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, November 2020

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. RMol performed pretesting of the panel. RMoe provided assistance in data reporting and data analysis. JS performed the analysis of the reported data with the assistance of AM and RMoe. JS wrote the first draft of the report and together with AM edited it for the final version. All authors reviewed the report before publication.

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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: 6, 8, 20, 21, 22 and 23.

Version 2: 26-01-2021: errata compared to version 1:
Page 4: Changed number (19>18) and percentage (11.6>11.0) of laboratories scoring a grade between 7 and 7.5.
Page 17: Changed in a sentence the number of workflows reporting either 1 (88>76), 2 (76>78) or 3 (10>8) target genes and changed in Figure 7 target gene 1 the number of E-gene SARS-CoV-2 specific (62>64).
Page 18: Changed 'gens' to 'genes' in the title of Figure 7.
Page 25: Changed the number of laboratories that reported 8 workflows (3>2) or 7 workflows without mPOCT (2>1) and added one laboratory reporting 7 or 6 workflows respectively.
Page 28+29: Made the same changes as on page 25 in Figures 22 and 23.
Page 30: Changed number (19>18) of laboratories scoring a grade between 7 and 7.5.
Page 43+44: Reporters for each laboratory removed due to 'Algemene verordening gegevensbescherming (AVG)'/General Data Protection Regulation (GDPR) rules for publication of names of persons from which no personal signed informed consent was obtained.

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 EQA. This report includes the first 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 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 October 2020 the LEQA panel was produced at the RIVM. It was pre-tested at both Dutch expertise centers (RIVM and Erasmus MC). After both centers obtained similar 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 November 2020. 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 1, 2, 4 and 7. 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 reduction 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.

Results

Out of the 164 reported workflows reported by 65 laboratories, 132 (80.5%) scored 100% correct results (score 8) and thus met all criteria set for reliable SARS-CoV-2 diagnostics, 18 (11.0%) scored between 7-7.5 out of 8, making it likely that only minor adjustments need to be made to meet all criteria and 14 (8.5%) workflows scored a 6 or lower, indicating that a lot of improvements still need to be made for these workflows to be reliable for clinical diagnostic settings and surveillance. For the SARS-CoV-2 negative core samples, no false positives have been reported, but some workflows gave indeterminate, equivocal or inconclusive results for non-SARS-CoV-2 containing samples (n=4). For the SARS-CoV-2-containing core samples, false negative results (n=24) and false indeterminate, equivocal or inconclusive results (n=13) were reported. Some workflows reported a negative result for SARS-CoV-2 presence in SARS-CoV-2-containing samples due to cutoff values used in the assay; up to 1.7% of each SARS-CoV-2 positive sample (range: 0% – 1.7%) 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. 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.

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. 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 November 2020 the first 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 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

In October 2020 the LEQA panel was produced at the RIVM after which it was pretested at both Dutch expertise centers (RIVM and Erasmus MC). After both centers obtained similar results per sample, all 70 laboratories (excluding the expertise centers) performing SARS-CoV-2 diagnostics in the Dutch network by November 2020 were contacted and notified of the distribution of the panel in the first week of November. 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 22nd of November to report their obtained results. After the 22nd of November 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 29th of November. 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 1, 2, 4 and 7.

2.2 Contents of LEQA panel

The LEQA 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 virus. SARS-CoV-2 was isolated from a clinical specimen 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 this stock 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 at 1.28*10^10 and 1.73*10^10 copies of E-gene and RdRp-gene positive strand RNA/ml, respectively. Because the virus was 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 (RIVM isolate) in various concentrations, hCoV-NL63 (kindly provided by Lia van der Hoek, Amsterdam University Medical Hospital), hCoV-229E (ATCC), hCoV-OC43 (ATCC), Influenza virus A(H3N2) (RIVM), SARS-CoV-1 (RNA) (kindly provided by Bart Haagmans, Erasmus MC) and a sample without any virus. 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 LEQA1 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
LEQA1_CoV20-1	SARS-CoV-2 (d1)	1.28*10^5	1.73*10^5	n/a	28.52 (4)	28.37 (4)	POSITIVE
LEQA1_CoV20-2	hCoV-NL63	-	-	28.10 (4)	Neg	Neg	Negative
ELQA1_CoV20-3	hCoV-229E	-	-	17.22 (4)	Neg	Neg	Negative
LEQA1_CoV20-4[5]	SARS-CoV-2 (d4)	1.28*10^2	1.73*10^2	n/a	36.95 (2)	35.59 (2)	Weakly POSITIVE
LEQA1_CoV20-5	SARS-CoV-2 (d3)	1.28*10^3	1.73*10^3	n/a	34.80 (4)	34.88 (4)	POSITIVE
LEQA1_CoV20-6	hCoV-OC43	-	-	27.77 (4)	Neg	Neg	Negative
LEQA1_CoV20-7	SARS-CoV-2 (d3)	1.28*10^3	1.73*10^3	n/a	34.68 (4)	34.74 (4)	POSITIVE
LEQA1_CoV20-8	Influenza virus A(H3N2)	-	-	22.76 (4)	Neg	Neg	Negative
LEQA1_CoV20-9	Negative	-	-	Neg	Neg	Neg	Negative
LEQA1_CoV20-10[6]	SARS-CoV-1	-	-	n/a	28.57 (4)	Neg	Negative[5]

[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 influenza virus A(H3N2) matrix gene; for hCoV-NL63 and hCoV-229E N-gene and hCoV-OC43 M-gene; n/a = not applicable.

[5] Educational specimen: repeats of this specimen may have the E-gene and/or RdRp-gene negative; only 38% of reported workflow reported this specimen positive for SARS-CoV-2.

[6] 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.

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 LEQA1_CoV20-4 and LEQA1_CoV20-10 (containing an educational load of SARS-CoV-2 and SARS-CoV-1 RNA, respectively) were deemed core samples (samples with clinically relevant amounts of 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 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 LEQA1_CoV20-01, LEQA1_CoV20-02, LEQA1_CoV20-04 and LEQA1_CoV20-07. 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. Workflows scoring 7.5 or 7 out of 8 might still be viable 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 LEQA1_CoV20-05 or LEQA1_CoV20-07 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

Seventy laboratories were contacted with the announcement of panel distribution for this first EQA round. Sixty-five (92.9%) of these laboratories reported their findings for 164 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=164)	SARS-CoV-2 detection workflow conclusion			
			№ Positive	№ Indeterminate, Equivocal or Inconclusive	№ Negative	Errors
LEQA1_CoV20-1	SARS-CoV-2 (d1)	162	161 (99.38%)	0	1 (0.62%)	False negative (n=1)
LEQA1_CoV20-2	hCoV-NL63	160	0	0	160 (100%)	None
ELQA1_CoV20-3	hCoV-229E	146	0	1 (0.68%)	145 (99.32%)	False indeterminate, equivocal or inconclusive (n=1)
LEQA1_CoV20-4	SARS-CoV-2 (d4)	162	78 (48.15%)	21 (12.96%)	63 (38.89%)	Not applicable, educational sample
LEQA1_CoV20-5	SARS-CoV-2 (d3)	148	131 (88.51%)	7 (4.73%)	10 (6.76%)	False negative (n=10), false indeterminate, equivocal or inconclusive (n=7)
LEQA1_CoV20-6	hCoV-OC43	145	0	0	145 (100%)	None
LEQA1_CoV20-7	SARS-CoV-2 (d3)	158	139 (87.97%)	6 (3.80%)	13 (8.23%)	False negative (n=13), false indeterminate, equivocal or inconclusive (n=6)
LEQA1_CoV20-8	Influenza virus A(H3N2)	144	0	0	144 (100%)	None
LEQA1_CoV20-9	Negative	144	0	3 (2.08%)	141 (97.92%)	False indeterminate, equivocal or inconclusive (n=3)
LEQA1_CoV20-10	SARS-CoV-1	147	63 (42.68%)	22 (14.97%)	62 (42.18%)	Not applicable, educational sample

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
8	3	4-7 (n=3)	1 (n=1)	1-4 (n=2)
6	2	2-6 (n=2)	4 (n=1)	0
5	3	5 (n=3)	0	0
4	9	2-4 (n=9)	1 (n=4)	1-2 (n=4)
3	6	1-3 (n=6)	2 (n=1)	0
2	18	1-2 (n=17)	1 (n=2)	1-2 (n=3)
1	24	1 (n=18)	1 (n=5)	1 (n=1)

Despite not all workflows obtained fully correct results with the core specimens (Table 2), nearly all laboratories (63/65; 96.9%) used at least one workflow with which a score 7-8 was obtained (Table 3). Except for one laboratory, 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 18/24 (75%) used a workflow with which a score of 8 was obtained. The main cause of obtaining a score of 7.5 or 7 for workflows that tested all specimens was reporting of an Indeterminate, Equivocal or Inconclusive result for samples LEQA1_CoV20-5 and LEQA1_CoV20-7 that contained the same viral load of SARS-CoV-2. If we consider such results acceptable 23/24 (95.8%) of laboratories using one workflow obtained fully correct or acceptable results. In the subsequent chapters a more detailed insight in the background of the results and the results themselves is 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 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 there 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).

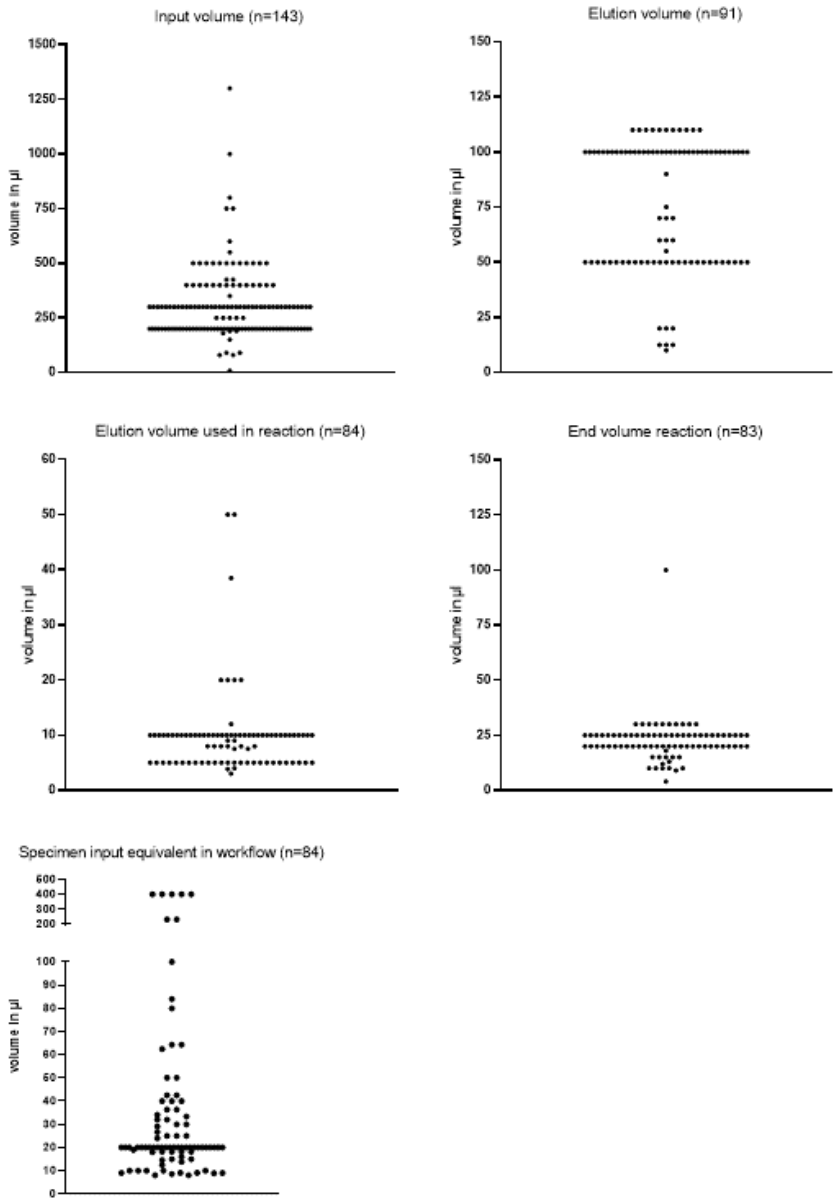


Figure 1: Volumes used during the RT-PCR of other NAATs described for the workflows used. Most commonly used volumes: 200 µl input volume; 100 µl elution volume; 10 µl elution input volume in reaction; 20 µl specimen input equivalent; 20 or 25 µl end volume in reaction.

Another factor that may determine the performance of the workflow 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.

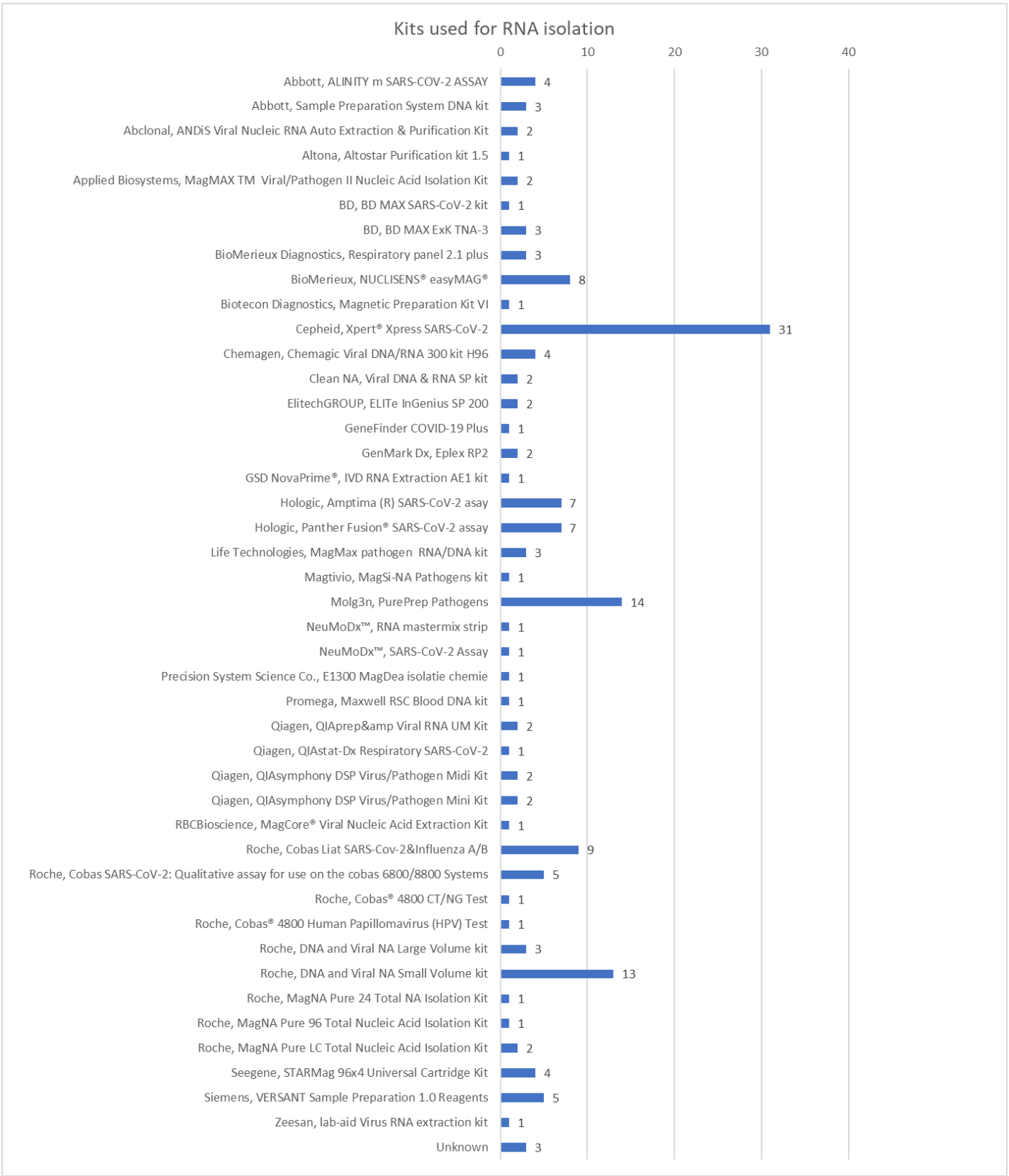


Figure 2: The RNA isolation kits used by workflows testing for SARS-CoV-2 together with the number of workflows per kit (n=164))

