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

Colophon

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Background

Since January 2020 different 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). Because of a heterogeneity in quality panels for checking SARS-CoV-2 Nucleic Acid Amplification Tests (NAAT) performance in the past, the fact that patchy quality checks were implemented only when workflows changed or laboratories were added to the network, and because the general importance to check the performance of the network of COVID-19 molecular diagnostic labs as a whole regularly 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'), a National External Quality Assessment (EQA) (Landelijk EQA; LEQA) program has been developed. Since the start of this program three rounds of LEQA have been performed. This report includes the third and most recent round of the LEQA program.

Objective

The goal of this LEQA round is to assess 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 variants of concern (VOC) SARS-CoV-2 B.1.351 20H/501Y.V2, SARS-CoV-2 P.1 20J/501Y.V3 and SARS-CoV-2 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. Of these 10 specimens, 8 were core specimen.

Materials and Methods

In April 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 second week of May 2021. Laboratories were asked to report their results via an online form. 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 that wanted to test these workflows with a limited panel were asked to indicate this. These laboratories were sent an email with instructions to limit testing for these workflows to samples 4, 6, 9 and 10.

Workflows were given a score of 8 for 100% correct results for the 8 core samples. The score was 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 4, 6, 9 and 10), 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 201 workflows reported by 77 participating laboratories, 160 (79.6%) scored a 100% correct score for all 8 core specimens (8 points) and thus met all criteria set for reliable SARS-CoV-2 diagnostics, 22 (10.9%) scored between 7-7.5, making it likely that only minor adjustments need to be made to meet all criteria and 19 (9.5%) workflows scored below 7. Unfortunately these numbers contain 9 workflows for which the panel was wrongly implemented. When excluding these workflows, the overall quality increases. Out of these 192 workflows, 160/192 (83.3%) scores an 8, 22/192 (11.5%) scores 7-7.5, 9/192 (5.2%) workflows score below 7. A score below 7 indicates that improvements need to be made for a workflow to be reliable for SARS-CoV-2 diagnostics in clinical diagnostic settings

and surveillance.
For the SARS-CoV-2 negative core samples no false positive or indeterminate, equivocal or inconclusive results were reported, confirming specificity of al testes work flows. For the 6 SARS-CoV-2 containing core samples, false negative results (n=34) and false indeterminate, equivocal or inconclusive results (n=8) were reported. Some workflows reported a negative result for SARS-CoV-2 presence in SARS-CoV-2 containing samples due to Cp cut-off values used in the assay; in one SARS-CoV-2 positive sample 0.7% of the total overall conclusions were reported as SARS-CoV-2 negative despite one (or more) of the target genes against SARS-CoV-2 in the assay generating a Cp value.

Variants of concern B.1.351 20H/501Y.V2, P.1 20J/501Y.V3 and B.1.1.7 20B/501Y.V1 were detected in 100%, 99.5% and 99.5% of the workflows, respectively.

Despite the wide variety of kits, equipment and enzymes that are used in the different implemented workflows, the influence on the quality of molecular diagnostics for SARS-CoV-2 was limited. Compared to the results obtained during the second round of quality control in this program (LEQA2), 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 could 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 the novel SARS-CoV-2 variants included in the panel (B.1.351 20H/501Y.V2, P.1 20J/501Y.V3 and B.1.1.7 20B/501Y.V1). 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 a wide variety 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 viral load components for checking SARS-CoV-2 Nucleic Acid Amplification Tests (NAAT) performance, they initially contained SARS-CoV-1 RNA, later replaced by 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 the performance of the network of COVID-19 molecular diagnostic labs as a whole is checked regularly, a National External Quality Assessment (EQA) (Landelijk EQA; LEQA) program has been developed. At this moment this program consists of three rounds of EQA. In the second week of May 2021 the third 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 three variants of concern (VOC) SARS-CoV-2 B.1.351 20H/501Y.V2, SARS-CoV-2 P.1 20J/501Y.V3 and SARS-CoV-2 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 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 variant of concern (VOC) containing samples the LEQA panel was produced at the RIVM in October 2020 and pretested at the RIVM and Erasmus MC. Both centres obtained similar results. In May 2021 aliquoted samples for LEQA3 were thawed and analysed again at RIVM. This confirmed that the samples were of unchanged quality. The samples containing VOC were prepared and pretested at RIVM in April 2021. A month later, the then 83 laboratories performing SARS-CoV-2 diagnostics in the Dutch network were contacted and notified of the distribution of the panel in the second week of May 2021. All laboratories were asked to report their findings via an online form using Formdesk software (Wassenaar, The Netherlands) to allow for a more streamlined method of data collection. Laboratories were given time until the 30th of May to report their obtained results. After the 30th of May laboratories that had not yet reported results were contacted and given one week grace time for reporting, after which the submission was closed on the 6th of June. 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 that wanted to test these workflows with a limited panel were asked to indicate this. These laboratories were sent an email with instructions to limit testing for these workflows to samples 4, 6, 9 and 10 (Table 1), covering three different concentrations of SARS-CoV-2 of which one was VOC SARS-CoV-2 B.1.1.7 20B/501Y.V1 and a hCoV-NL63 containing sample.

2.2 Contents of LEQA3 panel

The LEQA3 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 or no virus. 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. 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 four concentrations, SARS-CoV-2 Alpha (variant B.1.1.7; 20B/501Y.V1; hCoV-19/Netherlands/NH-RIVM-20432/2020), SARS-CoV-2 Beta (variant B.1.351; 20H/501Y.V2; hCoV-19/Netherlands/NH-RIVM-10159/2021) and SARS-CoV-2 Gamma (variant P.1; 20J/501Y.V3; hCoV-19/Netherlands/NH-RIVM-10915/2021), hCoV-NL63 (kindly provided by Lia van der Hoek, Amsterdam University Medical Center), 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 Cp 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 LEQA3 together with the target specific expected Cp 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 Cp[4]	E-gene (Sarbeco) Cp	RdRP-gene (SARS-CoV-2) Cp	Conclusion SARS-CoV-2
LEQA3_CoV20-1	No virus	-	-	n/a	Neg	Neg	Negative
LEQA3_CoV20-2[4, 5]	SARS-CoV-1 (RNA)	-	-	n/a	28.47 (4/4)	Neg	Negative[3]
LEQA3_CoV20-3[2]	SARS-CoV-2 (d1)	1.28*10^5	1.73*10^5	n/a	28.02 (4/4)	28.32 (4/4)	POSITIVE
LEQA3_CoV20-4	hCoV-NL63	-	-	28.10 (4/4)	Neg	Neg	Negative
LEQA3_CoV20-5	SARS-CoV-2 B.1.351 20H/501Y.V2	1.15*10^4	1.14*10^4	n/a	31.09 (4/4)	31.69 (4/4)	POSITIVE
LEQA3_CoV20-6[2, 6]	SARS-CoV-2 (d4)	1.28*10^2	1.73*10^2	n/a	35.04 (3/4)	35.70 (2/4)	Weak POSITIVE
LEQA3_CoV20-7[2]	SARS-CoV-2 (d3)	1.28*10^3	1.73*10^3	n/a	34.25 (4/4)	34.17 (4/4)	POSITIVE
LEQA3_CoV20-8	SARS-CoV-2 P.1 20J/501Y.V3	8.77*10^3	6.60*10^3	n/a	31.58 (4/4)	32.37 (4/4)	POSITIVE
LEQA3_CoV20-9[2]	SARS-CoV-2 (d3)	1.28*10^3	1.73*10^3	n/a	34.65 (4/4)	34.64 (4/4)	POSITIVE
LEQA3_CoV20-10	SARS-CoV-2 B.1.1.7 20B/501Y.V1	3.39*10^4	2.32*10^4	n/a	29.94 (4/4)	30.95 (4/4)	POSITIVE

[1] The expected Cp 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 Cp 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*. 2020; the RdRP primers and probes are modified from those published by Corman *et al*. (2020) 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 N-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 Cp 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 54.0% of reported workflows having tested this specimen reported this specimen positive for SARS-CoV-2

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 LEQA3_CoV20-2 and LEQA3_CoV20-6 (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. The terms Indeterminate, Equivocal and Inconclusive are used interchangeably by laboratories and generally mean: Unclear if positive or negative.

As the panel consisted of 8 core samples, each workflow started with a total of 8 points. 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 a smaller subset of samples specimens to enable making a limited statement about the sensitivity of detection of SARS-CoV-2 with the mPOCT used. These workflows only had to test LEQA3_CoV20-4, LEQA3_CoV20-6, LEQA3_CoV20-9 and LEQA3_CoV20-10. 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. [1] Workflows scoring 7 or 7.5 out of 8 might are 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 LEQA3_CoV20-7 or LEQA3_CoV20-9 samples is less severe than detection of SARS-CoV-2 targets in specimens which were SARS-CoV-2 negative (false positive). Any workflow 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-three laboratories were contacted with the announcement of panel distribution for this third EQA round. Seventy-eight (94.0%) of these laboratories reported their findings for a total of 202 workflows. Of these workflows, 201 workflows were used for regular diagnostics and/or SARS-CoV-2 variant determination assay. One of the 202 workflows was used as a SARS-CoV-2 variant determination assay only. Unfortunately, one laboratory did not test the LEQA3 panel, but another panel due to a misunderstanding. This laboratory sent their findings of this non-LEQA3 panel obtained using their only reported workflow. Concerning the results obtained for the LEQA3 panel, their results cannot be included in this report. All other data of this workflow were included in this report. The results of the remaining 77 laboratories and their 201 workflows on which the LEQA3 panel was tested were processed. The workflow results reported for each panel sample are summarized in Table 2 ('Not tested' results 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 LEQA3 panel sample.

