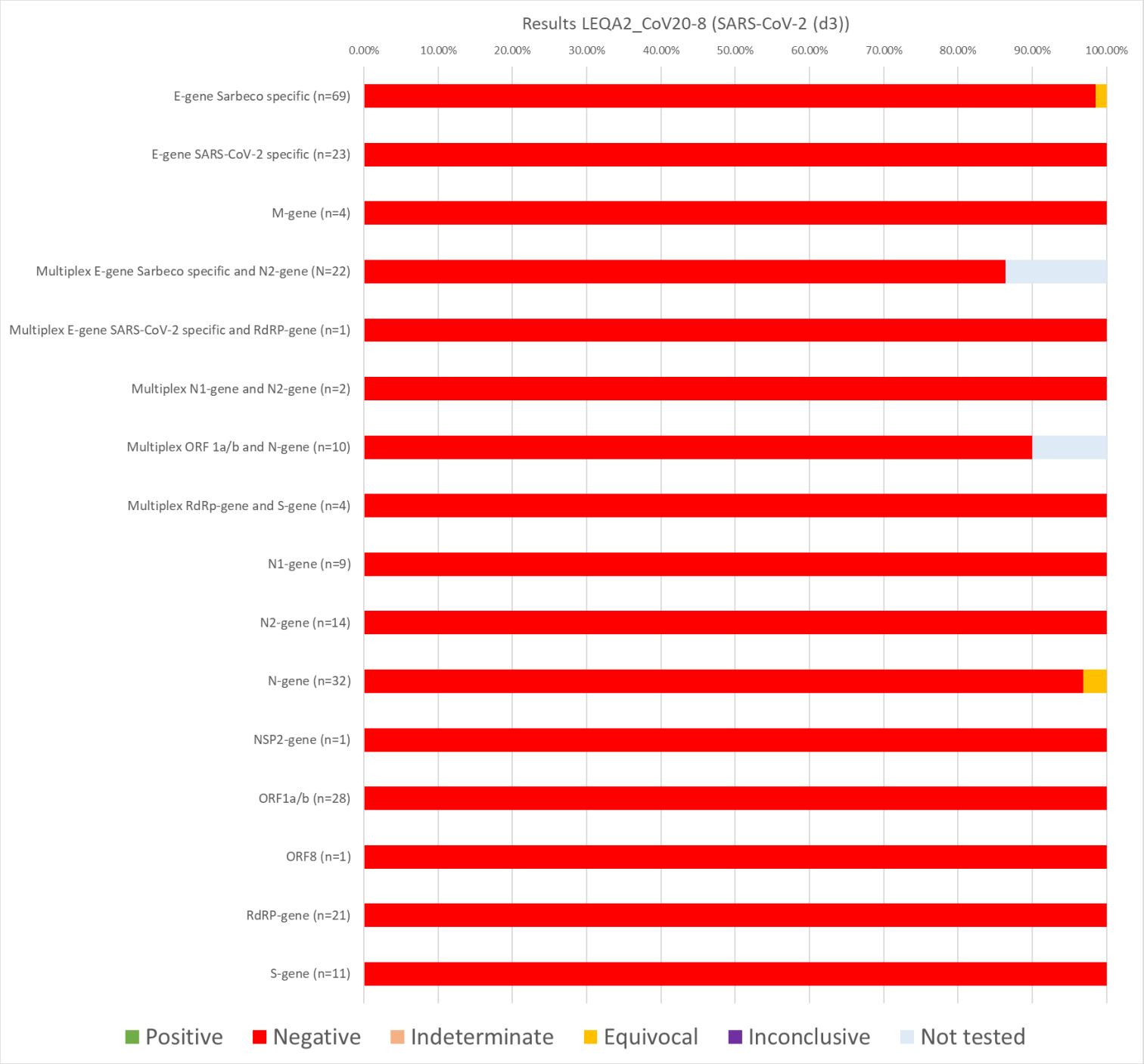
Supplemental Figure 5: The percentages of the various scores obtained for LEQA2_CoV20-5 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