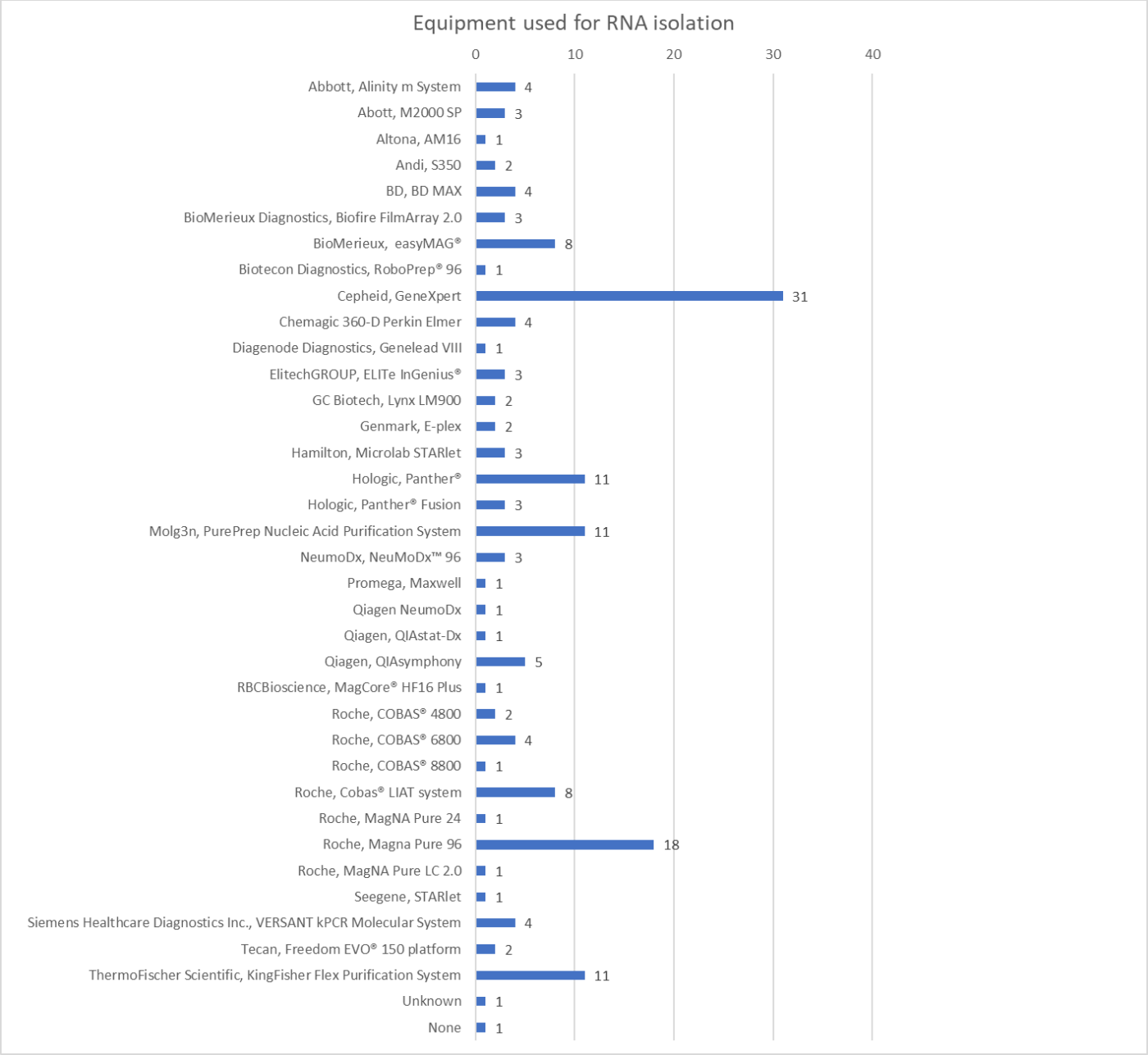


Figure 3: The RNA isolation equipment used by workflows testing for SARS-CoV-2 together with the number of workflows per machine (n=164)

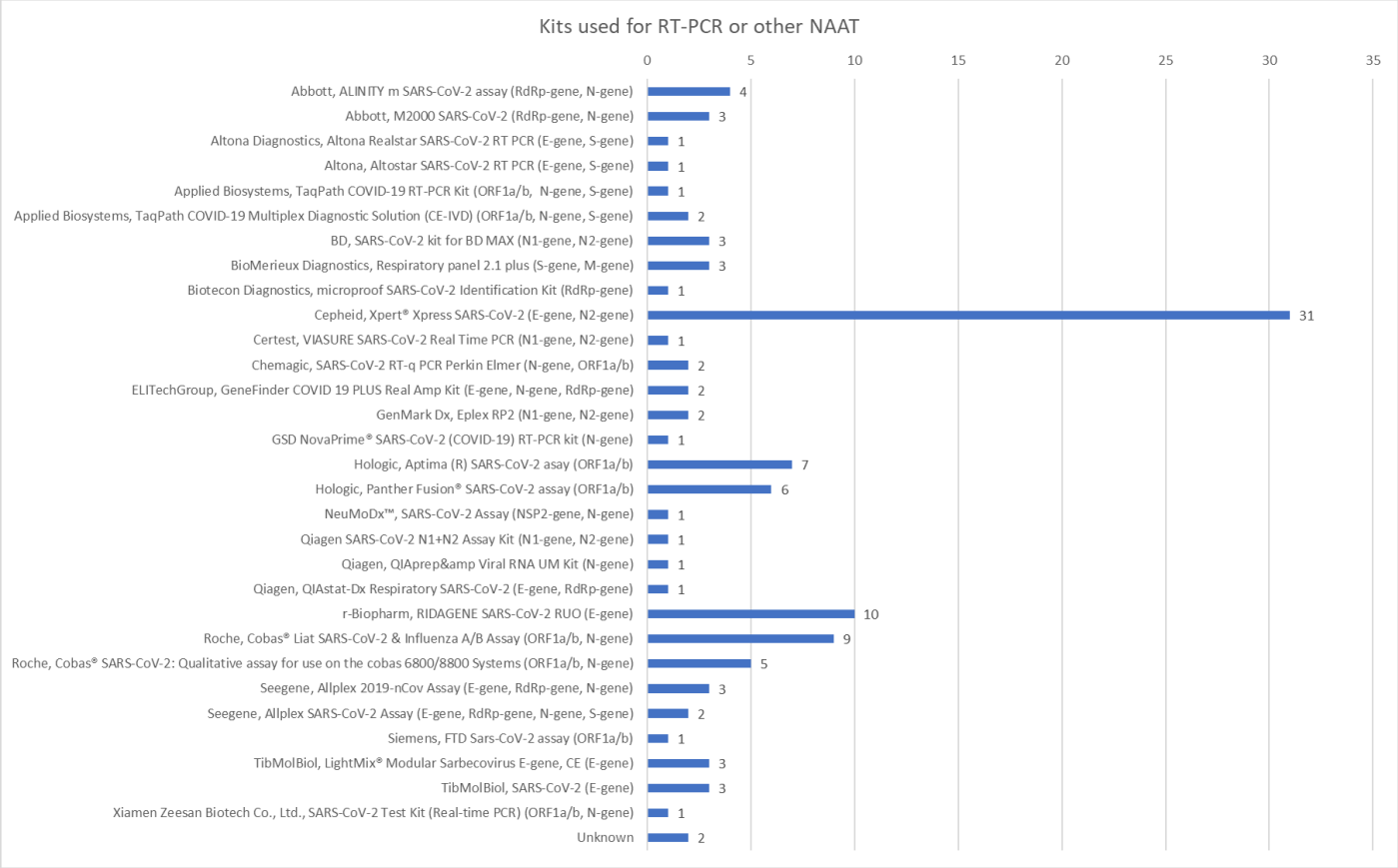


Figure 4: The RT-qPCR or other NAAT kits used by workflows testing for SARS-CoV-2 together with the number of workflows per kit (n=114). Not all workflows use kits for their RT-qPCR or other NAAT, so the total N is not equal to the amount of workflows tested. For each kit the used target genes are listed. Workflows using separate enzymes and primers and probe are listed in Figure 5.

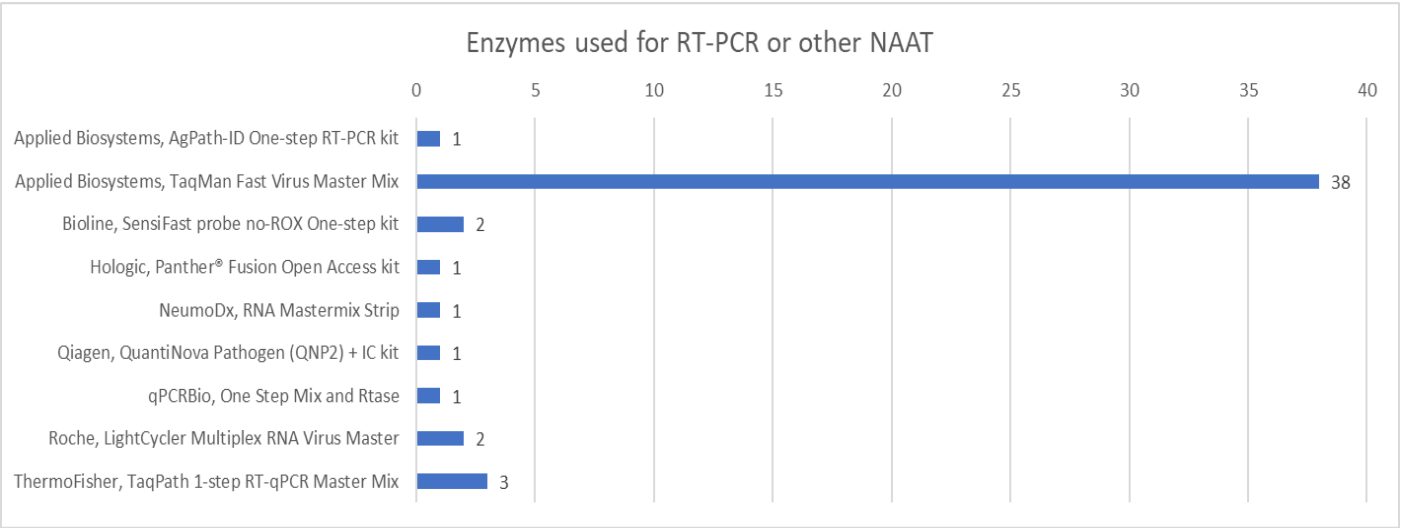


Figure 5: The enzymes used for performing RT-PCR or other NAAT by workflows testing for SARS-CoV-2 together with the number of workflows per enzyme (n=50). Not all workflows use separate enzymes for their RT-PCR or other NAAT, so the total N is not equal to the amount of workflows tested. In total 38/50 of the above mentioned workflows use 1 target gene to test for SARS-CoV-2 presence (E-gene Sarbeco specific (n=26); E-gene SARS-CoV-2 specific (n=6); N-gene (n=3); RdRp-gene (n=3)) and 12/50 workflows use 2 target genes to test for SARS-CoV-2 presence (E-gene Sarbeco specific + E-gene SARS-CoV-2 specific (n=1); E-gene Sarbeco specific + N-gene (n=1); E-gene Sarbeco specific + N1-gene (n=5); E-gene Sarbeco specific + RdRp-gene (n=3); E-gene SARS-CoV-2 specific + N1-gene (n=2)).

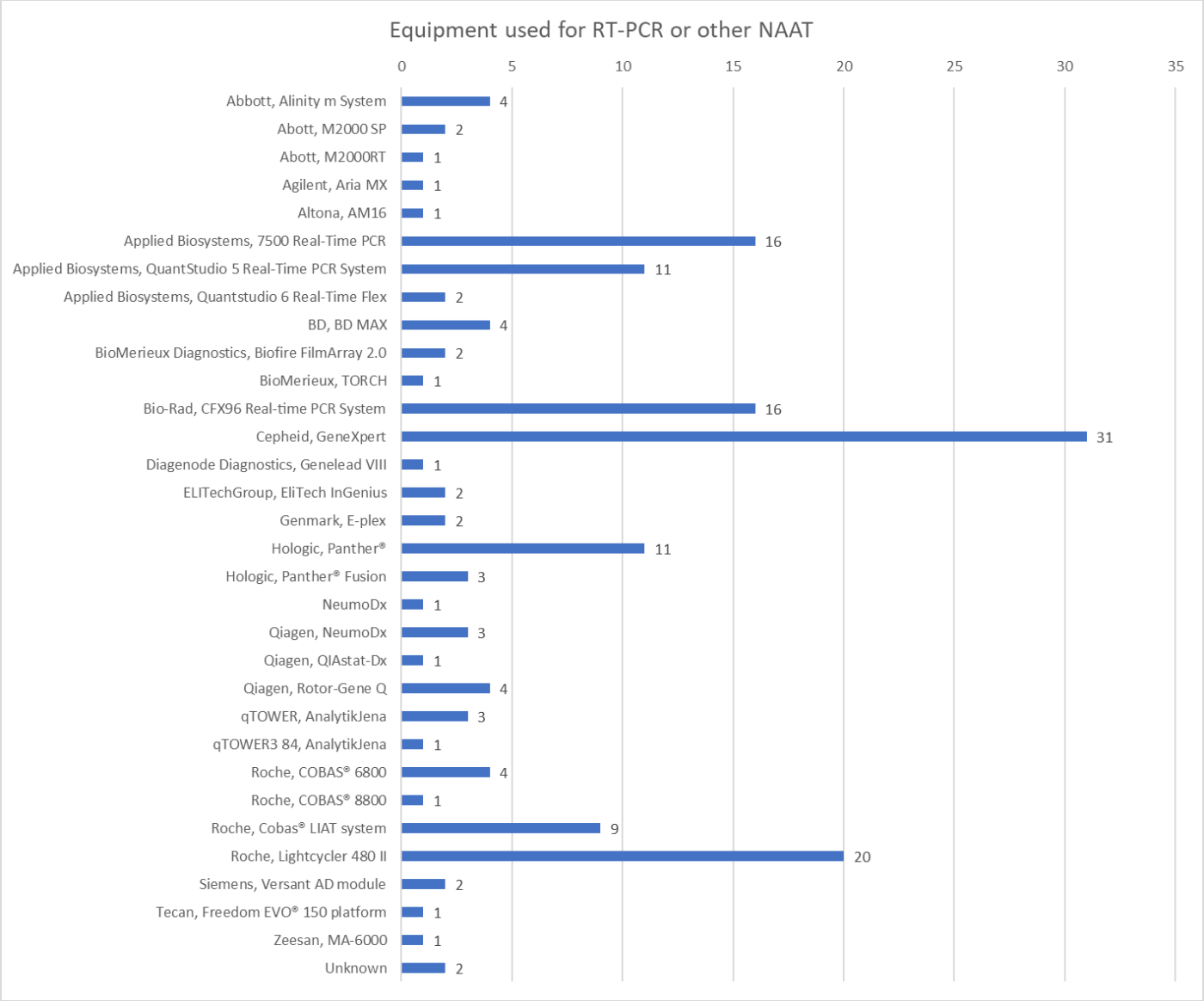


Figure 6: The RT-PCR or other NAAT equipment used by workflows testing for SARS-CoV-2 together with the number of workflows per machine (n=164)

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 164 workflows a total of 76 workflows used 1 target gene, 78 workflows used 2 target genes, 8 workflows used 3 target genes and 2 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.