Panel sample	Content	№ of workflows with test result reported (n=201)[1]	SARS-CoV-2 detection workflow conclusion			
			№ Positive	№ Indeterminate, equivocal or inconclusive	№ Negative	Errors
LEQA3_CoV20-1	No virus	182	0	0	182 (100%)	None
LEQA3_CoV20-4	hCoV-NL63	197	0	0	197 (100%)	None
LEQA3_CoV20-2[2]	SARS-CoV-1 (RNA)	183	73 (39.9%)	12 (6.6%)	98 (53.6%)	Not applicable, educational sample
LEQA3_CoV20-3	SARS-CoV-2 (d1)	183	183 (100%)	0	0	None
LEQA3_CoV20-7[2]	SARS-CoV-2 (d3)	186	170 (91.4%)	4 (2.2%)	12 (6.5%)	False indeterminate, equivocal or inconclusive (n=4), false negative (n=12)
LEQA3_CoV20-9	SARS-CoV-2 (d3)	197	173 (87.8%)	4 (2.0%)	20 (10.2%)	False indeterminate, equivocal or inconclusive (n=4), false negative (n=20)
LEQA3_CoV20-6	SARS-CoV-2 (d4)	200	108 (54.0%)	12 (6.0%)	80 (40.0%)	Not applicable, educational sample
LEQA3_CoV20-10	SARS-CoV-2 B.1.1.7 20B/501Y.V1	196	195 (99.5%)	0	1 (0.5%)	False negative (n=1)
LEQA3_CoV20-5	SARS-CoV-2 B.1.351 20H/501Y.V2	183	183 (100%)	0	0	None
LEQA3_CoV20-8	SARS-CoV-2 P.1 20J/501Y.V3	182	181 (99.5%)	0	1 (0.5%)	False negative (n=1)

[1] 11 of the 201 workflows tested were mPOCT testing samples 4, 6, 9 and 10 only. For 9 of 201 workflows laboratories made another limited selection of panel samples to test. Therefore the number of workflows with test result per sample is less than 201.

[2] Sum of percentages not 100 due to rounding for this 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	2	4-6 (n=2)	2 (n=1)	2 (n=2)
7	1	6 (n=1)	0	1 (n=1)
6	4	2-6 (n=4)	1-3 (n=3)	1 (n=1)
5	3	2-5 (n=3)	0	3 (n=1)
4	11	1-4 (n=11)	1-3 (n=4)	1 (n=3)
3	14	2-3 (n=14)	1 (n=1)	1 (n=5)
2	11	1-2 (n=11)	1 (n=2)	0
1	31	1 (n=26)	1 (n=3)	1 (n=2)

Despite not all workflows obtained fully correct results with the core specimens (Table 2), nearly all laboratories (75/77; 97.4%) used at least one workflow with which a score 7 to 8 was obtained (Table 3). When including only laboratories that correctly implemented the panel, all laboratories (75/75, 100%) have at least one workflow that scored 7 to 8 points. 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 26/31 (83.9%) used a workflow with which a score of 8 was obtained**.** In the subsequent chapters a more detailed insight in the results and their background is presented.

3.2 Target genes used for RT-PCR or other NAAT

As the sensitivity of a workflow may also depend on the used target gene(s), for all workflows the target genes used were inventoried based on the online report form. Workflows used up to 4 target genes. Table 4 shows the scores obtained by all workflows testing LEQA3 sorted by the number target genes used. From the 202 workflows a total of 88 workflows used 1 target gene, 94 workflows used 2 target genes, 14 workflows used 3 target genes and 6 workflows used 4 target genes. Combinations of genes used by number of workflows are listed in Table 4. Some workflows using more than one target gene do not generate separate result for each independent gene but rather a composite conclusion. In Supplemental Figure 1, the target genes in the order reported for each workflow are shown. Supplemental Figure 2 shows the combinations of target genes used in the various workflows.

Table 4. Scores obtained by all workflows sorted by number of target genes used

№ of workflows per lab	№ of workflows	№ of workflows with indicated score		
		Score 8	Score 7 or 7.5	Score < 7
1	88	71 (80.7%)	12 (13.6%)	5 (5.7%)
2	93	72 (77.4%)	7 (7.5%)	14 (15.1%)
3	14*	12 (85.7%)	2 (14.3%)	0
4	6	5 (83.3%)	1 (16.7%)	0

* One workflow was used to test another panel than LEQA3. Therefore the workflow could not be scored on quality and is excluded from this table.

Table 5. 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	16
	N-gene	9
	NSP-2	1
	NSP-15	1
	ORF1a/b	20
	RdRP-gene	8
	S-gene	1
2	E-gene Sarbeco specific; N1-gene	6
	E-gene Sarbeco specific; N2-gene	42
	E-gene Sarbeco specific; N-gene	3
	E-gene Sarbeco specific; ORF1a/b	2
	E-gene Sarbeco specific; RdRP-gene	2
	E-gene Sarbeco specific; S-gene	1
	E-gene SARS-CoV-2 specific; RdRP-gene	2
	M-gene; S-gene	4
	N-gene; RdRP-gene	9
	N1-gene; N2-gene	8
	N-gene; ORF1a/b	14
	ORF8; RdRP-gene	1
3	E-gene Sarbeco specific; N-gene; RdRP-gene	7
	E-gene Sarbeco specific; N-gene; S-gene	1
	N-gene; ORF1a/b; S-gene	6
4	E484K; N501Y; del69/70; L452R *	1
	E-gene Sarbeco specific; N-gene; RdRP-gene; S-gene	4
	E-gene SARS-CoV-2 specific; RdRP-gene; S-gene P1 specific; S-gene	1

* This workflow was only used as a SARS-CoV-2 variant determination assay

3.3 Performance of the workflows

As described before, all workflows were graded using a point system from 0 (being the lowest grade) up to 8 (highest grade). One point was given for each of the core specimen in the complete panel. Two points for each of the core specimen was given when testing the reduced (mPOCT) panel. 160 workflows were given an '8', six workflows scored a '7.5', 16 workflows scored a '7', nine workflows scored a "6", two workflow scored a '5', four workflows scored a '4', one workflow scored a '3', one workflow scored a '1' and two workflows scored a '0'. It should be noted that all but one of the 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 1 shows all grades given to the reported workflows. As mentioned in paragraph 1, all laboratories using two or more workflows had at least one workflow with which a score of 8 and only 3 and 2 laboratories using one workflow only scored between 7-7.5 or below 7 respectively.

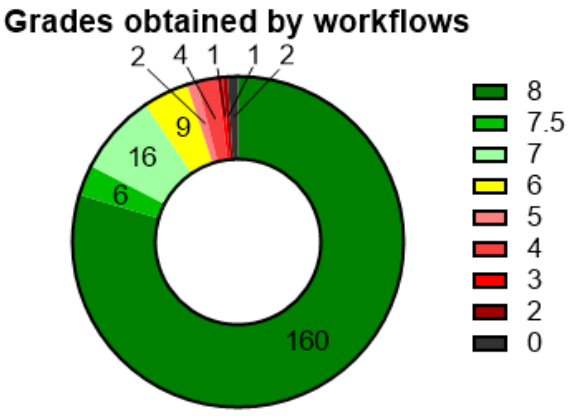


Figure 1: All grades obtained by the reported workflows out of the maximum of 8 points (n=201).

An overview containing the results obtained per target gene per panel sample for workflows reporting Cp values is shown in Figure 2. In this figure for each of the target genes used (shown in the order in which they were reported) the Cp values are shown for each of the tested samples. Each laboratory submitted up to 4 target genes per workflow. Target gene 1-4 can be any combinations of target genes as mentioned in Table 5. Also shown are the number of tested samples with the target genes used, the percentage of reported positive results, the percentage of reported negative results and the percentage of reported negative results (likely due to implemented cut-off values) with Cp < 50.

The obtained scores per workflow are also coupled to the used extraction kit or method, the performed PCR or other NAAT test and the used number of target genes 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 Supplemental Figure 29.