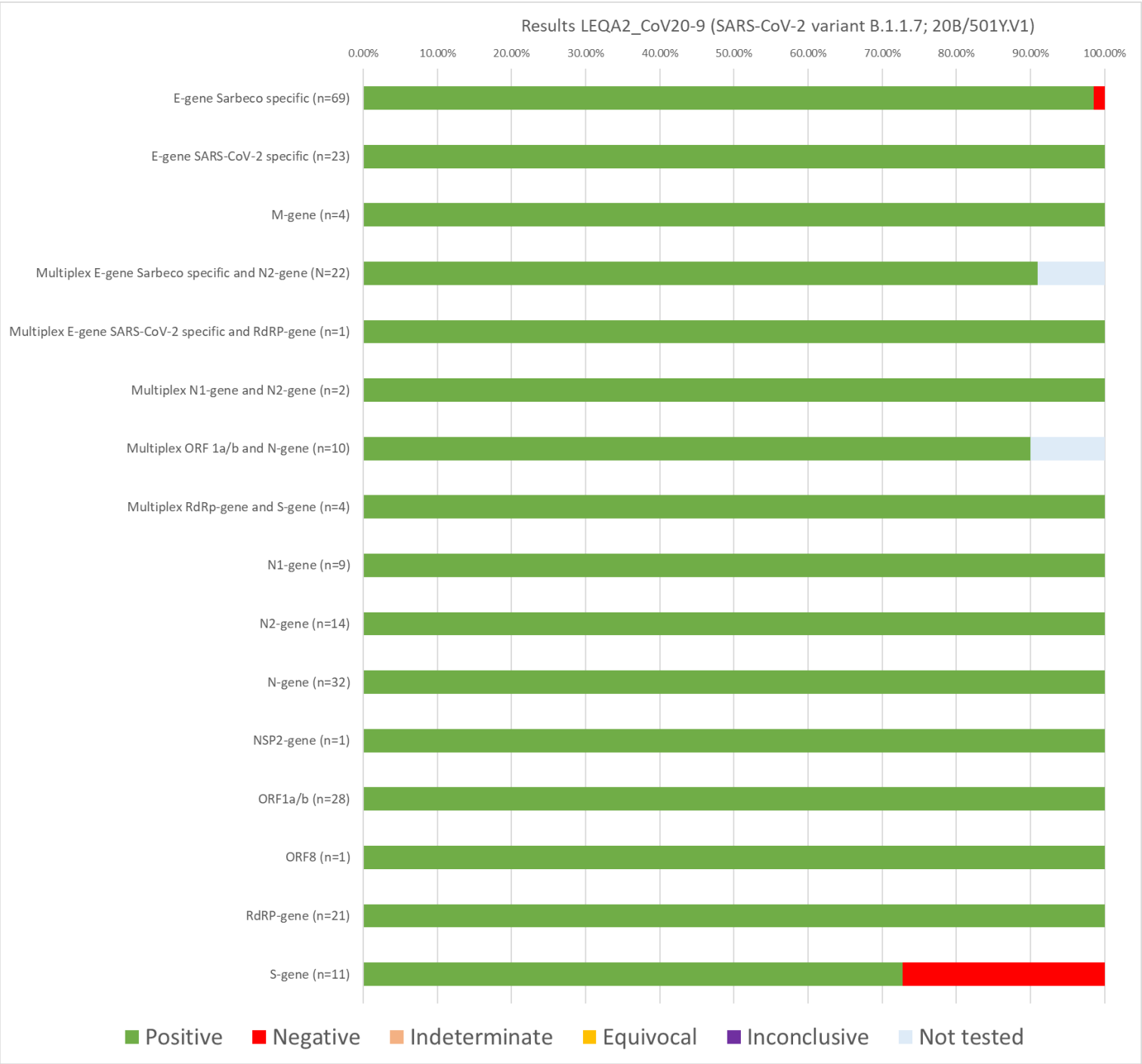
Supplemental Figure 6: The percentages of the various scores obtained for LEQA2_CoV20-6 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



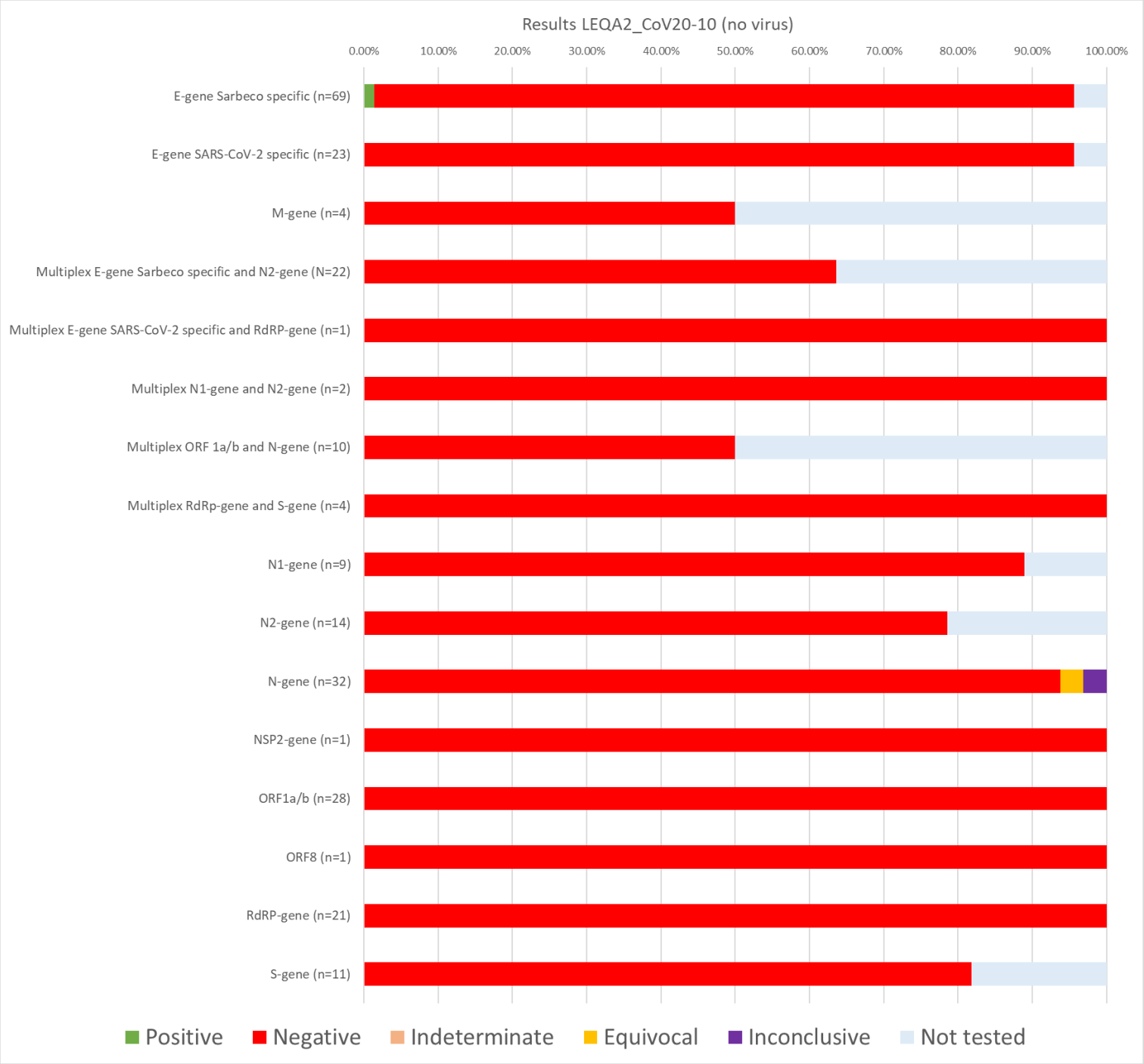
Supplemental Figure 7: The percentages of the various scores obtained for LEQA2_CoV20-7 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample.