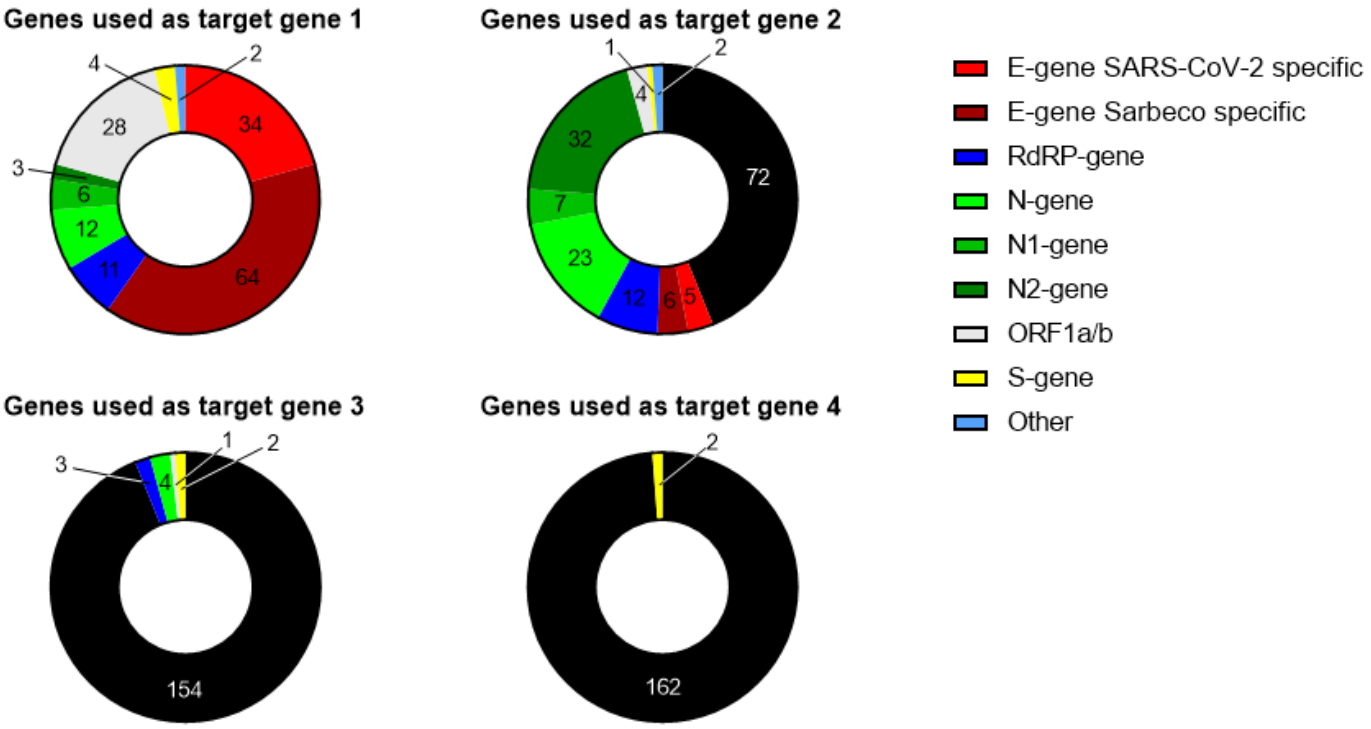


Figure 7: Target genes used in the workflows as reported in the questionnaire (n=164). Color coding genes is shown in the legend. Black color indicates workflows that do not contain a 2nd, 3rd or 4th gene target.

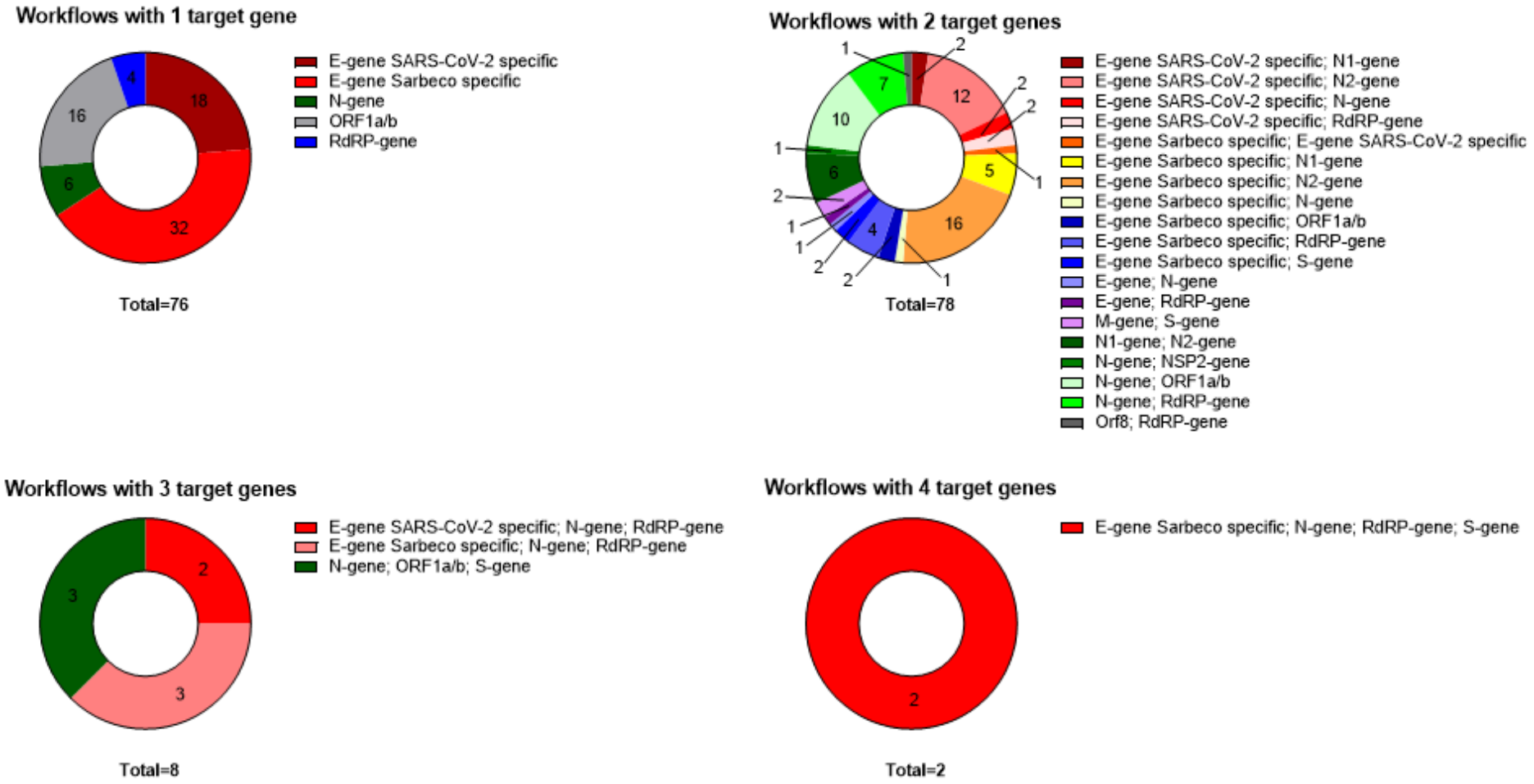


Figure 8: Combinations of target genes used in the workflows as reported in the questionnaire (n=164). Color coding genes is shown in the legend.

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	32
	E-gene SARS-CoV-2 specific	18
	ORF1a/b	16
	N-gene	6
	RdRp-gene	4
2	E-gene Sarbeco specific; N2-gene	16
	E-gene SARS-CoV-2 specific; N2-gene	12
	N-gene; ORF1a/b	10
	N-gene; RdRp-gene	7
	N1-gene; N2-gene	6
	E-gene Sarbeco specific; N1-gene	5
	E-gene Sarbeco specific; RdRp-gene	4
	E-gene Sarbeco specific; ORF1a/b	2
	E-gene Sarbeco specific; S-gene	2
	E-gene SARS-CoV-2 specific; N1-gene	2
	E-gene SARS-CoV-2 specific; N-gene	2
	E-gene SARS-CoV-2 specific; RdRp-gene	2
	M-gene; S-gene	2
	E-gene Sarbeco specific; E-gene SARS-CoV-2 specific	1
	E-gene Sarbeco specific; N-gene	1
	E-gene; N-gene	1
	E-gene; RdRp-gene	1
	N-gene; NSP2-gene	1
	Orf8; RdRp-gene	1
3	E-gene Sarbeco specific; N-gene; RdRp-gene	3
	E-gene SARS-CoV-2 specific; N-gene; RdRp-gene	2
	N-gene; ORF1a/b; S-gene	3
4	E-gene Sarbeco specific; N-gene; RdRp-gene; S-gene	2

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 LEQA1_CoV20-01, LEQA1_CoV20-02, LEQA1_CoV20-04 and LEQA1_CoV20-07. Figure 9 – Figure 18 show the obtained results for LEQA1_CoV20-01 up to LEQA1_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.

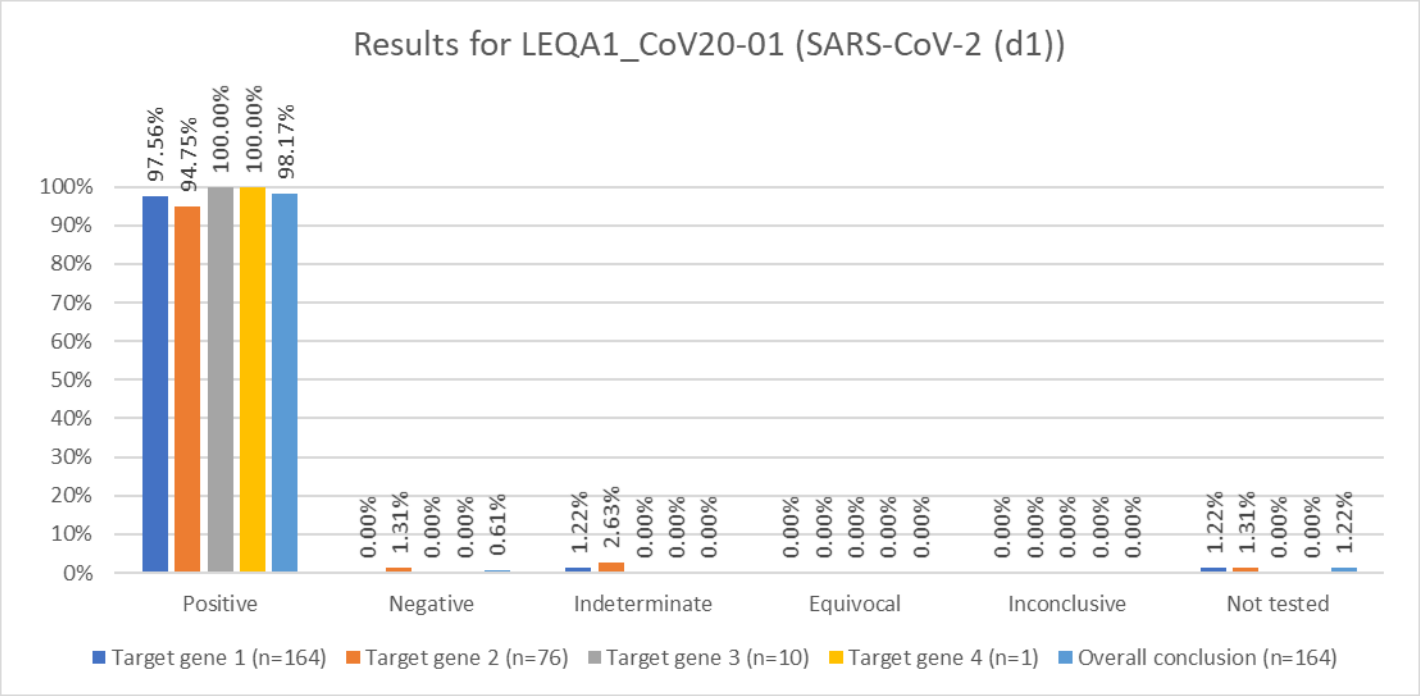


*Figure 9: Results obtained for LEQA1_CoV20-01 containing SARS-CoV-2 (with 1.28*10^5 copies E target/ml specimen (determined with dPCR) and 1.73*10^5 copies RdRp target/ml specimen (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to 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. Of all 162 workflows that reported an overall conclusion 161 (99.38%) identified SARS-CoV-2 in this sample correctly. 1/162 workflows reported incorrectly a negative result.*