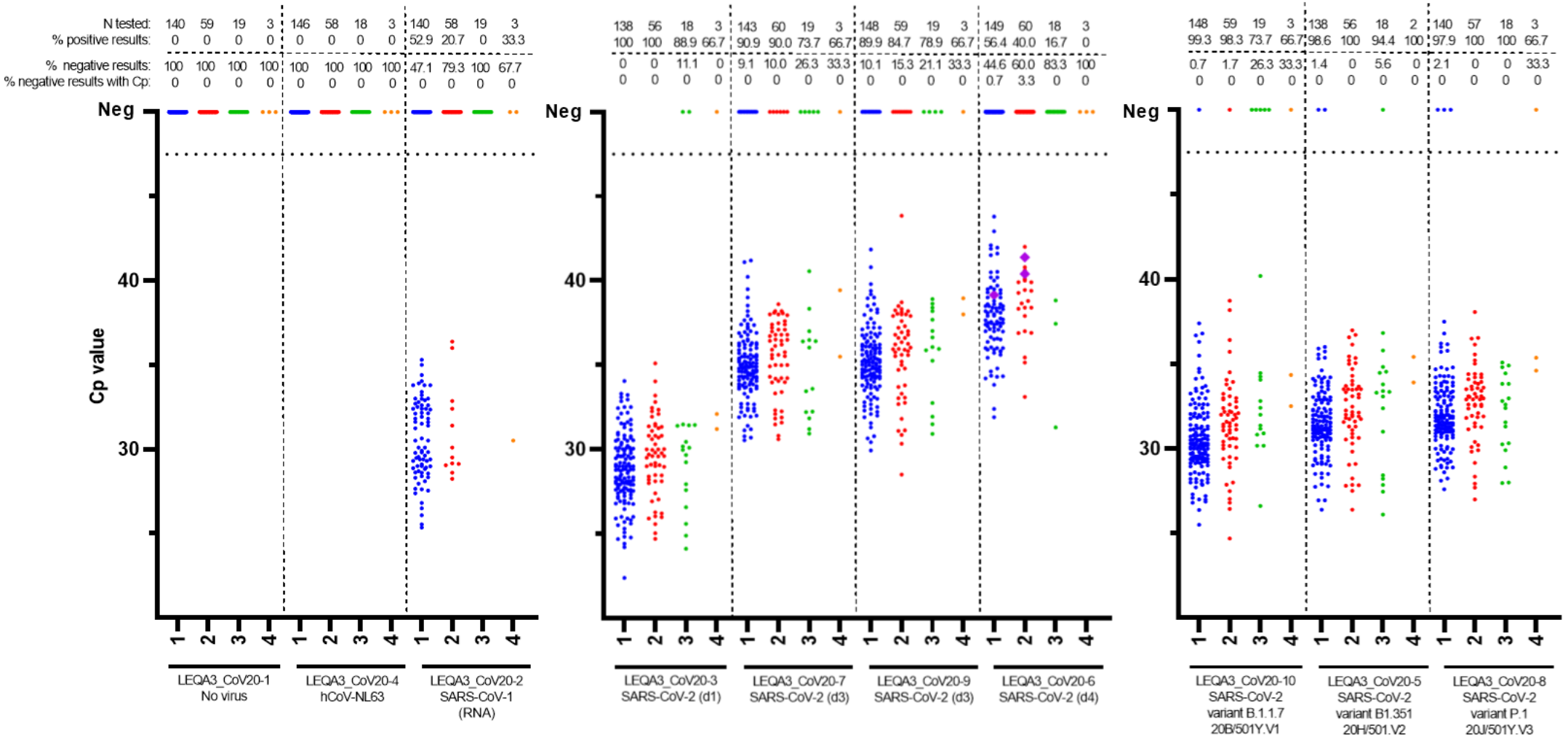


Figure 2: Results obtained per target gene per panel sample for workflows reporting Cp values. The numbers on the X-axis indicate which target gene (in order in which they were reported) is used for the detection of each sample. Underneath these numbers the contents of the sample are shown. All negative values for which no Cp value was given by the reporters have been given an artificial Cp value of 50. Not all negative results have a Cp value of 50. Some results with Cp below 50 are deemed negative by laboratories, likely due to Cp cutoff values used in the interpretation of an obtained result. Above the graph the number of tests (N) and the percentage of Cp 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 Cp value below 50 is shown as well per sample per target gene. Samples deemed negative with a Cp value below 50 are indicated with a purple diamond inside the graph.

3.4 Comparing results LEQA3 to LEQA2

Whereas 79 laboratories reported data for 180 workflows for LEQA2, 78 laboratories reported data for 202 workflows for LEQA3. This is a decrease of 1.3% of reporting laboratories and an increase of 12.2% of reported workflows. The results obtained from one workflow are excluded as the wrong panel was tested. It should be noted that 5/8 core samples were exactly the same in both panels. Compared to LEQA2 the following specimens were removed: hCoV-229E, hCoV-OC43 and SARS-CoV-2 variant B.1.1.7/20B/501Y.V1 (at 3.39*10^5 copies E gene and 2.32*10^5 copies RdRP gene target/ml specimen). Instead the following specimens were introduced in LEQA3: SARS-CoV-2 B.1.351 20H/501Y.V2 (at 1.15*10^4 copies E gene and 1.14*10^4 copies RdRP gene target/ml specimen), SARS-CoV-2 P.1 20J/501Y.V3 (at 8.77*10^3 copies E gene and 6.60*10^3 copies RdRP gene target/ml specimen) and SARS-CoV-2 B.1.1.7/20B/501Y.V1 (at 3.39*10^4 copies E gene and 2.32*10^4 copies RdRP gene target/ml specimen). The grades obtained by the workflows for both LEQA2 and LEQA3 are listed in Table 5.

Table 5. Overview of grades obtained by the workflows having tested LEQA2 and LEQA3. All workflows receiving scores below 6 are grouped in <6

	Grade obtained						
	8	7.5	7	6.5	6	<6	Total workflows tested
LEQA2	148 (82.2%)	5 (2.7%)	13 (7.2%)	1 (0.1%)	8 (4.4%)	5 (2.7%)	180
LEQA3	160 (79.6%)	6 (3.0%)	16 (8.0%)	0	9 (4.5%)	10 (5.0%)	201

The shift in grades obtained by the workflows tested between LEQA2 and LEQA3 are shown in Table 6. Not all workflows were tested for both LEQA2 and LEQA3. These workflows are labelled 'Not Tested' or 'NT' abbreviated. This data is also visualized in Figure 3.

Table 6. Overview of scores obtained by workflows that tested LEQA2 and how they scored testing LEQA3. Green cells indicate workflows performing better in LEQA3 than in LEQA2. Red cells indicate workflows performing worse in LEQA3 than in LEQA2. White cells indicate workflows performing equally in LEQA3 and LEQA2. All workflows receiving scores below 6 are grouped in <6

		Grade obtained for LEQA2							
		<6	6	6.5	7	7.5	8	NT[1]	Subtotal
Grade obtained for LEQA3	<6	4	1				3	2	10
	6						3	6	9
	6.5								0
	7				1	1	8	6	16
	7.5				1		1	4	6
	8		3		4	4	90	59	160
	NT[2]	1	4	1	7		43		
	Subtotal	5	8	1	13	5	148		201 / 180

[1] Short for 'Not Tested'. These workflows were not used to test LEQA2, but were used to test LEQA3

[2] Short for 'Not Tested'. These workflows were not used to test LEQA3, but were used to test LEQA2

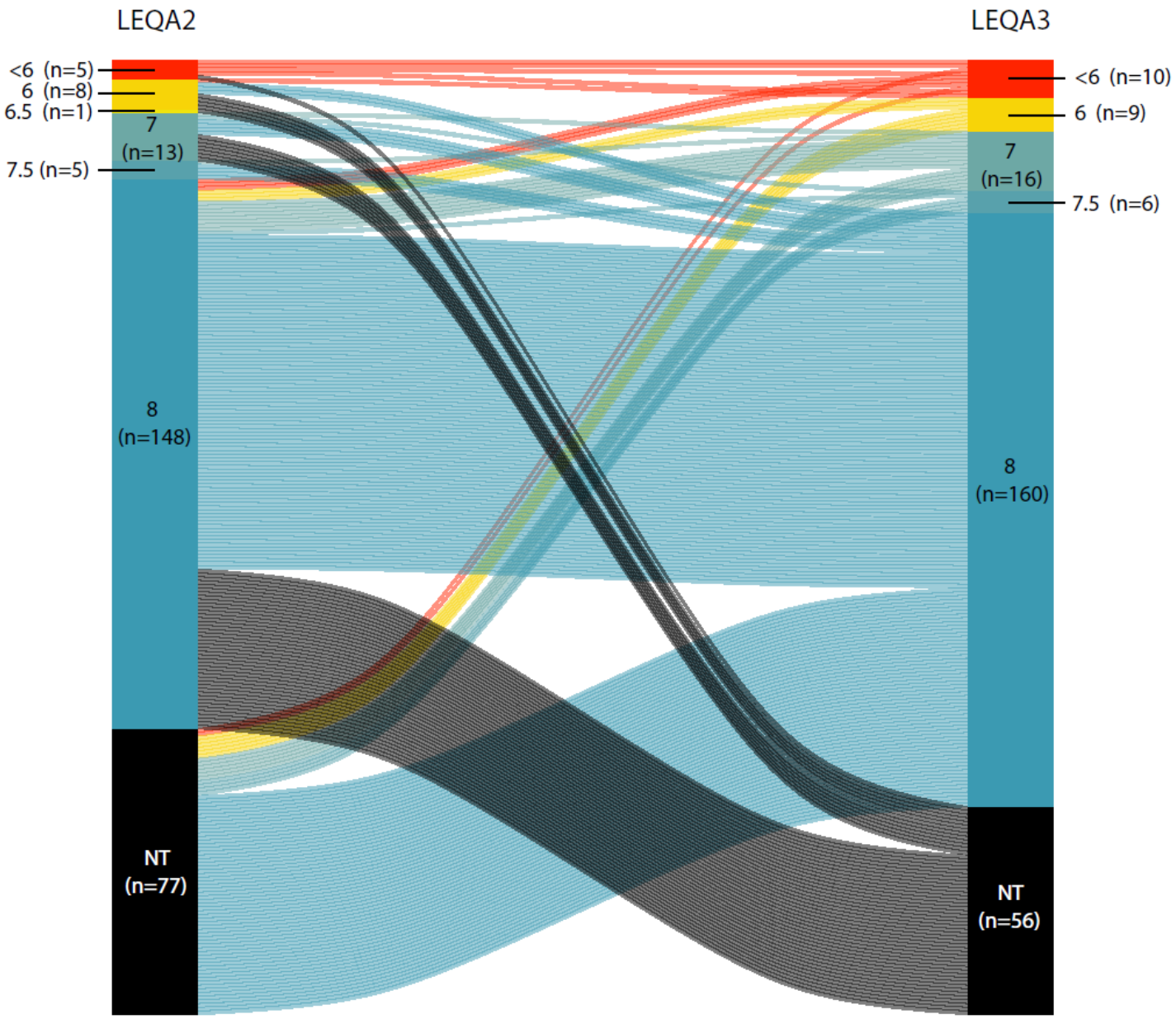


Figure 3: Overview of grades obtained by workflows that tested LEQA2 and how they scored testing LEQA3. 'NT' stands 'Not Tested'. These workflows were not used to test LEQA2, but have tested LEQA3 or vice versa. Color of trails per workflow are based on the grade obtained for LEQA3. All workflows receiving grades below 6 are grouped in <6.

In total 56 workflows were not used to test LEQA3 but were used to test LEQA2. Fourteen of these workflows were mPOCT assays (25.0%), 23 workflows used 'ready to use' kits for RT-qPCR (41.1%), one workflow was a loop-mediated isothermal amplification (LAMP) assay (1.8%) and 18 workflows were in-house assays (32.1%).

Of the 77 newly implemented workflows in LEQA3 (which were not used to test LEQA2 by the same laboratory), 29 were mPOCT assays (37.7%), 30 workflows used 'ready to use' kits for their RT-qPCR (39.0%), 2 workflows were LAMP assays (2.6%) and 16 workflows were in-house assays (20.8%). Although there are 77 newly implemented workflows by laboratories, 50 of these were already included in LEQA2 by other laboratories. The other 27 newly implemented workflows are not tested by any laboratory in LEQA2. These 27 new entries all are RT-PCR based assays.

In total 98/179 (54.7%) of the workflows tested in LEQA2 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 108/200 (54.0%) of the workflows tested in LEQA3 could detect SARS-CoV-2 in this sample.