Supplemental Figure 8: The percentages of the various scores obtained for LEQA2_CoV20-8 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



Supplemental Figure 9: The percentages of the various scores obtained for LEQA2_CoV20-9 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample.



Supplemental Figure 10: The percentages of the various scores obtained for LEQA2_CoV20-10 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample. This sample was not deemed a core sample from the LEQA panel.

6.2 Participating laboratories

All participating laboratories are listed below. We would like to thank colleagues from these laboratories for their participation in this round of LEQA for the Dutch SARS-CoV-2 diagnostics laboratory network.

Laboratory
Admiraal de Ruyter Ziekenhuis
Alrijne Zorggroep
ArminLabs GmbH
Atalmedial
Brightlabs B.V.
Canisius Wilhelmina Ziekenhuis
Catharina Ziekenhuis
Certe
Deventer Ziekenhuis
Diagnostiek voor U
Diakonessenhuis Utrecht
Elisabeth Tweesteden Ziekenhuis
Erasmus Medisch Centrum
Eurofins Biomnis France
Eurofins Genomics Europe Applied Genomics GmbH
Eurofins NMDL-HVL
Eurofins NMDL-LCPL
Fenelab Consortium - Mérieux Nutrisciences
Fenelab Consortium - NofaLab B.V.
Fenelab Consortium - Normec Biobeheer
Fenelab Consortium - Nutreco Nederland B.V. - MasterLab
Fenelab Consortium - NutriControl
Fenelab Consortium - Nutrilab B.V.
Fenelab Consortium - SGS Nederland B.V.
Fenelab Consortium - Triskelion
Franciscus Gasthuis & Vlietland
Gelre Ziekenhuizen Apeldoorn
GGD Amsterdam Streeklaboratorium
Groene Hart Ziekenhuis
Haaglanden Medisch Centrum
Hagaziekenhuis
IJssellandziekenhuis
Ikazia ziekenhuis
inBiome
Isala
Izore
Jeroen Bosch ziekenhuis
LabMicTA
Labor Dr Wisplinghoff
Laurentius ziekenhuis

Laboratory
Leiden University Medical Center
Maasstad ziekenhuis
MeanderMC
Medizinisches Versorgungszentrum Dr. Stein + Kollegen
Microbe & Lab
Microvida
Mozand B.V.
Noordwest Ziekenhuisgroep Alkmaar
Novogenia GmbH
OLVG Lab B.V.
Pro Health Medical
RadboudUMC
Reinier Haga MDC
Rijnstate
RLM Dordrecht-Gorinchem
Royal GD
Saltro, locatie Hudsondreef
Saltro, locatie Mississippidreef
Sanquin, NSS
St. Antonius ziekenhuis
Star-shl
Stichting PAMM
Streeklab Haarlem
SYNLAB Heppignies
Synlab MVZ Weiden
SYNLAB Jena Oncoscreen
SYNLAB Laboratoire Collard
SYNLAB MVZ Trier
Tergooi - CBSL
TLR International Laboratories
TNO
Universitair Medisch Centrum Groningen
Universitair Medisch Centrum Utrecht
VieCuri MC
Wageningen Bioveterinary Research
Ziekenhuis Gelderse Vallei
Ziekenhuis Rivierenland Tiel
Ziekenhuis StJansdal
Zuyderland Medisch Centrum