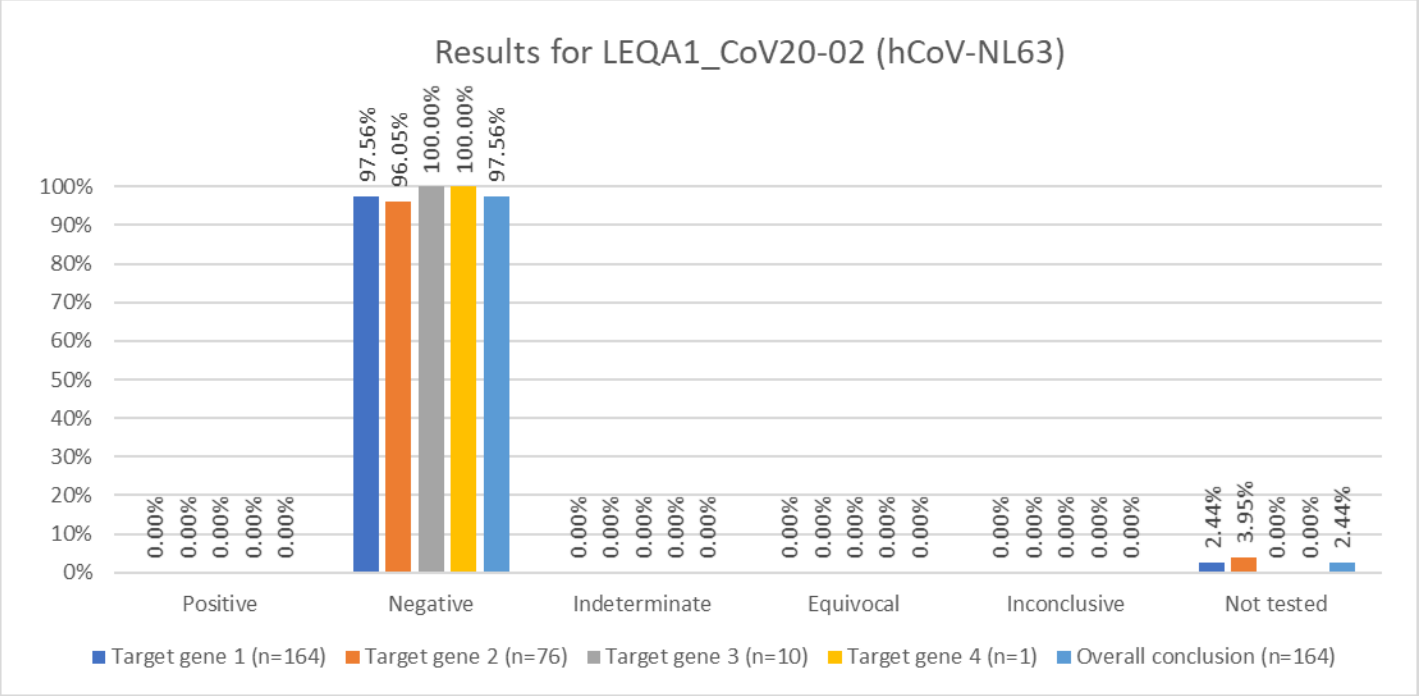


Figure 10: Results obtained for LEQA1_CoV20-02 containing hCoV-NL63 combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive 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. Overall, all 160 workflows that reported an overall conclusion did correctly not detect SARS-CoV-2 in this sample.

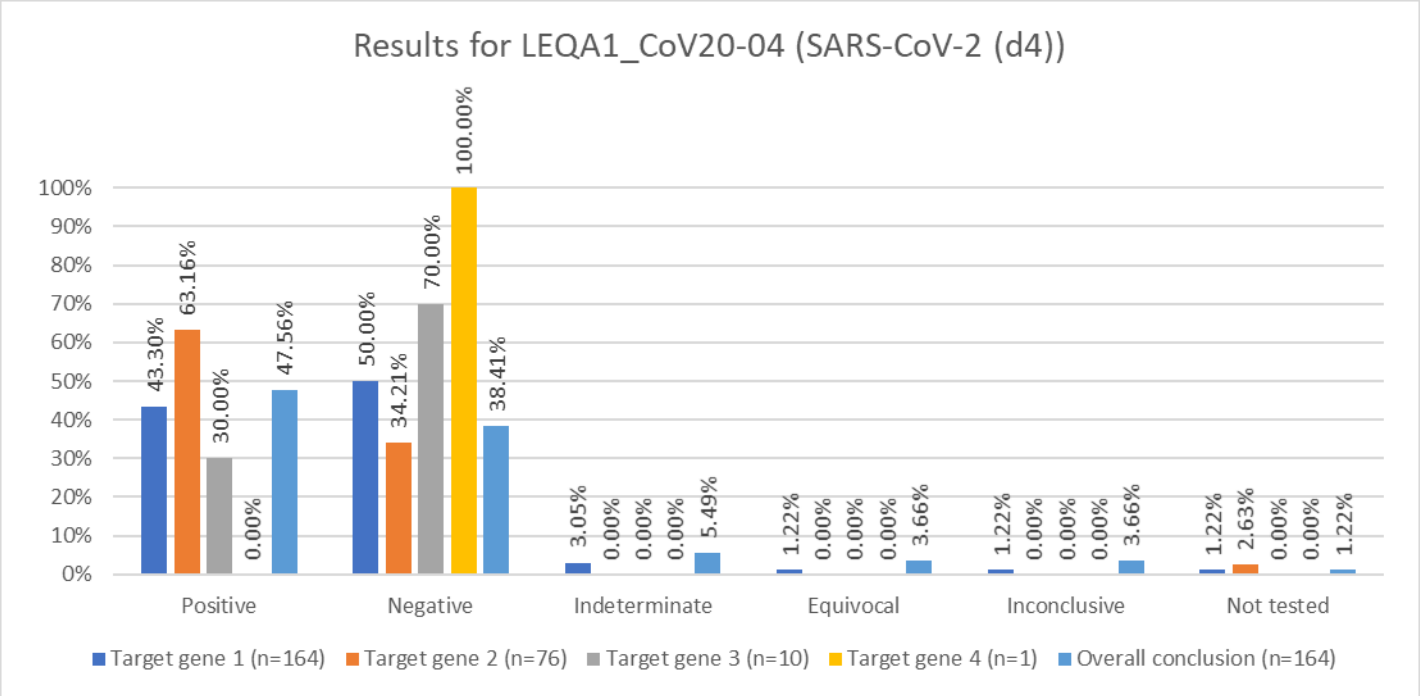


*Figure 11: Results obtained for LEQA1_CoV20-04 containing SARS-CoV-2 (with 1.28*10^2 copies E target/ml specimen (determined with dPCR) and 1.73*10^2 copies RdRp target/ml specimen (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to 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 162 workflows that reported an overall conclusion only 78 (48.15%) identified SARS-CoV-2 in this sample correctly and a further 21 (12.96%) with an indeterminate, equivocal or inconclusive result. 63/162 (38.89%) workflows reported a negative overall result.*

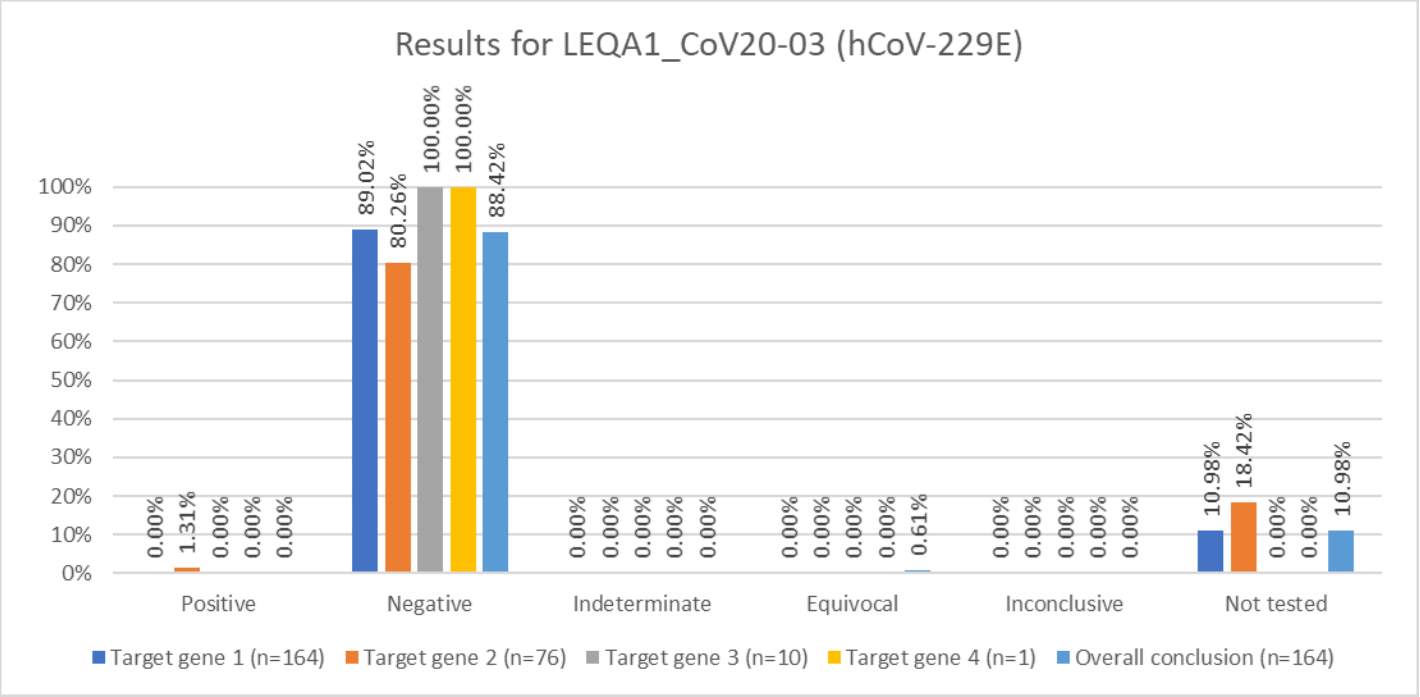


Figure 12: Results obtained for LEQA1_CoV20-02 containing hCoV-229E combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive 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 146 workflows that reported an overall conclusion 145 (99.32%) did correctly not detect SARS-CoV-2 in this sample whereas one workflow reported an equivocal overall conclusion.

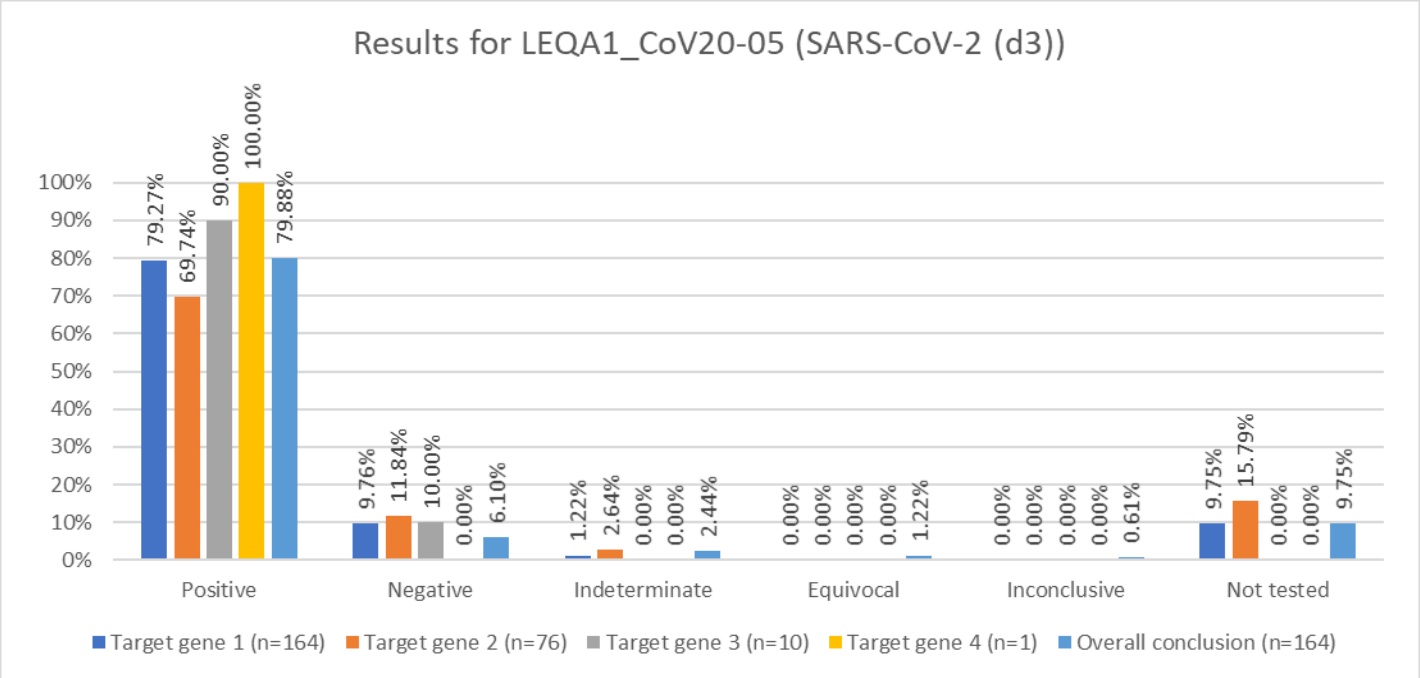


*Figure 13: Results obtained for LEQA1_CoV20-05 containing SARS-CoV-2 (with 1.28*10^3 copies E target/ml specimen (determined with dPCR) and 1.73*10^3 copies RdRp target/ml specimen (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to 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 148 workflows that reported an overall conclusion 131 (88.51%) identified SARS-CoV-2 in this sample correctly and a further 7 (4.73%) with an indeterminate, equivocal or inconclusive overall conclusion. 10/148 (6.76%) workflows reported incorrectly a negative result.*

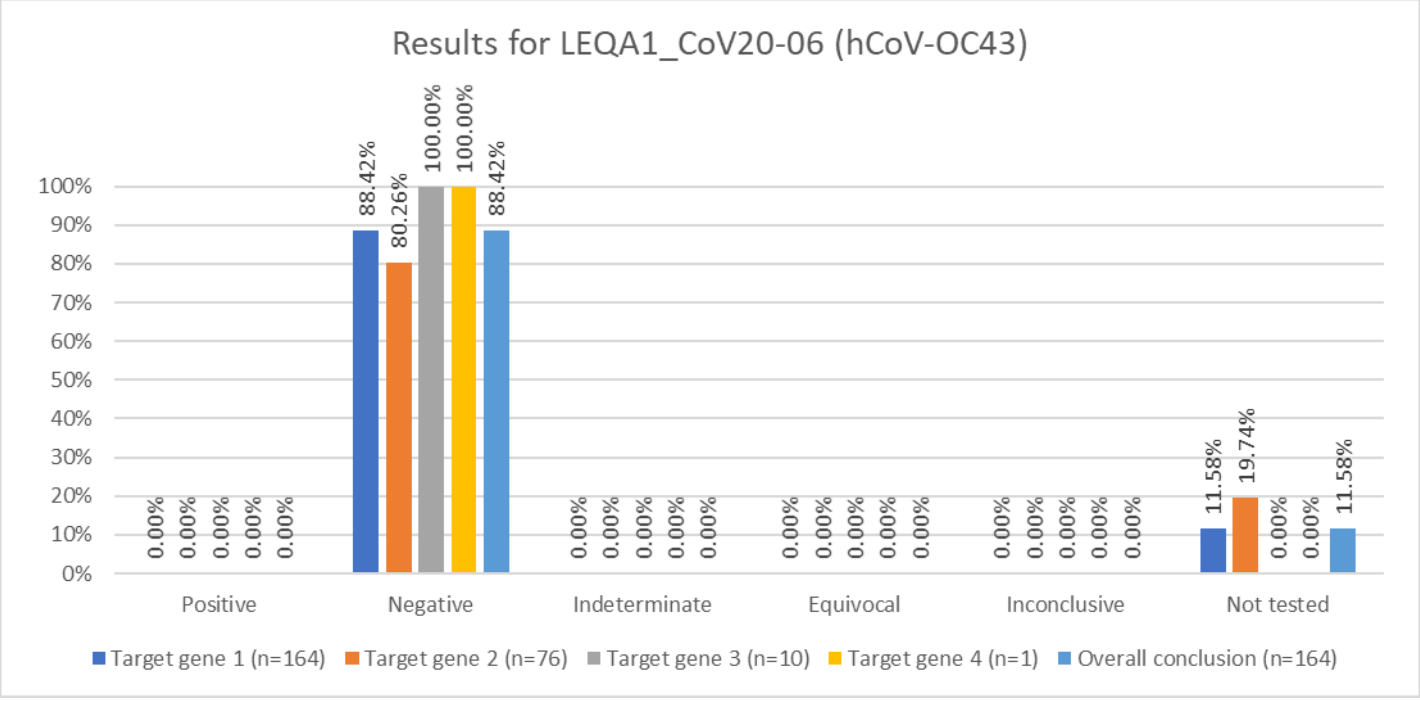


Figure 14: Results obtained for LEQA1_CoV20-06 containing hCoV-OC43 combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive 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, all 145 workflows that reported an overall conclusion did correctly not detect SARS-CoV-2 in this sample.