Both LEQA2_CoV20-6 and LEQA2_CoV20-8 contained SARS-CoV-2 at a concentration of 128 and 173 copies E gene and RdRP gene target/ml specimen, respectively (dilution d3, see Table 1). LEQA2_CoV20-6 was measured positive for SARS-CoV-2 by a total of 147/162 (90.7%) of the workflows. For LEQA2_CoV20-8 157/176 (89.2%) of the workflows tested these samples positive for SARS-CoV-2. In LEQA3 identical samples with the same SARS-CoV-2 load but with different labelling were put in the panel: LEQA3_CoV20-7 and LEQA3_CoV20-9 respectively. LEQA3_CoV20-7 was scored positive for SARS-CoV-2 by 170/186 (91.4%) workflows. An increase of 0.7% compared to LEQA2_CoV20-6. LEQA3_CoV20-9 was scored positive by 173/197 (87.8%) workflows. A decrease of 1.4% compared to LEQA2_CoV20-8.
Another set of specimens with the same SARS-CoV-2 loads in both LEQA panels were LEQA2_CoV20-2 and LEQA3_CoV20-3. 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). LEQA2_CoV20-2 was scored positive for SARS-CoV-2 by 160/160 (100%) workflows, whereas LEQA3_CoV20-3 was scored positive by 183/183 (100%) workflows. So, at the highest SARS-CoV-2 concentration (d1), all workflows could detect this sample in both LEQA rounds. The percentage of positive results for both specimens with the intermediate SARS-CoV-2 load (d3) changed from 89.2%/90.7% to 91.4%/87.8%. Lastly the specimens with the lowest SARS-CoV-2 load (d4) of both panels show a slight decrease in positive results from 54.7% to 54.0%. Taken together, for each of the samples with different SARS-CoV-2 concentrations, the workflows included in LEQA3 showed similar results as those in LEQA2.
On a laboratory level, when testing LEQA2, six laboratories had only workflows with grades below 7. Two of these laboratories (laboratory 13 and 20 in Supplemental Figures 30 and 31) have improved their workflows in the meantime and now no longer score below 7 for LEQA3. Two other laboratories (laboratory 76 and 77) unfortunately still score below 7 with their workflows. This is at least in part caused by not testing the full LEQA3 panel nor the reduced mPOCT panel for these workflows, leading to a lower grade. The two remaining laboratories which scored below 7 only in LEQA2 unfortunately did not submit any data for LEQA3.
From the workflows initially scoring a grade <6 for LEQA2, 1/5 (20.0%) were not retested with LEQA3. Similarly, from 4/8 (50.0%) workflows initially scoring a 6, 1/1 (100%) of the workflows initially scoring a 6.5, 7/13 (53.8%) workflows initially scoring a 7, 0/5 (0%) workflows initially scoring a 7.5 and 43/148 (29.1%) workflows initially scoring an 8 were not retested with LEQA3. The relative amount of LEQA2 workflows not re-tested in LEQA3 is bigger for the poorer scoring workflows (<7; 42.9% not re-tested) compared to the better scoring workflows (7-8; 30.1% not re-tested) in LEQA3.
A number of workflows included in LEQA3 scored lower than in LEQA2 (a total of 17 workflows). Of all these workflows scoring an 8 in LEQA2, 3/15 (20.0%) scored a 4 due to not testing the full panel or the reduced panel, 3/15 (20.0%) scored a 6, 8/15 (53.3%) scored a 7 and 1/15 (6.7%) scored a 7.5. One workflow scoring a 7.5 in LEQA2 scored a 7 in LEQA3. Another workflow scored a 6 in LEQA2 and <6 in LEQA3. The latter workflow is a LAMP-based assay of which lower sensitivity is often reported compared to conventional (RT-PCR based) workflows.
In total, 124 workflows used to test LEQA2 were also used to test LEQA3. A detailed description of a comparison of the performance with these workflows is shown in Table 6.

4. Discussion and conclusion

Her we describe the results of the third national LEQA panel. 77 laboratories, using in total 201 workflows have reported their findings. Only one workflow scored below 6 when testing either the full panel or the reduced mPOCT panel. Out of the 201 workflows reported, 160/201 (79.6%) scored a maximum score for all 8 core specimens (8 points) and thus met all criteria set for reliable SARS-CoV-2 diagnostics. 22/201 (10.9%) scored between 7-7.5, making it likely that only minor adjustments need to be made to meet all criteria and 19/201 (9.5%) workflows scored a 6.5 or lower. All but one of the workflows scoring below 6 (n=10; 5.0%) seemed to have obtained a low score due to testing not the recommended reduced panel for mPOCT testing, but a different smaller subset of the core samples**.** Due to this, a lot of samples received a score of "Not tested" and thus decreased the overall grade of the test. The workflows on which neither the full nor reduced panel were tested artificially reduce the overall quality of the Dutch SARS-CoV-2 diagnostic field. When the workflows on which only the full panel or reduced mPOCT panel were tested are taken into account (n=192), the overall quality increases. Out of these 192 workflows, 160/192 (83.3%) scores an 8, 22/192 (11.5%) scores 7-7.5, 9/192 (4.7%) workflows score a 6 and 1/192 (0.5%) scores below 6. When scoring below 6, it is an indication that major improvements need to be made for these workflows to be reliable for SARS-CoV-2 diagnostics in clinical diagnostic settings and surveillance. In LEQA1 and LEQA2 was shown that the workflows showed very little specificity issues, and that most issues were found in sensitivity of SARS-CoV-2 detection. [2, 3] As the number of SARS-CoV-2 variant infections are rising, the new specimens are a valuable addition to the LEQA3 panel. Although the composition of the panels has changed, it is still valuable to compare the scores obtained between LEQA2 and LEQA3.

When considering all workflows each laboratory has access to and has reported LEQA3 results for, two laboratories have workflows which do not have a grade of 7 or above. When including only laboratories that correctly implemented the panel, all laboratories have at least one workflow that scored 7 to 8 points. Only four laboratories have solely access to mPOCT based assays, limiting their maximal daily throughput of clinical specimens. Two of these laboratories had at least one workflow scoring an 8. The other two laboratories did not (due to not testing the full panel or reduced mPOCT panel).

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 (118 out of 201 workflows).

When comparing all workflows, there appears to be a small correlation between the number of target genes used and the score obtained by the workflow, indicating a small increased sensitivity when using multiple targets. When leaving out all workflows on which neither the full panel nor the mPOCT panel were tested, the average scores obtained are 7.76, 7.80, 7.89 and 7.92 out of 8 for workflows containing one, two, three and four target genes, respectively. Another argument in favour of multiple target genes is that it decreases the chance of false negative test results when testing (novel) SARS-CoV-2 variants. Mutations in SARS-CoV-2 variant B.1.1.7/20B/501Y.V1 can cause an S-gene dropout in certain assays. [4] If an assay were to use only an S-gene as target gene, there is a bigger chance on a false negative result than in assays with multiple target genes. It is likely that other novel SARS-CoV-2 variants cause target gene dropouts as primer/probe sets are not optimized for that particular genetic variant.

No false indeterminate/inconclusive/equivocal results have been obtained for any of the core samples of LEQA3. Additionally, many workflows reported the SARS-CoV-1 containing sample (LEQA3_CoV20-2) 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 20% of the workflows SARS-CoV-2

specific E-gene primers/probe are reported whilst it still detects SARS-CoV-1 (Supplemental Figure 14). It appears most likely that for these workflows the specificity of E-gene primers/probe is wrongly labelled as SARS-CoV-2 specific rather than Sarbeco specific. Less likely is that these primer/probe sets have a strong cross-reaction to SARS-CoV-1. 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.

Whereas no 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 target genes generating a Cp value in some workflows, the target gene result was deemed negative for SARS-CoV-2 presence, possibly related to a laboratory specific determined cut off value or other criterium, e.g. shape of the amplification curve. In one SARS-CoV-2 positive sample (dilution d4, see Table 1) 0.7% of the total overall conclusions were reported as SARS-CoV-2 negative despite one (or more) of the target genes against SARS-CoV-2 in the assay generating a Cp value.

For all LEQA3 samples tested with a workflow providing Cp values as an output, a broad range of Cp values for the individual SARS-CoV-2 containing samples has been reported. The biggest range of reported Ct values was found for LEQA3_CoV20-9. The average Ct value was 35.67 (SD 2.69) where the lowest Cp value reported was 28.50 and the highest value was 43.84 (making a 15.35 difference in Cp value), indicating a wide spread of Cp values reported for the same sample, generated by the large variety of workflows used. Despite this wide range of Cp values for the same sample by different workflows, it did not seem to affect the sensitivity of the workflows. This finding indicates that comparing Cp values between workflows and laboratories should not be done without calibration with 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 and LEQA2. [2, 3] 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. [5] 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 novel variants of SARS-CoV-2. Therefore it is encouraging that in the current LEQA3, SARS-CoV-2 variants of concern B.1.351 20H/501Y.V2, P.1 20J/501Y.V3 and B.1.1.7 20B/501Y.V1 were detected by 100%, 99.5% and 99.5% of the workflows, respectively. SARS-CoV-2 variant P.1 20J/501Y.V3 was likely did not detect by one (0.5%) of workflow due to sensitivity issues. It is unclear why one (0.5%) of the workflows did not detect variant B.1.1.7 20B/501Y.V1 as similar workflows implemented by other laboratories tested this sample positive for SARS-CoV-2.

Comparing this third round of quality assessment with the second round, one less laboratory reported their findings. The total number of reported workflows increased with 22 (+12.2%) compared to the earlier assessment. The relative amount of LEQA2 workflows not re-tested in LEQA3 is bigger for the poorer scoring workflows (<7; 42.9% not re-tested) compared to the better scoring workflows (7-8; 30.1% not re-tested) in LEQA3. Between LEQA1 and LEQA2 a similar pattern is visible as 64.3% of the poorer scoring workflows (score <7) and 37.3% of the better scoring workflows (score 7-8) were not retested. [2, 3] This data seems to indicate that laboratories use these quality assessments as a way to improve their performance in SARS-CoV-2 diagnostics. In total 14 workflows were tested in both LEQA1 and LEQA3, but not in LEQA2. There seems to be a trend showing a disuse of poorer 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 third LEQA round.