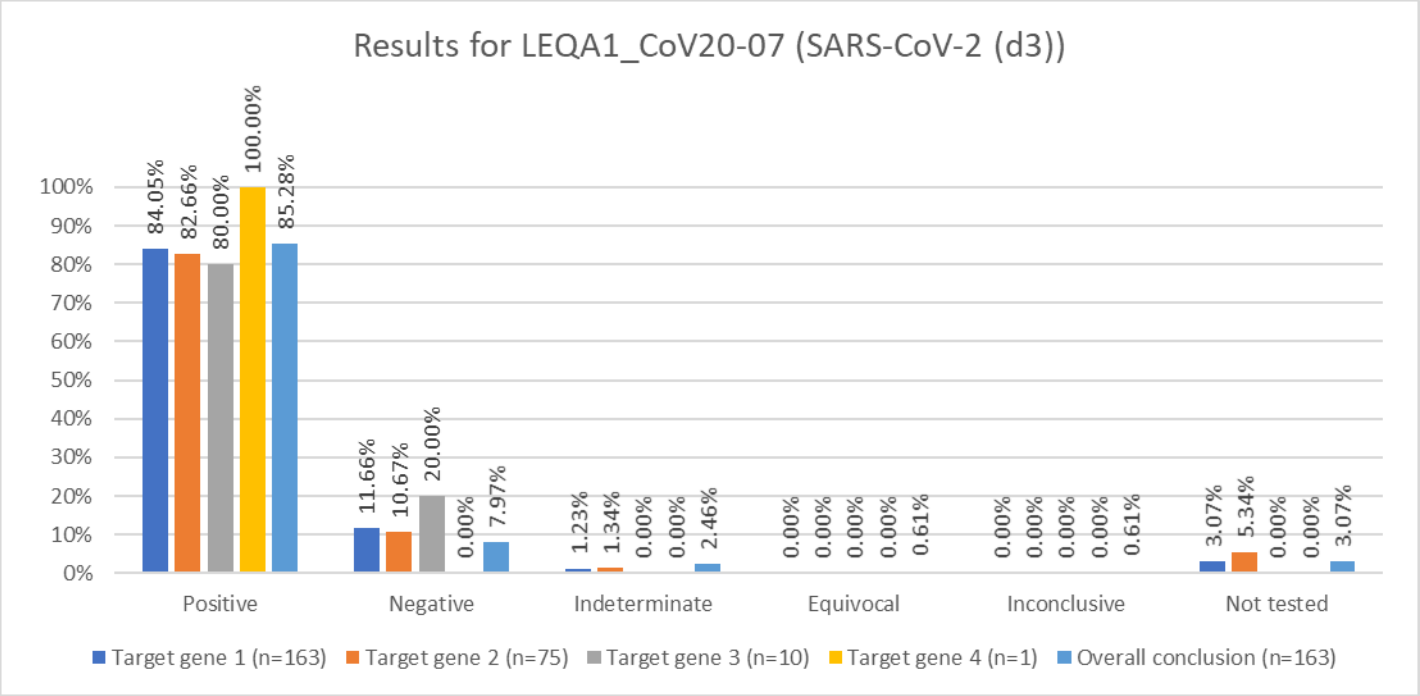


*Figure 15: Results obtained for LEQA1_CoV20-07 containing SARS-CoV-2 (with 1.28*10^3 copies E target/ml specimen (determined with dPCR) and 1.73*10^3 copies RdRp target/ml specimen (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were asked to 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 159 workflows that reported an overall conclusion 140 (88.05%) identified SARS-CoV-2 in this sample correctly and a further 6 (3.77%) with an indeterminate, equivocal or inconclusive overall conclusion. 13/159 (8.18%) workflows reported incorrectly a negative result.*

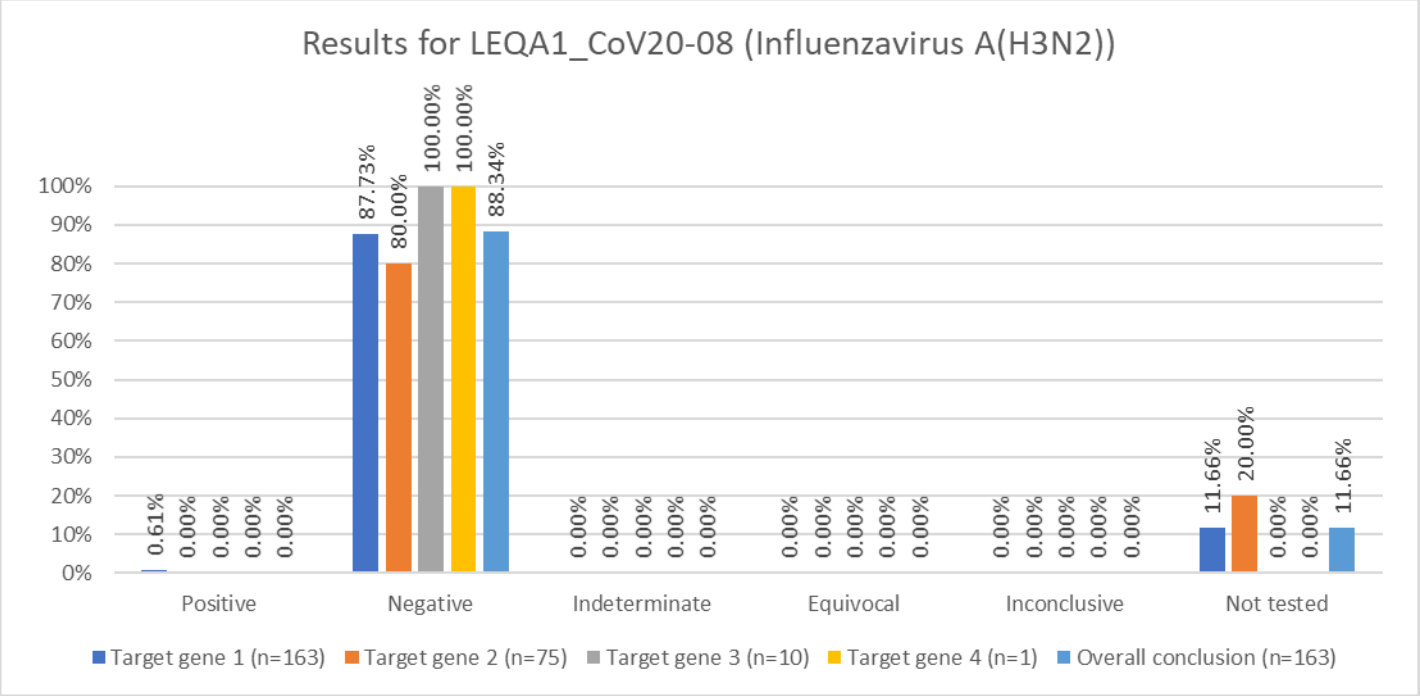


Figure 16: Results obtained for LEQA1_CoV20-08 containing Influenza virus A(H3N2) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, 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 145 workflows that reported an overall conclusion did correctly not detect SARS-CoV-2 in this sample.

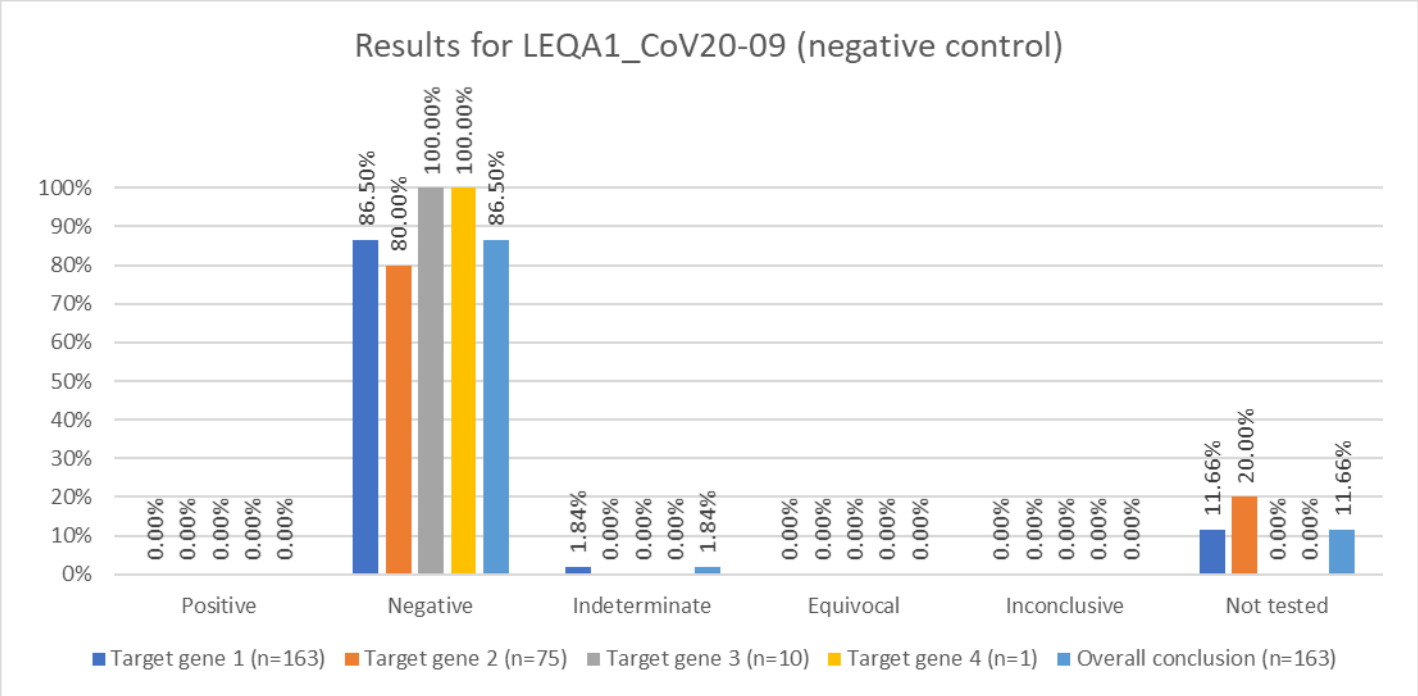


Figure 17: Results obtained for LEQA1_CoV20-09 containing no virus (negative control) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, 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 145 workflows that reported an overall conclusion 142 (97.93%) did correctly not detect SARS-CoV-2 in this sample. 3/145 (2.07%) workflows reported an incorrect indeterminate result.

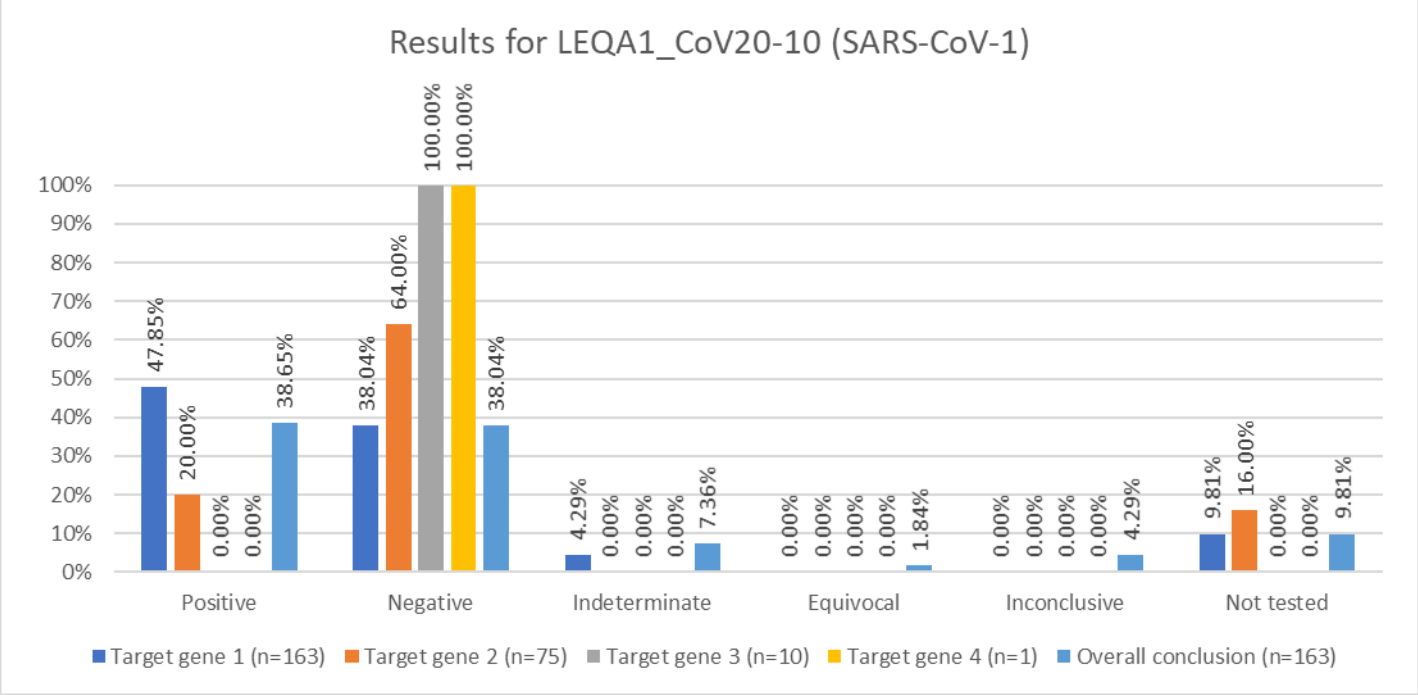


Figure 18: Results obtained for LEQA1_CoV20-10 containing SARS-CoV-1 combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, Inconclusive 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 148 workflows that reported an overall conclusion 62 (41.89%) did correctly not detect SARS-CoV-2 in this sample. 22/148 (14.86%) workflows reported an indeterminate, equivocal or inconclusive result and 63/148 (42.57%) reported a positive result, highly likely because in the current epidemiological situation no other Sarbeco virus than SARS-CoV-2 would be expected.