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

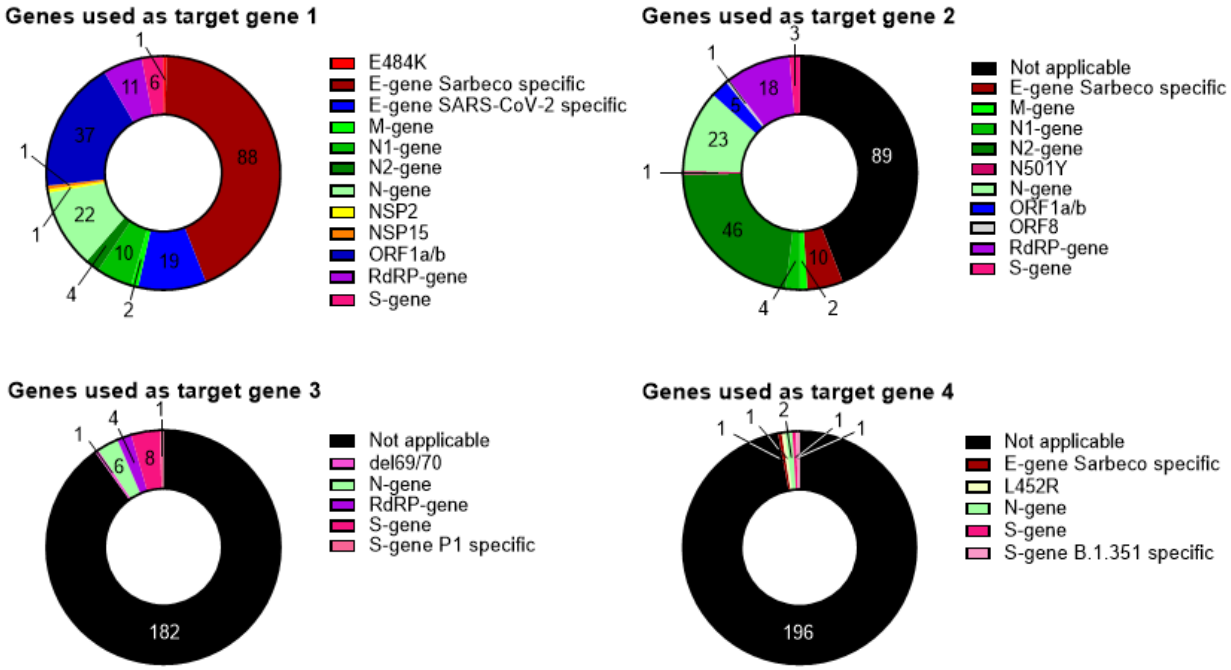
5. References

1. RIVM. Aanvullende informatie diagnostiek COVID-19. 2021 [cited 2021 28-6-2021]; 24: Available from: https://lci.rivm.nl/covid-19/bijlage/aanvullend.
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/documenten/external-quality-assessment-of-laboratories-performing-sars-cov-2-diagnostics-for-dutch
3. J. Sluimer *et al.*, "External Quality Assessment of laboratories Performing SARS-CoV-2 Diagnostics for the Dutch Population, February 2021", RIVM report. Available from: https://www.rivm.nl/documenten/external-quality-assessment-of-laboratories-performing-sars-cov-2-diagnostics-for-0
4. Kidd, M., et al., *S-Variant SARS-CoV-2 Lineage B1.1.7 Is Associated With Significantly Higher Viral Load in Samples Tested by TaqPath Polymerase Chain Reaction.* J Infect Dis, 2021. **223**(10): p. 1666-1670.
5. 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.

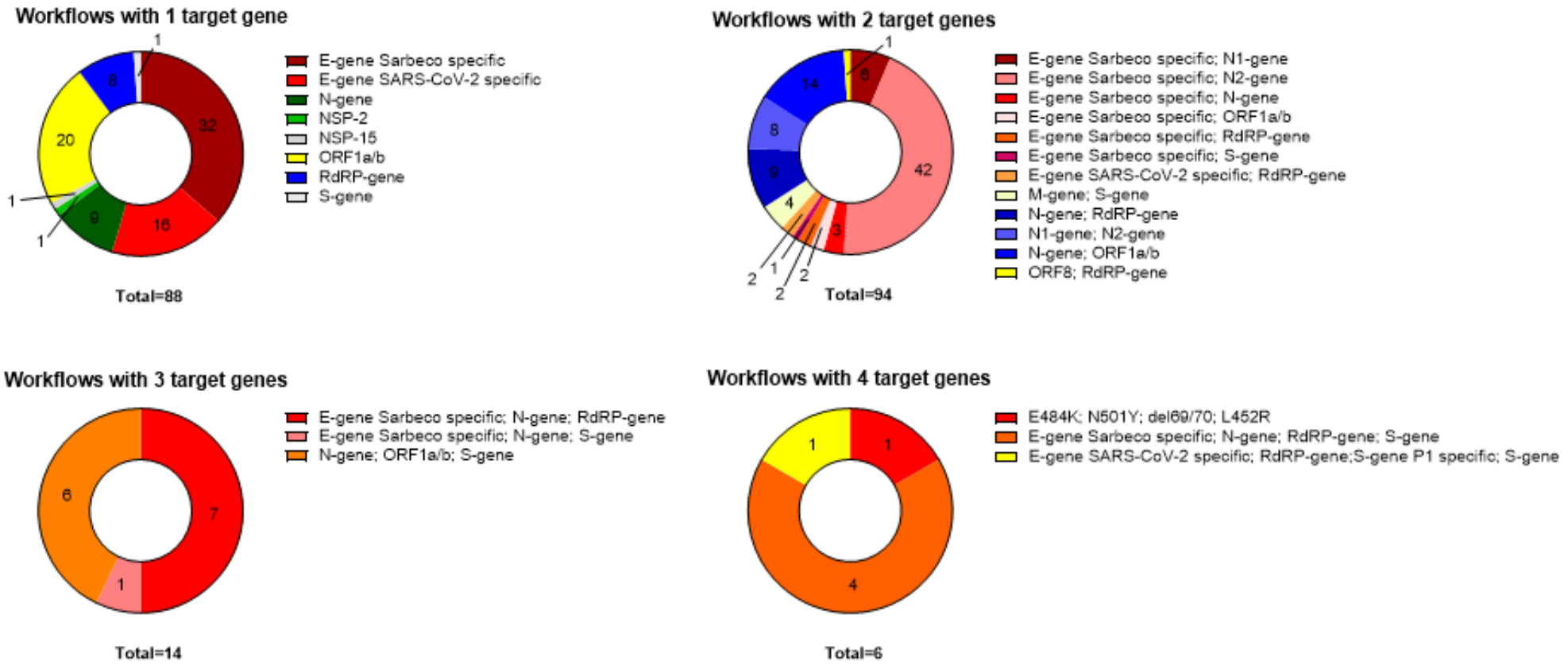
6. Supplemental material

6.1 Target genes used by workflows

Some workflows used up to 4 target genes. From the 202 workflows a total of 88 workflows used 1 target gene, 94 workflows used 2 target genes, 14 workflows used 3 target genes and 6 workflows used 4 target genes. 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, the target genes used in the order reported for each workflow are shown. Supplemental Figure 2 shows the combinations of target genes used in the workflows.



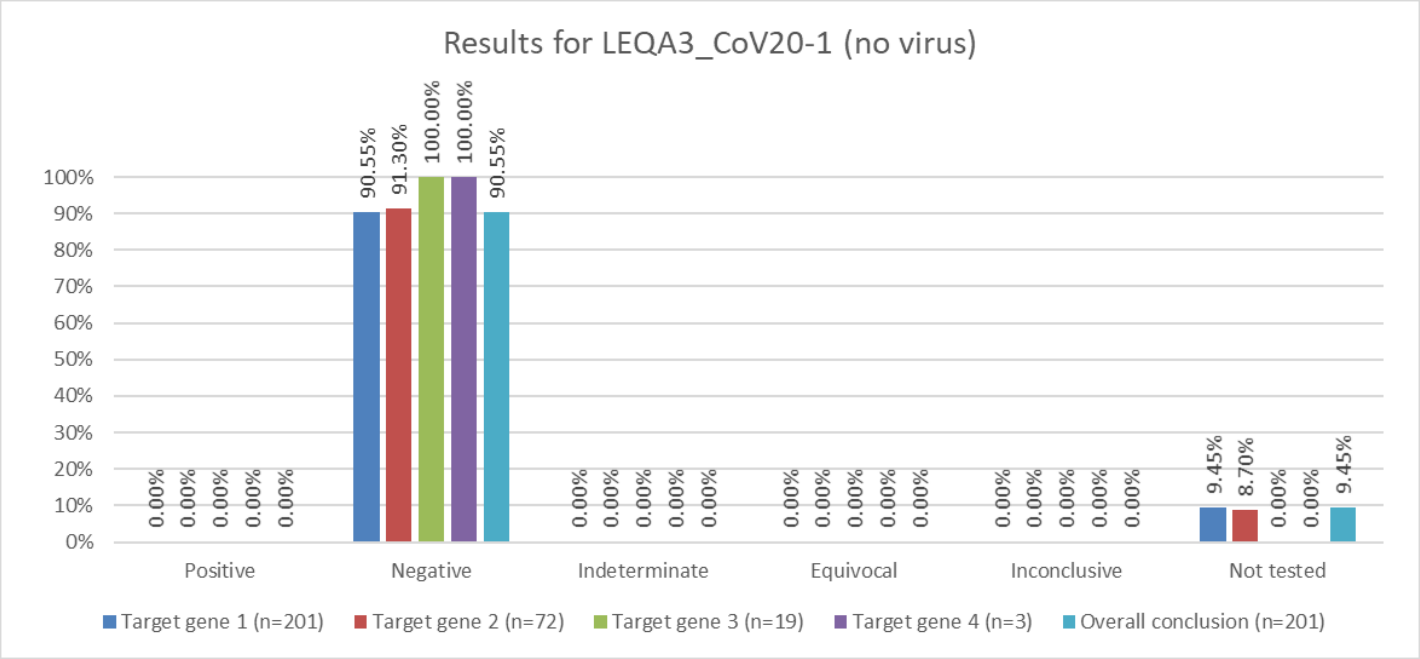
Supplemental Figure 1: Target genes used in the workflows as reported in the questionnaire (n=202). The colour coding of the used target genes is shown in the legends. Black colour indicates workflows that do not contain a 2nd, 3rd or 4th gene target.