As described before all workflows were graded using a point system from 0 (being the lowest grade) up to 8 (highest grade). 132 workflows were given an “8”, seven workflows scored a “7.5”, 11 workflows scored a “7”, eight workflows scored a “6”, one workflow scored a “4.5”, one workflow scored a “4”, one workflow scored a “2”, one workflow scored a “1” and two workflows scored a “0”. 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.

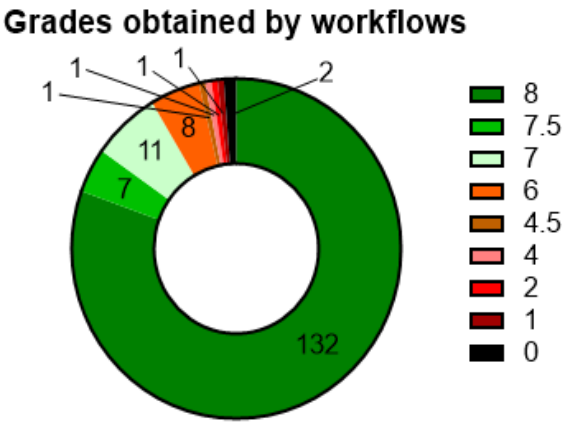


Figure 19: All grades obtained by the reported workflows out of the maximum of 8 points (n=164).

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 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 are shown.

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 84/164 workflows and therefore this factor is not included in Figure 21. For the 84 workflows for which it was calculated the specimen equivalent volume was median 20 µl (range 8 µl – 400 µl). For the 143 workflows for which specimen input volume in extraction was reported the median volume was 300 µl (range 8 µl – 1300 µl). The median reaction volume reported for 83 workflows was 20 µl (range 4 µl – 100 µl).

In total two laboratories reported data from 8 workflows, one laboratory reported data from 7 workflows, two laboratories reported data from 6 workflows, three laboratories reported data from 5 workflows, nine laboratories reported data from 4 workflows, six laboratories reported data from 3 workflows, eighteen laboratories reported data from 2 workflows and twenty-four laboratories reported data from 1 workflow. There are only two 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 65 laboratories, 58 laboratories reported at least one workflow with fully correct results. When excluding all mPOCT assays from the analysis, one laboratory reported data from 7 workflows, one laboratory reported data from 6 workflows, one laboratory reported data from 5 workflows, four laboratories reported data from 4 workflows, seven laboratories reported data from 3 workflows, eighteen laboratories reported data from 2 workflows, thirty-one laboratories reported data from 1 workflow and two laboratories do not report any other workflows than mPOCT assays. Two 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”.

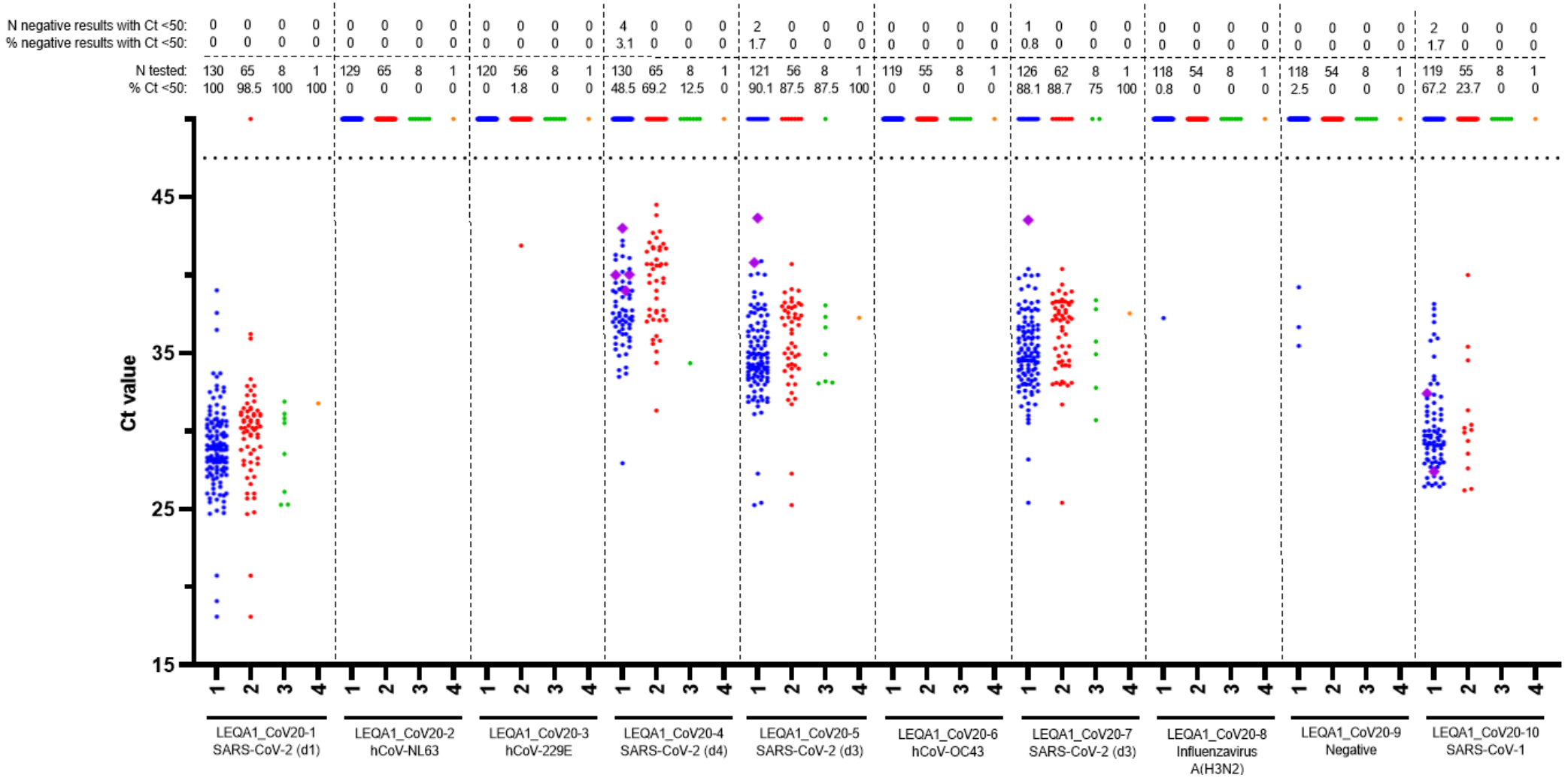
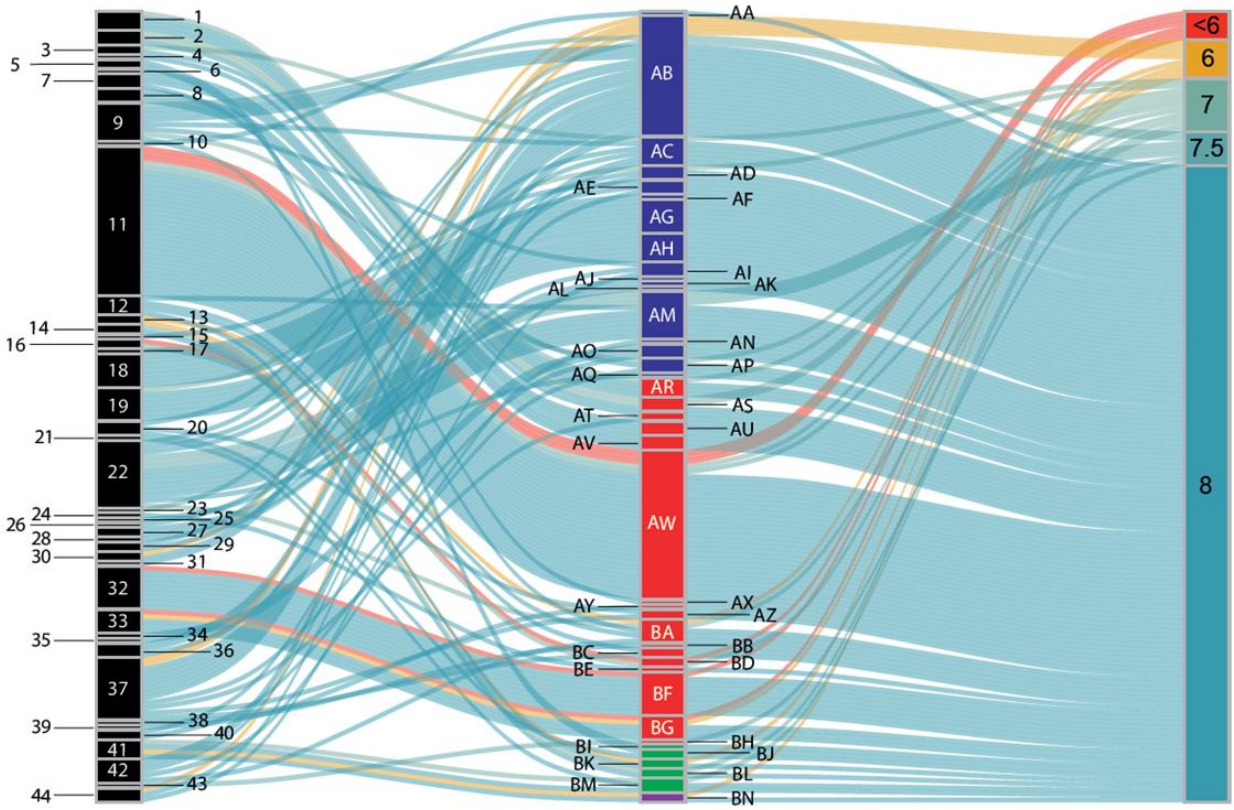


Figure 20: Results obtained per target gene per panel sample for workflows reporting Ct values. The numbers on the X-axis indicate which target gene (in order in which they were reported) is used for the detection of each sample. Underneath these numbers the contents of the sample are shown. All negative 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.



Extraction kit	
Abbott, ALINITY m SARS-CoV-2 ASSAY	1
Abbott, Sample Preparation System DNA kit	2
Abclonal, ANDiS Viral Nucleic RNA Auto Extraction & Purification Kit	3
Altona, Altostar Purification kit 1.5	4
Applied Biosystems, MagMAX TM Viral/Pathogen II Nucleic Acid Isolation Kit	5
BD, BD MAX SARS-CoV-2 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
Cepheid, Xpert® Xpress SARS-CoV-2	11
Chemagen, Chemagic Viral DNA/RNA 300 kit H96	12
Clean NA, Viral DNA & RNA SP kit	13
ElitechGROUP, ELITe InGenius SP 200	14
GeneFinder COVID-19 Plus	15
GenMark Dx, Eplex RP2	16
GSD NovaPrime®, IVD RNA Extraction AE1 kit	17
Hologic, Aptima (R) SARS-CoV-2 assay	18
Hologic, Panther Fusion® SARS-CoV-2 assay	19
Life Technologies, MagMax pathogen RNA/DNA kit	20
Magtivio, MagSi-NA Pathogens kit	21
Molg3n, PurePrep Pathogens	22
NeuMoDx™, RNA mastermix strip	23
NeuMoDx™, SARS-CoV-2 Assay	24
Precision System Science Co., E1300 MagDea isolatie chemie	25
Promega, Maxwell RSC Blood DNA kit	26
Qiagen, QIAprep&amp Viral RNA UM Kit	27
Qiagen, QIAstat-Dx Respiratory SARS-CoV-2	28
Qiagen, QIAsymphony DSP Virus/Pathogen Midi Kit	29
Qiagen, QIAsymphony DSP Virus/Pathogen Mini Kit	30
RBCBioscience, MagCore® Viral Nucleic Acid Extraction Kit	31
Roche, Cobas Liat SARS-Cov-2&Influenza A/B	32
Roche, Cobas SARS-CoV-2: Qualitative assay for use on the cobas 6800/8800 Systems	33
Roche, Cobas® 4800 CT/NG Test	34
Roche, Cobas® 4800 Human Papillomavirus (HPV) Test	35
Roche, DNA and Viral NA Large Volume kit	36
Roche, DNA and Viral NA Small Volume kit	37
Roche, MagNA Pure 24 Total NA Isolation Kit	38
Roche, MagNA Pure 96 Total Nucleic Acid Isolation Kit	39
Roche, MagNA Pure LC Total Nucleic Acid Isolation Kit	40
Seegene, STARMag 96x4 Universal Cartridge Kit	41
Siemens, VERSANT Sample Preparation 1.0 Reagents	42
Zeesan, lab-aid Virus RNA extraction kit	43
Unknown	44

PCR test	
Tests with 1 target gene	
Biotecon Diagnostics, microproof SARS-CoV-2 Identification Kit	AA
Corman et al. (2020) E-gene Sarbeco specific	AB
Corman et al. (2020) E-gene SARS-CoV-2 specific	AC
Corman et al. (2020) RdRP-gene	AD
E-gene Sarbeco specific (Unknown sequence)	AE
GSD, NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR kit	AF
Hologic, Aptima (R) SARS-CoV-2 assay	AG
Hologic, Panther Fusion® SARS-CoV-2 assay	AH
Lu et al. (2020) N-gene	AI
Qiagen, QIAprep&amp Viral RNA UM Kit	AJ
Qiagen, QIAstat-Dx Respiratory SARS-CoV-2	AK
Qiagen, SARS-CoV-2 N1+N2 Assay Kit	AL
r-Biopharm, RIDAGENE SARS-CoV-2 RUO	AM
Siemens, FTD Sars-CoV-2 assay	AN
TibMolBio, LightMix® Modular Sarbecovirus E-gene, CE	AO
TibMolBio, SARS-CoV-2	AP
Unknown	AQ
Tests with 2 target genes	
Abbott, ALINITY m SARS-CoV-2 ASSAY	AR
Abbott, M2000 SARS-CoV-2	AS
Altona Diagnostics, Altona Realstar SARS-CoV-2 RT PCR	AT
BD, SARS-CoV-2 kit	AU
BioMerieux Diagnostics, Respiratory panel 2.1 plus	AV
Cepheid, Xpert® Xpress SARS-CoV-2	AW
Certest, VIASURE SARS-CoV-2 Real Time PCR	AX
Corman et al. (2020) E-gene Sarbeco specific; Corman et al. (2020) E-gene SARS-CoV-2 specific	AY
Corman et al. (2020) E-gene Sarbeco specific; Corman et al. (2020) RdRP-gene	AZ
Corman et al. (2020) E-gene Sarbeco specific; Lu et al. (2020) N1-gene	BA
Corman et al. (2020) E-gene Sarbeco specific; Lu et al. (2020) N-gene	BB
Corman et al. (2020) E-gene SARS-CoV-2 specific; Lu et al. (2020) N1-gene	BC
GenMark Dx, Eplex RP2	BD
NeuMoDx™, SARS-CoV-2 Assay	BE
Roche, Cobas Liat SARS-Cov-2&Influenza A/B	BF
Roche, Cobas SARS-CoV-2: Qualitative assay for use on the cobas 6800/8800 Systems	BG
Xiamen Zeesan Biotech Co., Ltd., SARS-CoV-2 Test Kit (Real-time PCR)	BH
Tests with 3 target genes	
Applied Biosystems, TaqPath COVID-19 RT-PCR Kit	BI
Applied Biosystems, TaqPath COVID-19 Multiplex Diagnostic Solution (CE-IVD)	BJ
Chemagic, SARS-CoV-2 RT-q PCR Perkin Elmer	BK
ELITechGroup, GeneFinder COVID 19 PLUS Real Amp Kit	BL
Seegene, Allplex 2019-nCov Assay	BM
Test with 4 target genes	
Seegene, Allplex SARS-CoV-2 Assay	BN

Figure 21: A flow diagram showing all workflows reported to have tested te 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.

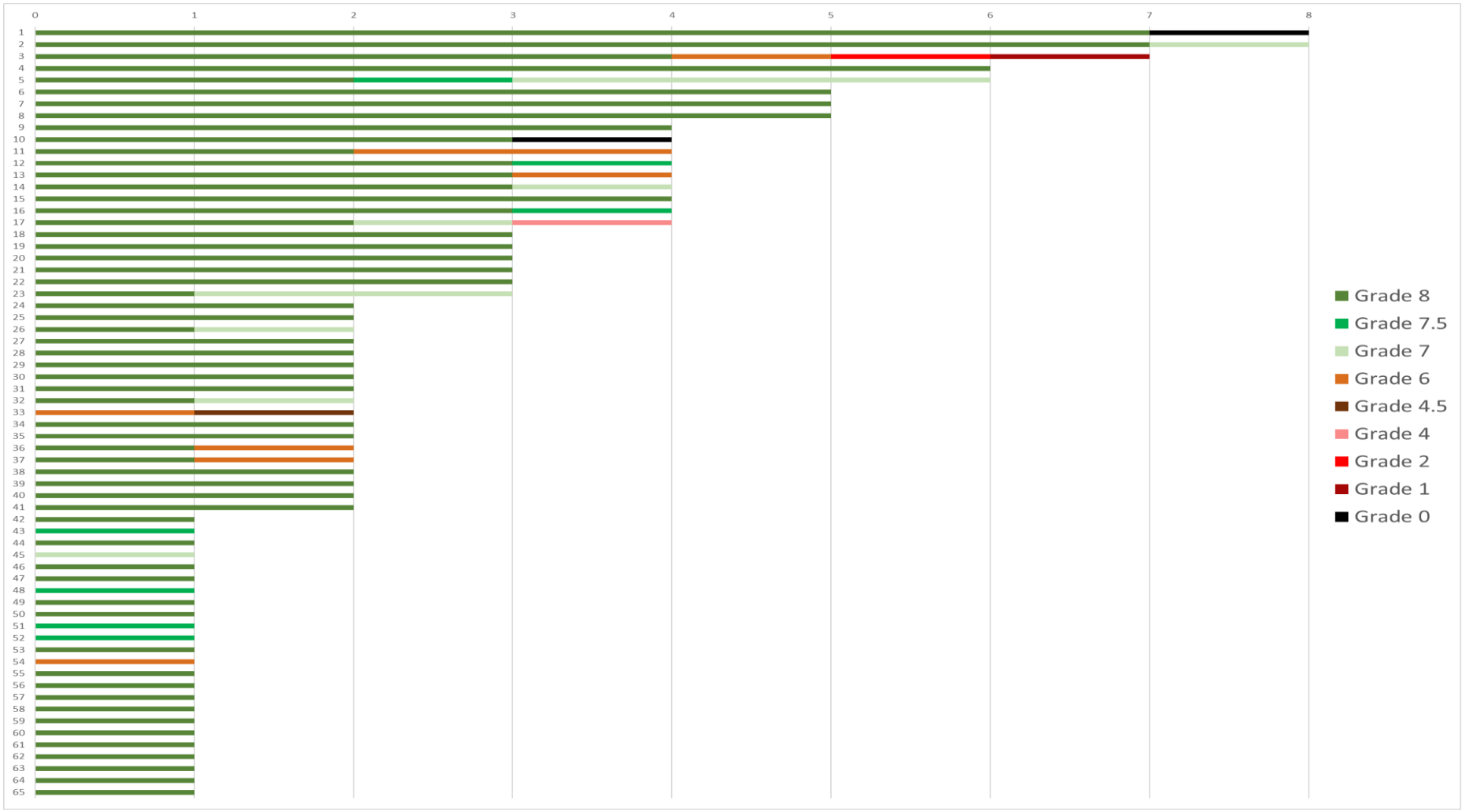


Figure 22: Grades obtained per workflow per laboratory (anonymized). For each of the laboratories the amount of reported workflows is shown on the X-axis together with their accompanying grades. In total 65 laboratories sent in data of their workflows. There are only two laboratories which only have 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.

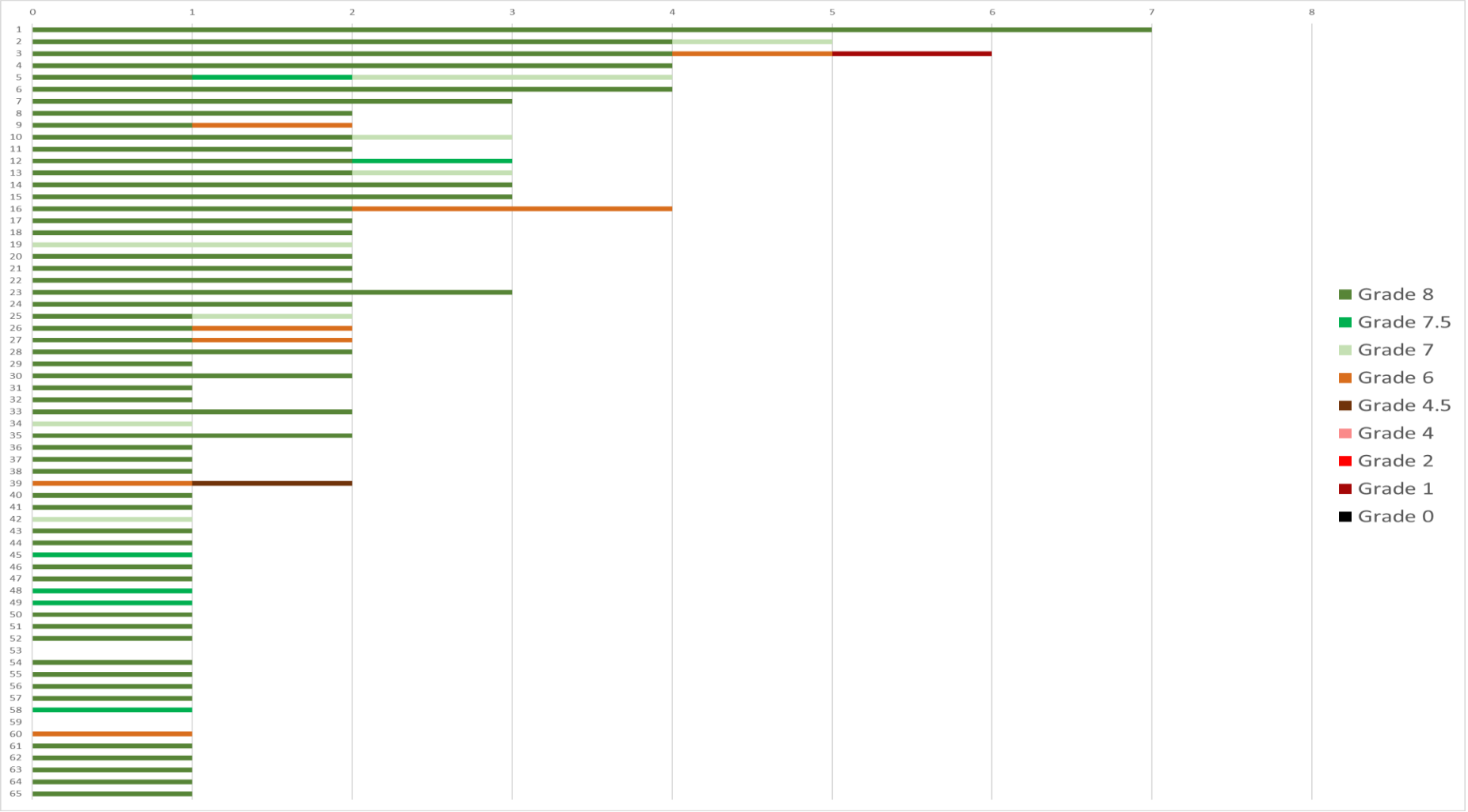


Figure 23: Grades obtained per workflow per laboratory excluding all mPOCT assays (anonymized). For each of the laboratories the amount of reported workflows is shown on the X-axis together with their accompanying grades. In total 65 laboratories sent in data of their workflows. There are only two laboratories which only have scores of < 7 for all reported workflows. Two 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.

4. Discussion and conclusion

Out of 164 workflows reported, 132 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 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=8) 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. 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 panels or reduced panels were tested are taken into account (n=156), none of the workflows submitted receive a grade below 6. When considering all workflows each laboratory has access to, only two laboratories have workflows which do not have a grade of 7 or above. Although it is not desirable to use a workflow scoring a 7 or 7.5 out of 8 for SARS-CoV-2 diagnostics due to its current relevance, it is likely that only minor adaptions of the concerned workflows are needed to perform within the desired criteria. Only two 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 (108 out of 164 workflows). There was no significant difference in performance between workflows using different single target genes or combinations of target genes.

No false positive results have been reported by the laboratories, except for the SARS-CoV-1 containing sample (LEQA1_CoV20-10). 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. What is striking, is that for a lot of workflows a SARS-CoV-2 specific E-gene is reported as the target gene which still detects SARS-CoV-1 (53.85%; Supplemental Figure 10). The average Ct value found for SARS-CoV-1 when the SARS-CoV-2 specific E-gene and Sarbeco specific E-gene are used as target genes are Ct 32.08 (SD 4.64; n=29; data not shown) and Ct 29.53 (SD 2.19; n=60; data not shown), respectively. It appears that for quite some workflows the E-gene as target gene is wrongly labeled as SARS-CoV-2 specific rather than Sarbeco specific or still has a strong cross-reaction to SARS-CoV-1 which is less likely.

Whereas no false positive samples 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 generating a Ct value by a target gene targeting SARS-CoV-2, the sample was deemed SARS-CoV-2 negative, possibly related to a used cutoff value or other criterium, e.g. shape of the amplification curve; up to 1.7% of each SARS-CoV-2 positive sample (range: 0% – 1.7%) 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 using Ct values as an output, a broad range of Ct values has been reported. The biggest range of reported Ct values was found for LEQA_CoV20-1. The average Ct value was 28.85 (SD 2.77; data not shown) where the lowest Ct value reported was 18.11 and the highest value was 39.02 (making a 20.91 difference in Ct value), indicating a wide spread of Ct values for the same sample throughout the workflows reported. Despite this wide range of Ct values for the same sample 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. 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. [1] 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 the recent UK and South-African variants of SARS-CoV-2. [2] In the next round of LEQA we therefore plan to include variants.

It seems that the workflows used for SARS-CoV-2 diagnostics for the Dutch population perform very well and provide a reliable network. 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

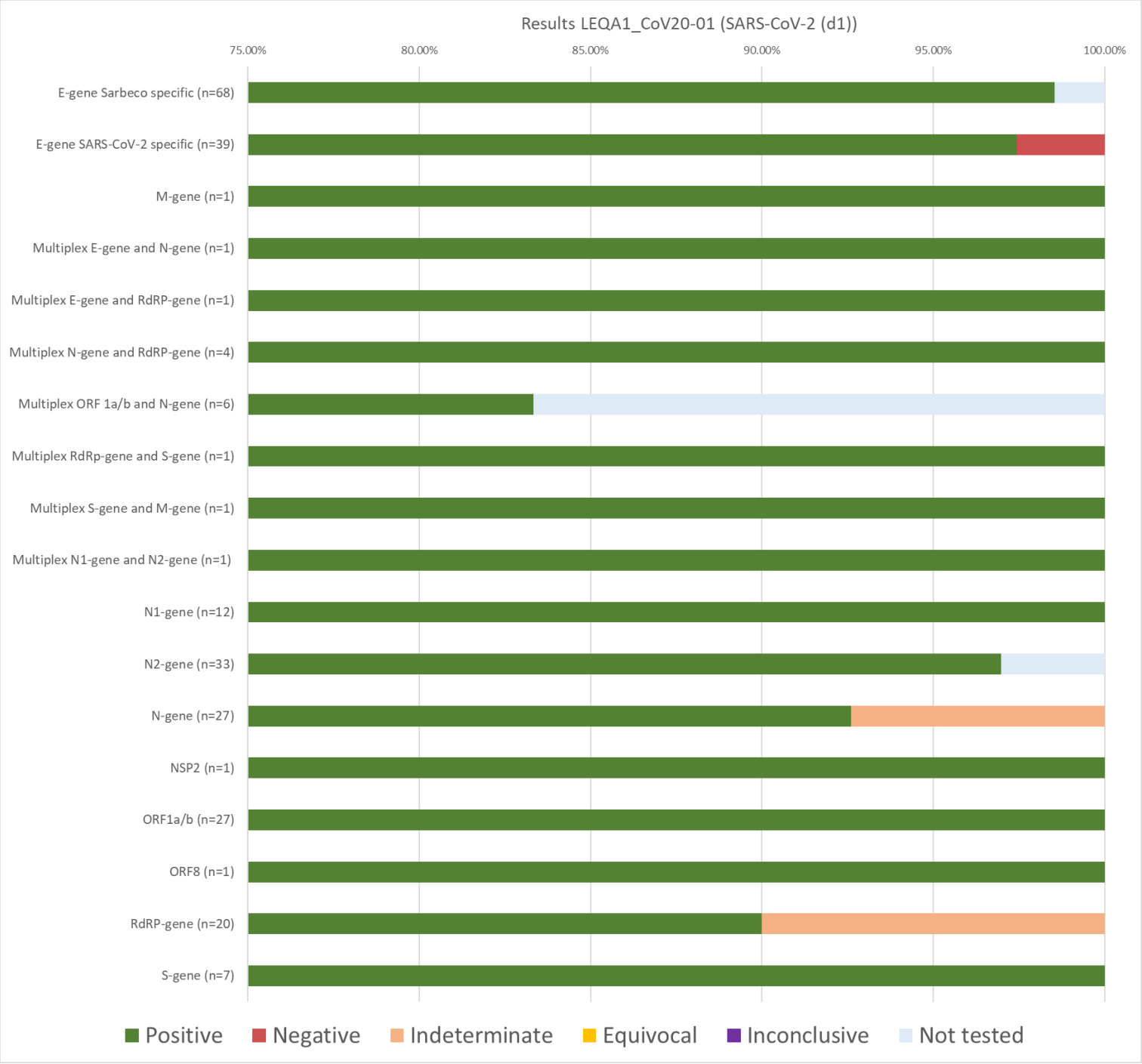
[1] A. Meijer *et al.*, “Preparing the outbreak assistance laboratory network in the Netherlands for the detection of the influenza virus A(H1N1) variant,” *J. Clin. Virol.*, vol. 45, no. 3, pp. 179–184, 2009.