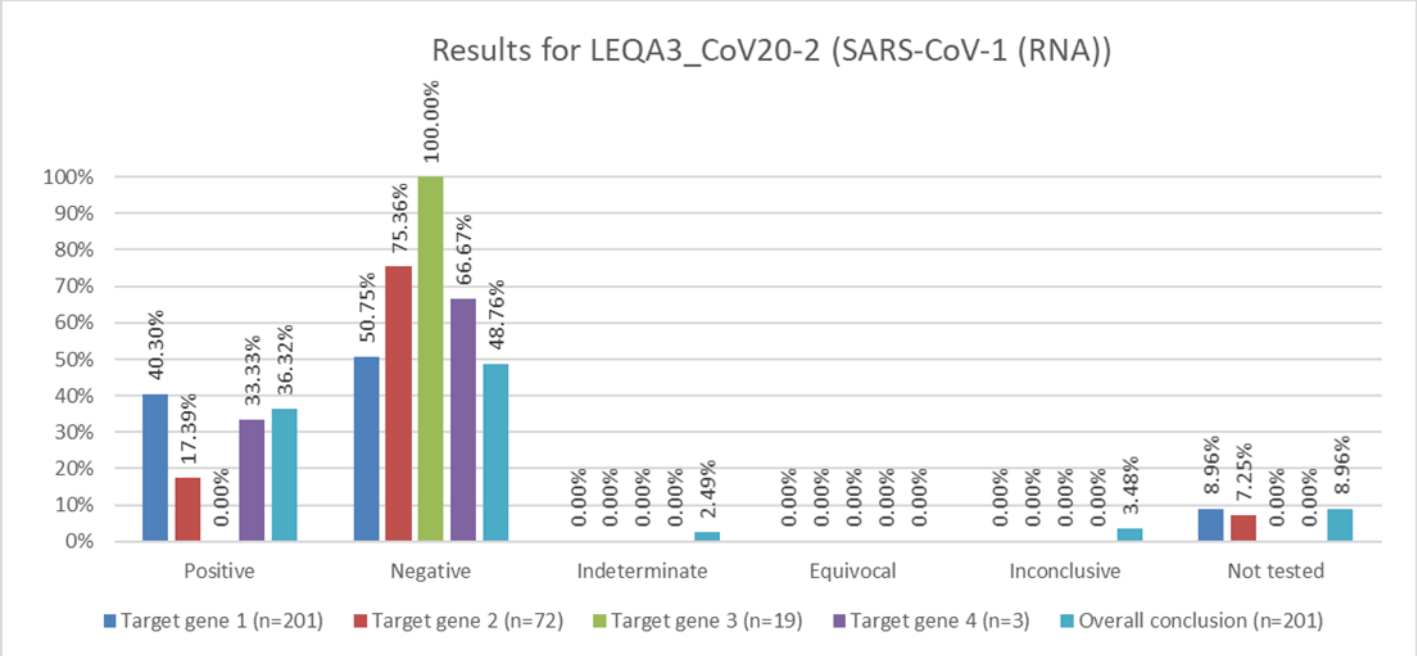
Supplemental Figure 2: Combinations of target genes used in the workflows as reported in the questionnaire (n=202). The colour coding of the used target genes is shown in the legends.

6.2 Results obtained per LEQA3 sample

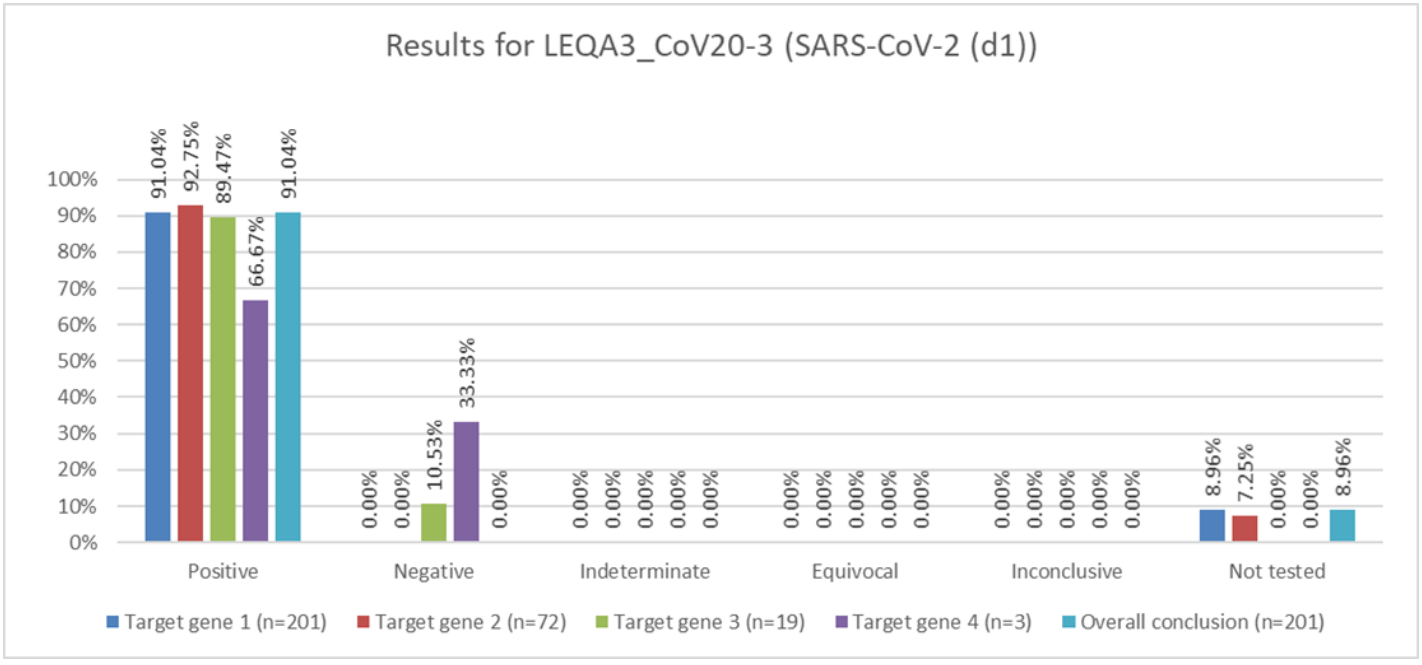
Supplemental Figure 3 – Figure 12 show the obtained results for LEQA3_CoV20-1 up to LEQA3_CoV20-10 for all target genes tested. The results per target gene are shown in the order in which the target genes were reported together with an overall conclusion. The target genes used are shown in Supplemental Figure 1. 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 Supplemental Figure 3 – Figure 12



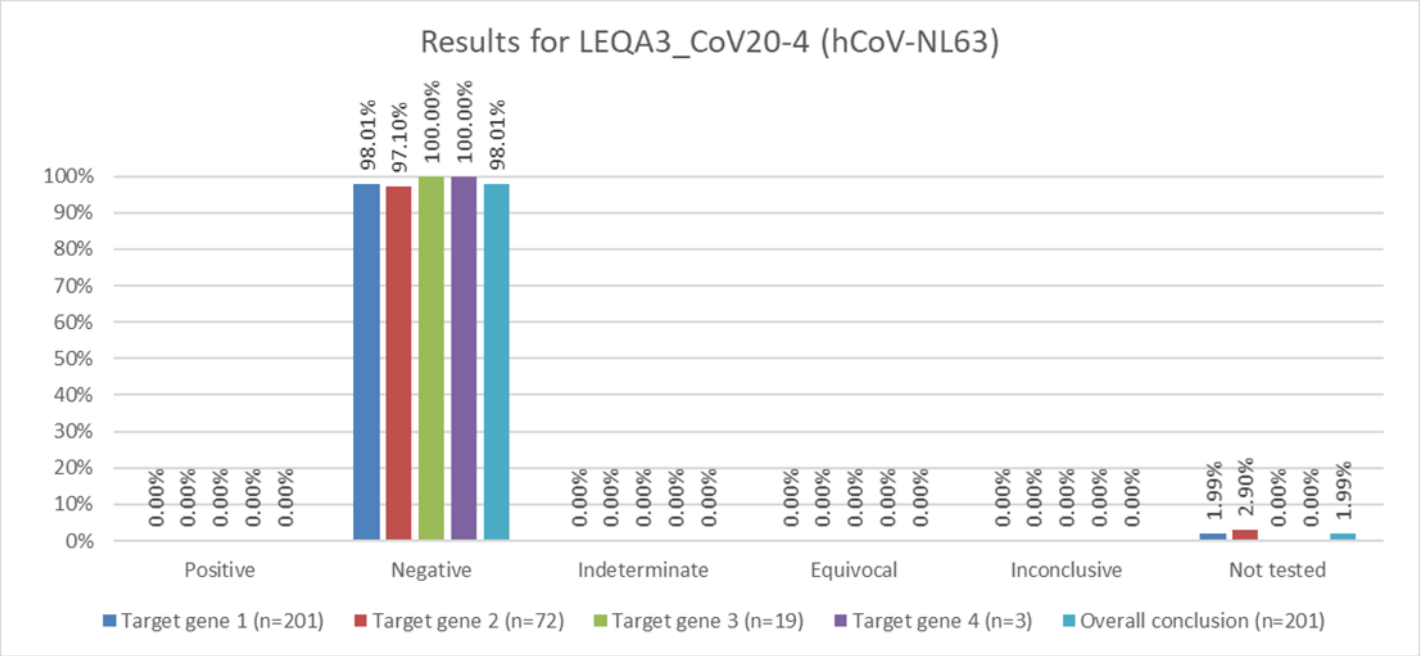
Supplemental Figure 3: Results obtained for LEQA3_CoV20-1 containing no virus combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, 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 182 workflows that reported an overall conclusion 182/182 (100%) did correctly not detect SARS-CoV-2 in this sample.



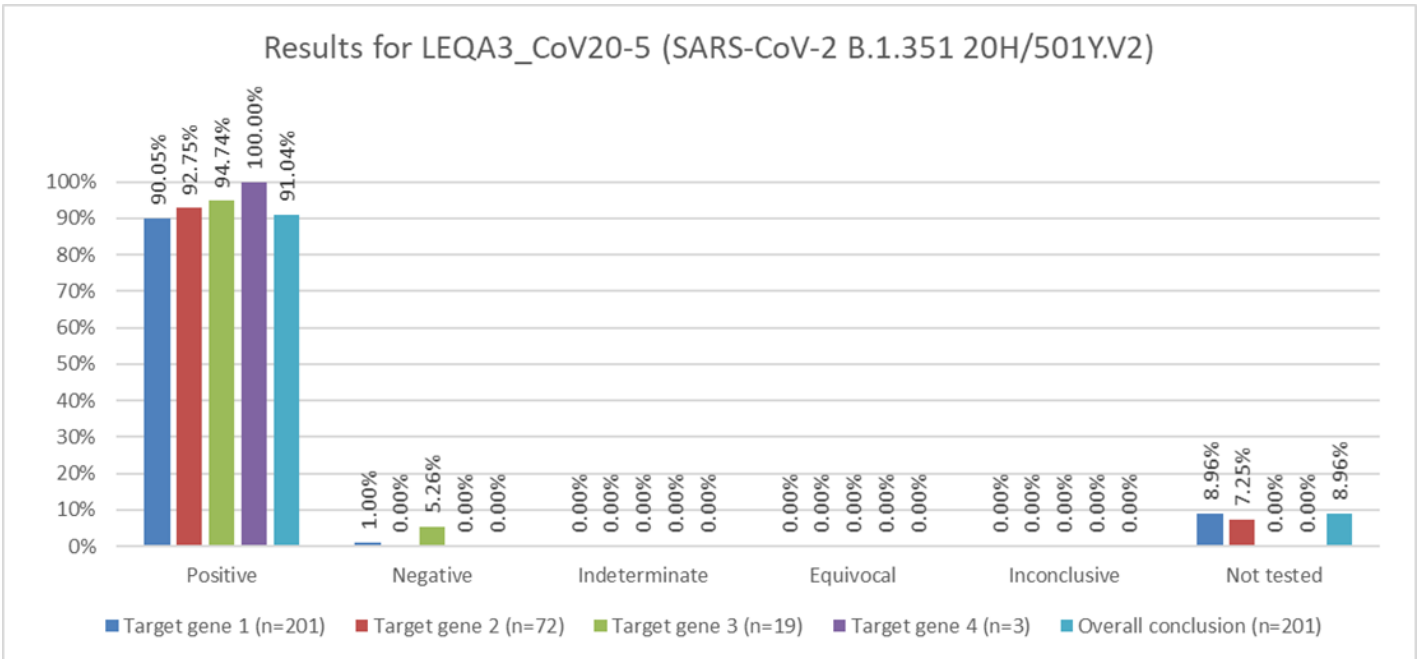
Supplemental Figure 4: Results obtained for LEQA3_CoV20-2 containing SARS-CoV-1 (RNA) combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, 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 183 workflows that reported an overall conclusion 98/183 (53.6%) did correctly not detect SARS-CoV-2 in this sample. 12/183 (6.6%) workflows reported an indeterminate, equivocal or inconclusive result and 73/183 (39.9%) reported a positive result, highly likely because in the current epidemiological situation no other Sarbeco virus than SARS-CoV-2 would be expected.



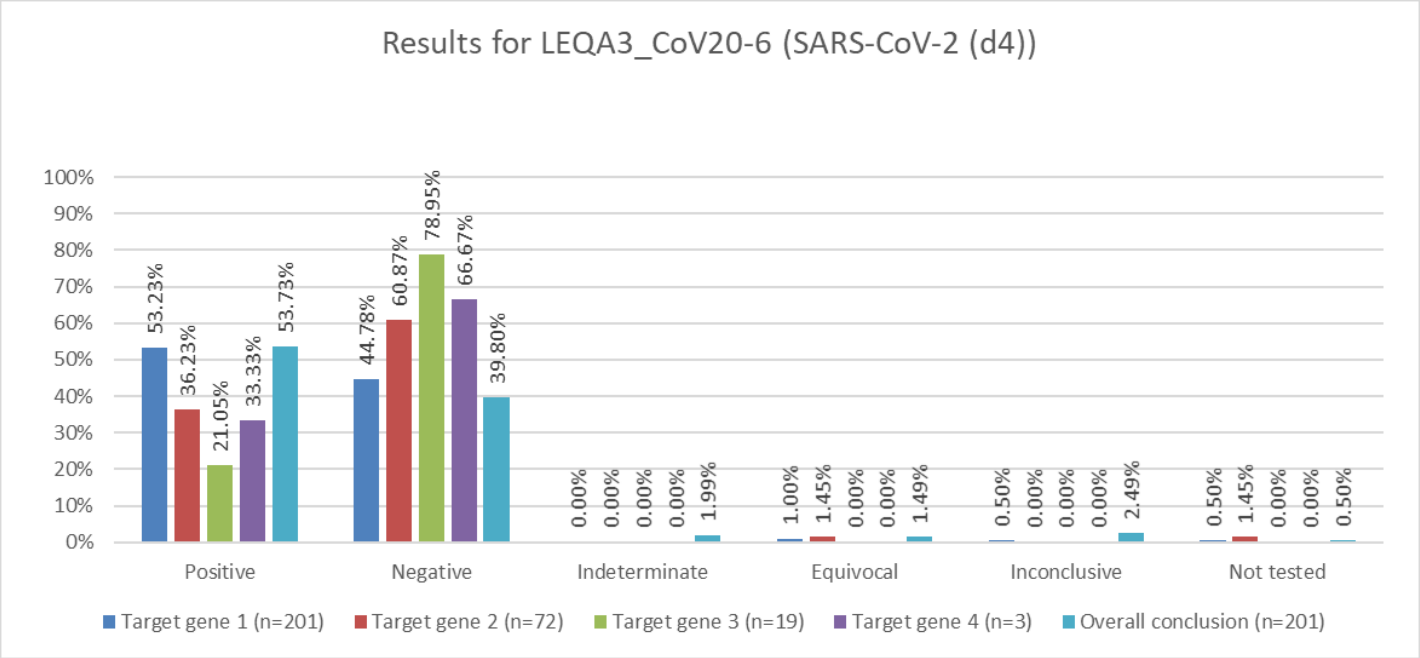
*Supplemental Figure 5: Results obtained for LEQA3_CoV20-3 containing SARS-CoV-2 (with 1.28*10^5 copies E target/ml sample (determined with dPCR) and 1.73*10^5 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were 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 183 workflows that reported an overall conclusion did correctly detect SARS-CoV-2 in this sample.*



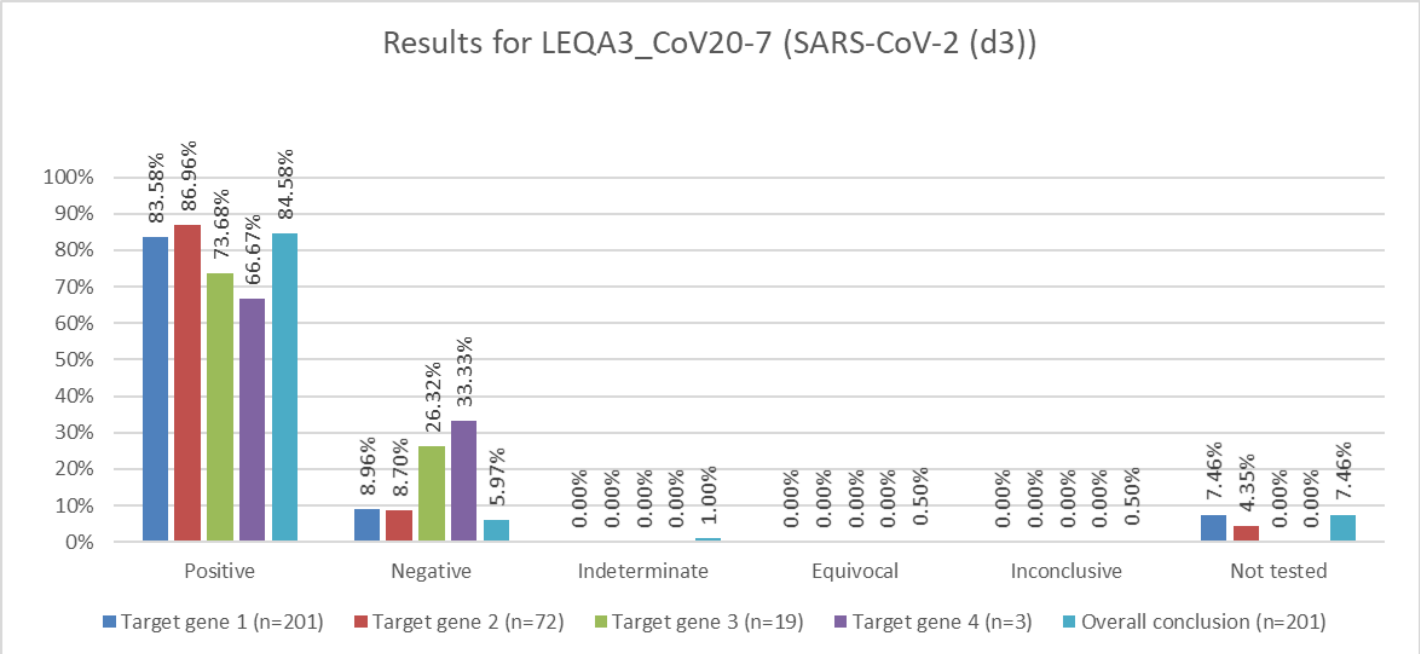
Supplemental Figure 7: Results obtained for LEQA3_CoV20-4 containing hCoV-NL63 combined from all workflows. All laboratories participating in testing of the LEQA were asked to score this sample for the presence of SARS-CoV-2 in any of these six categories: Positive, Negative, Indeterminate, Equivocal, 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 deemed a core sample in the panel. Overall, all 197 workflows that reported an overall conclusion did correctly not detect SARS-CoV-2 in this sample.