[2] WHO (2020). "SARS-CoV-2 Variants." Retrieved 13-01-2021, 2021, from https://www.who.int/csr/don/31-december-2020-sars-cov2-variants/en/.

6. Supplemental figures

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.

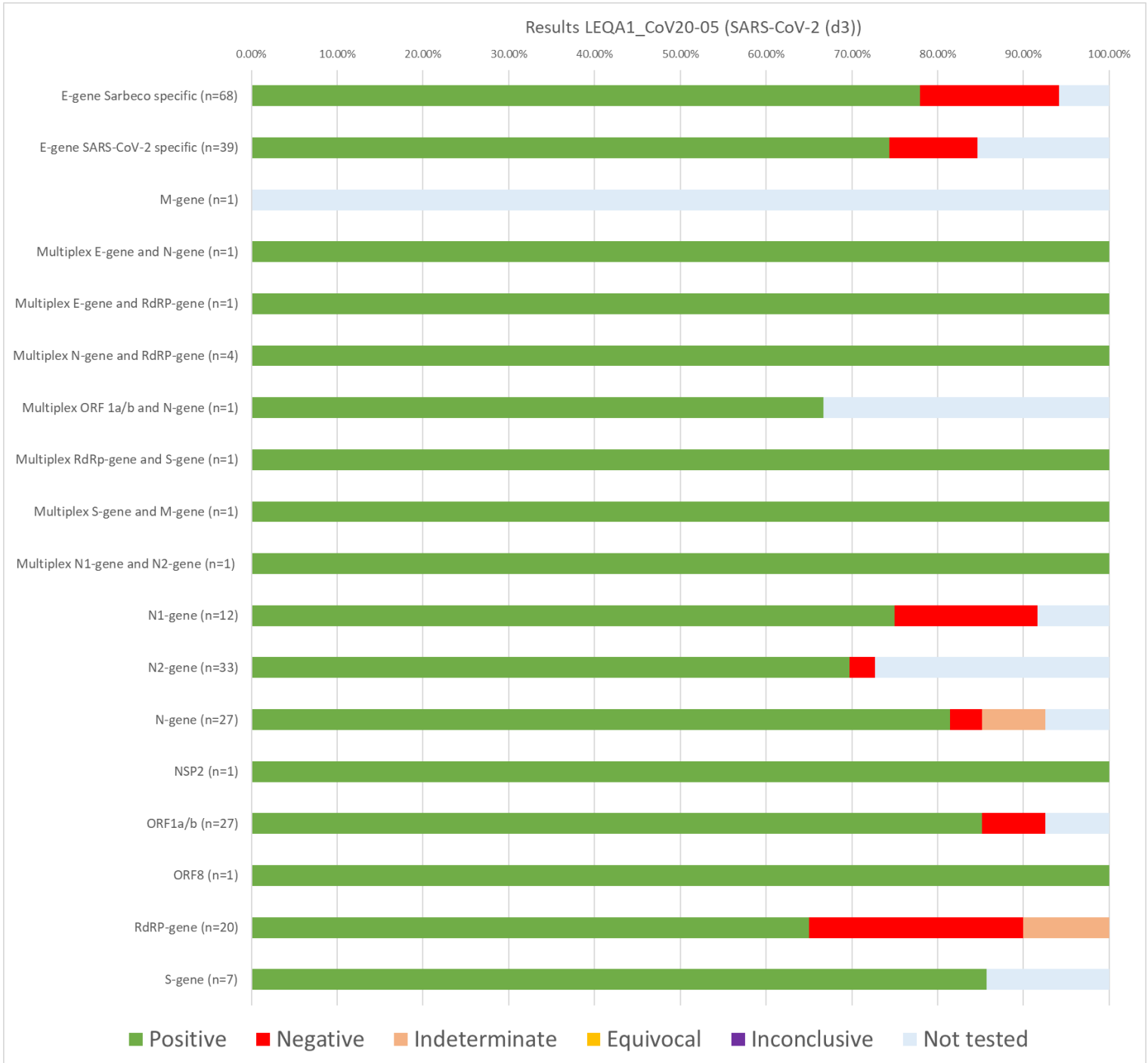


Supplemental Figure 1: The percentages of the various scores obtained for LEQA1_CoV20-01 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample

Supplemental Figure 2: The percentages of the various scores obtained for LEQA1_CoV20-02 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

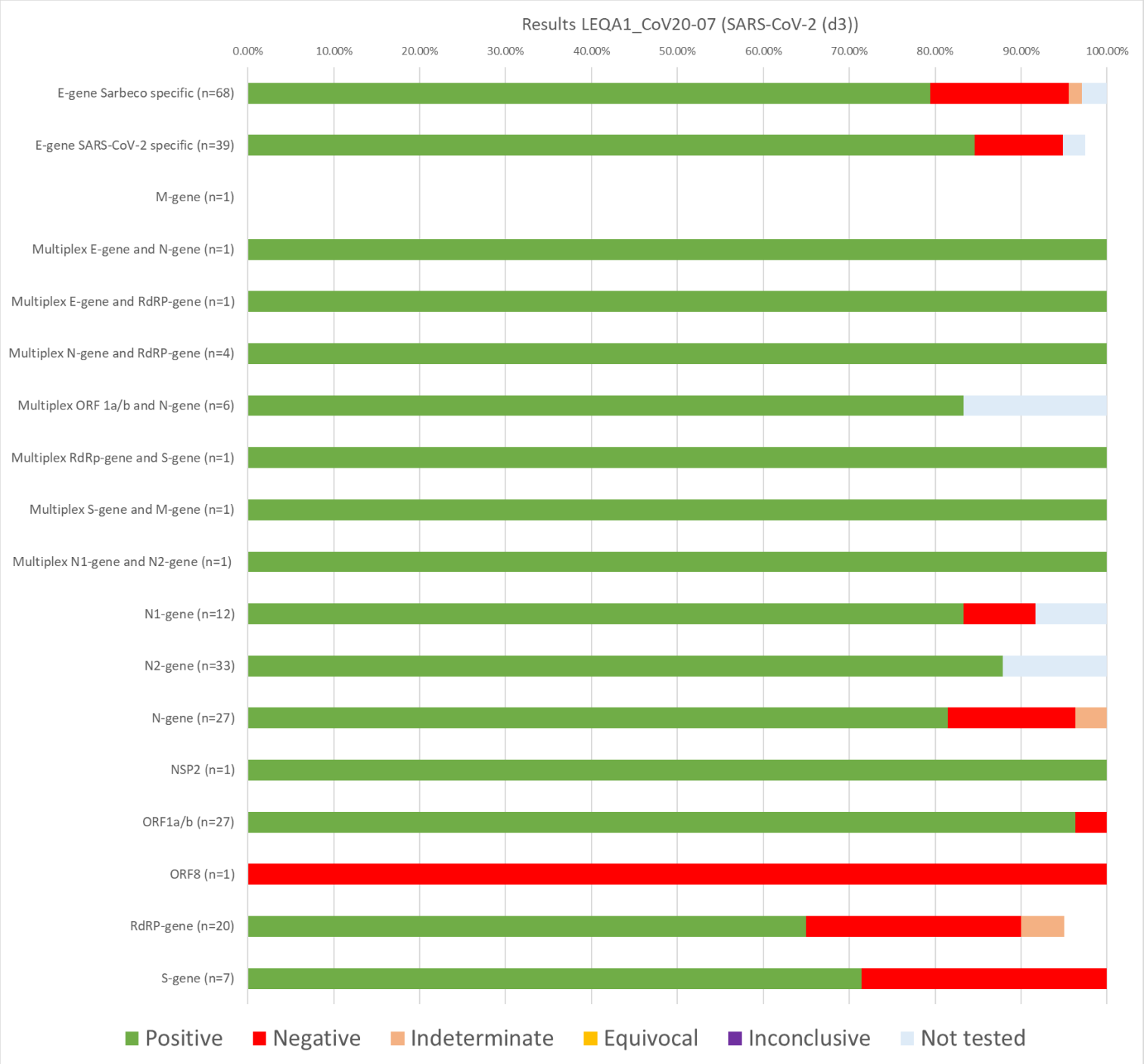
Supplemental Figure 3: The percentages of the various scores obtained for LEQA1_CoV20-03 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample

Supplemental Figure 4: The percentages of the various scores obtained for LEQA1_CoV20-04 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 LEQA1_CoV20-05 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

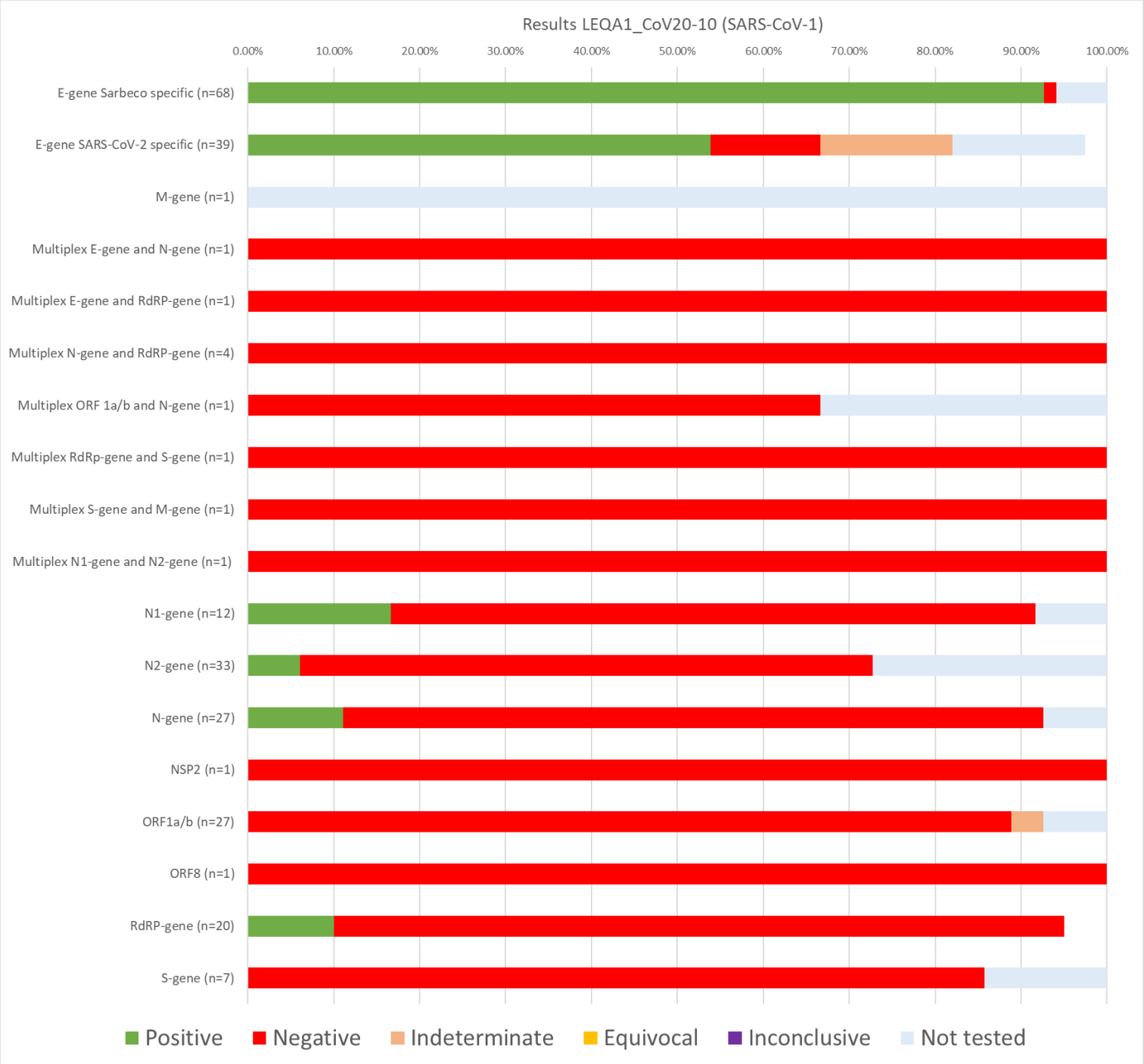
Supplemental Figure 6: The percentages of the various scores obtained for LEQA1_CoV20-06 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



Supplemental Figure 7: The percentages of the various scores obtained for LEQA1_CoV20-07 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. Not all results of the used target genes have a total sum of 100%, due to incomplete reports for some workflows.

Supplemental Figure 8: The percentages of the various scores obtained for LEQA1_CoV20-08 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 LEQA1_CoV20-09 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. Not all results of the used target genes have a total sum of 100%, due to incomplete reports for some workflows.



Supplemental Figure 10: The percentages of the various scores obtained for LEQA1_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. Not all results of the used target genes have a total sum of 100%, due to incomplete reports for some workflows.

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
St. Antonius Ziekenhuis Nieuwegein
Atalmedial
Brightlabs B.V.
Canisius Wilhelmina Ziekenhuis
Certe
Deventer Ziekenhuis
Diagnostiek voor U
Diakonessenhuis Utrecht
Elisabeth Tweesteden Ziekenhuis
Eurofins Genomics Europe Applied Genomics GmbH
Eurofins NMDL-LCPL
Fenelab Consortium - Masterlab
Fenelab Consortium - Mérieux NutriSciences
Fenelab Consortium - Nofalab BV
Fenelab Consortium - Normec Biobeheer
Fenelab Consortium - NutriControl
Fenelab Consortium - Nutrilab B.V.
Fenelab Consortium - SGS
Fenelab Consortium - Triskelion
Franciscus gasthuis en vlietland
Gelre ziekenhuizen
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
Leids Universitair Medisch Centrum
Maasstadziekenhuis
Maastricht Universitair Medisch Centrum
Meander MC
Microvida

Laboratory
MVZ Labor Stein & Kollegen
Noordwest Ziekenhuisgroep Alkmaar
Onze Lieve Vrouwe Gasthuis B.V.
Pro Health Medical
RadboudUMC
Rijnstate
RLM Dordrecht-Gorinchem
Royal GD
Saltro
Sanquin, NSS lab
Slingeland Ziekenhuis Doetinchem
Star-shl
Stichting PAMM
Streeklab Haarlem
SYNLAB Germany Leverkusen
SYNLAB Heppignes
SYNLAB Laboratoire Collard
Tergooi Ziekenhuis
TLR International Laboratories
Universitair Medisch Centrum Groningen
Universitair Medisch Centrum Utrecht
VieCuri MC Venlo
Wageningen Bioveterinary Research
Ziekenhuis St Jansdal
Zuyderland MC