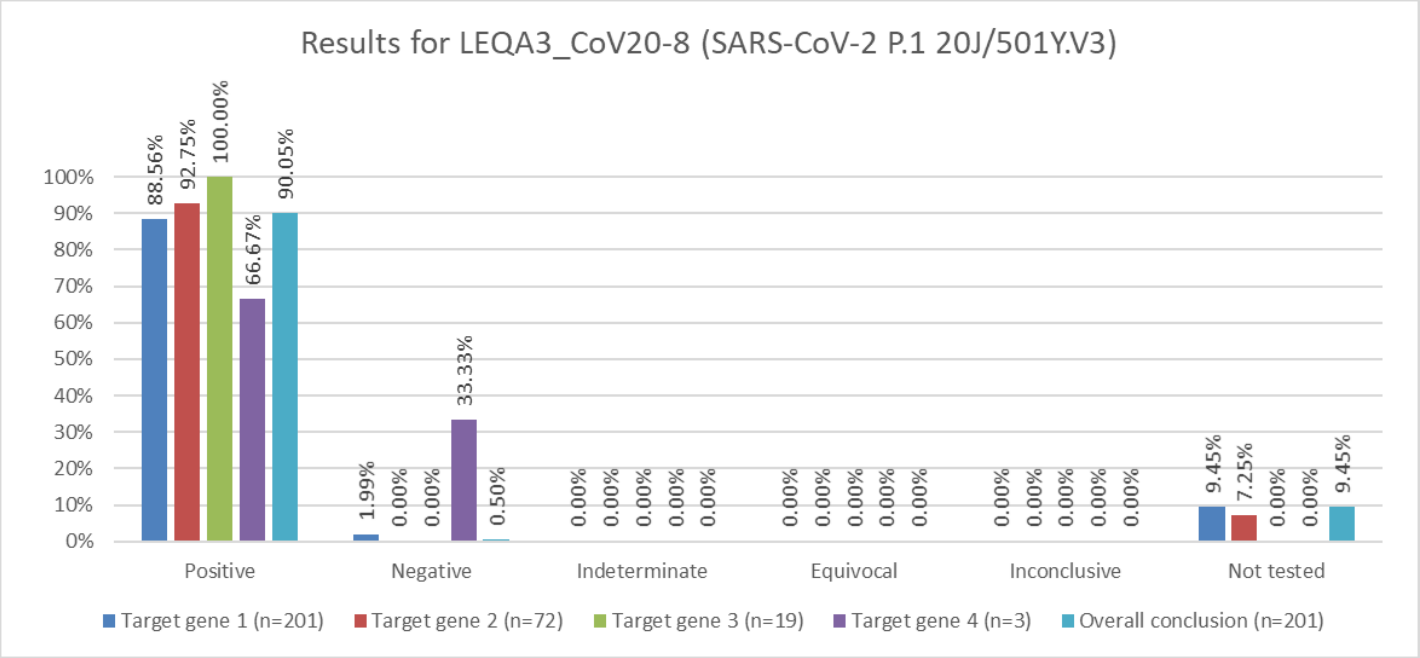
*Supplemental Figure 6: Results obtained for LEQA3_CoV20-05 containing SARS-CoV-2 (SARS-CoV-2 variant B.1.351 20H/501Y.V2.V1) (with 1.15*10^4 copies E target/ml sample (determined with dPCR) and 1.14*10^4 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories 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 183 workflows that reported an overall conclusion did correctly detect SARS-CoV-2 in this sample*



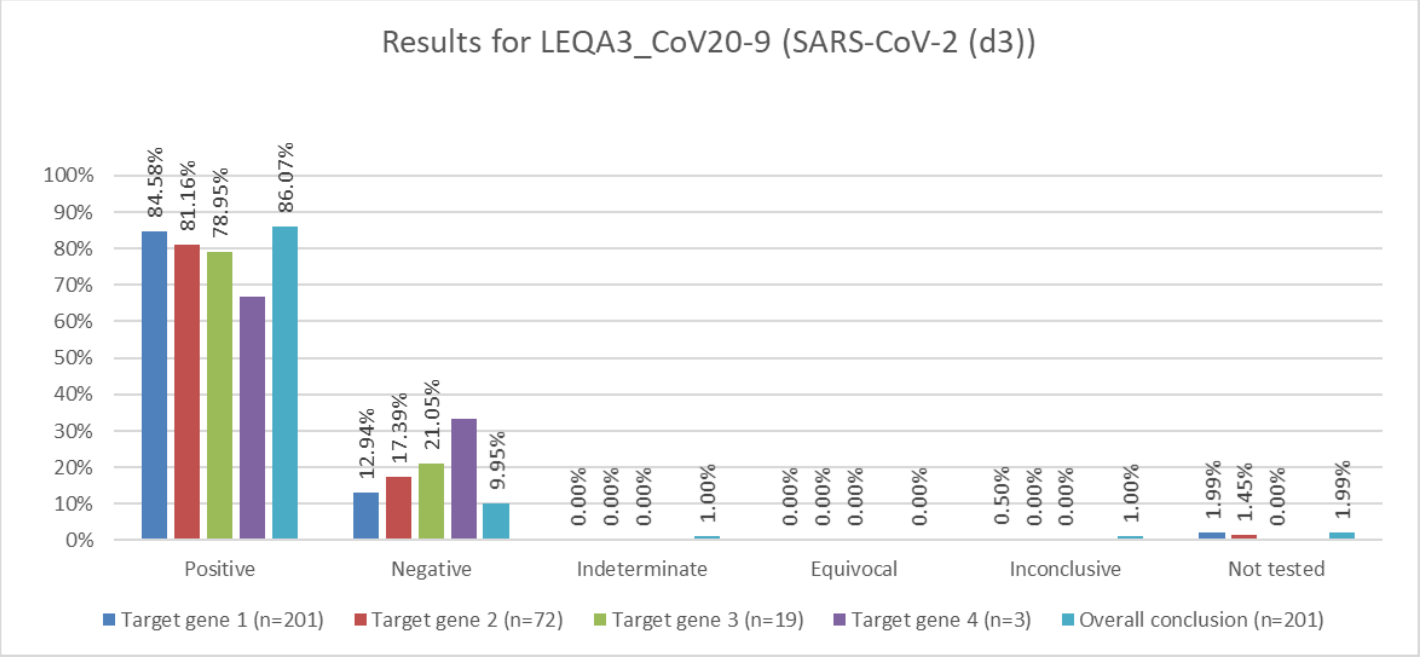
*Supplemental Figure 9: Results obtained for LEQA3_CoV20-6 containing SARS-CoV-2 (with 1.28*10^2 copies E target/ml sample (determined with dPCR) and 1.73*10^2 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were 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. Overall, of all 200 workflows that reported an overall conclusion 108/200 (54.0%) correctly identified this sample as SARS-CoV-2 positive and 12/200 (6.0%) workflows gave an indeterminate, equivocal or inconclusive overall conclusion. 80/200 (40.0%) workflows reported incorrectly a negative result.*



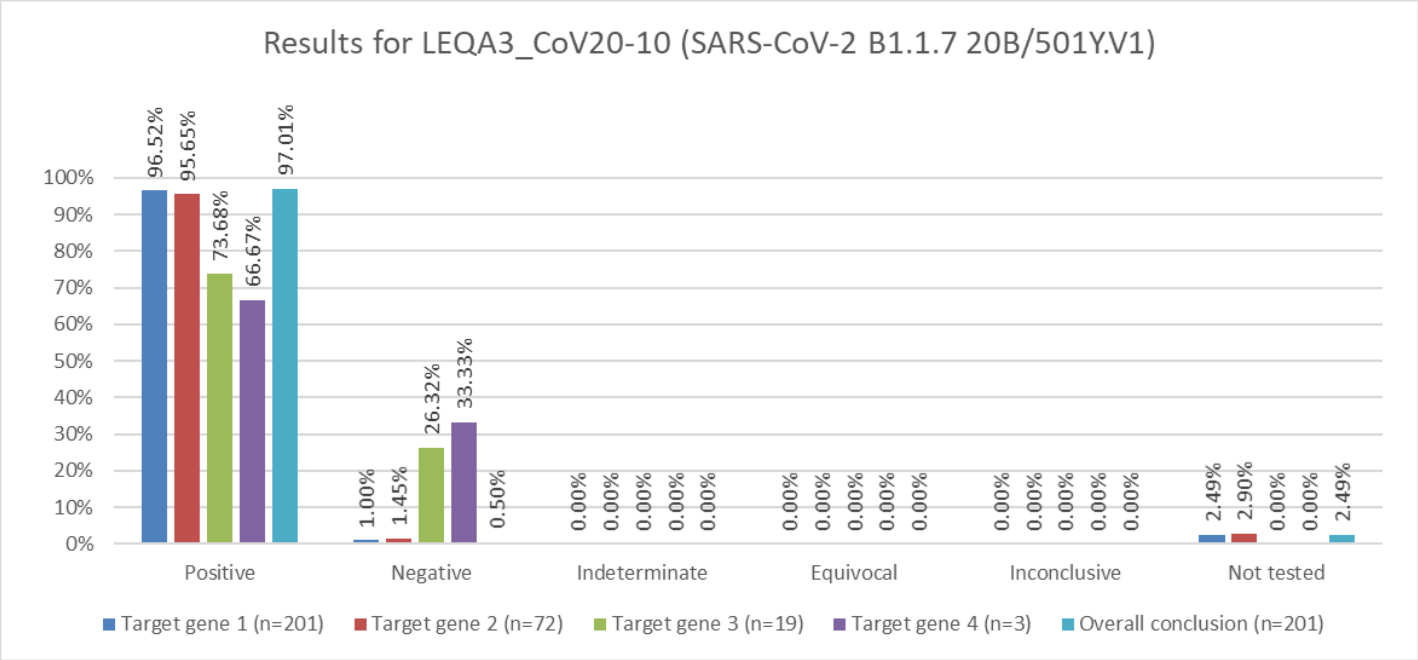
*Supplemental Figure 8: Results obtained for LEQA3_CoV20-7 containing SARS-CoV-2 (with 1.28*10^3 copies E target/ml sample (determined with dPCR) and 1.73*10^3 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were 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 170/186 (91.4%) correctly identified this sample as SARS-CoV-2 positive and 4/186 (2.2%) workflows gave an indeterminate, equivocal or inconclusive overall conclusion. 12/186 (6.5%) workflows reported incorrectly a negative result.*



*Supplemental Figure 11: Results obtained for LEQA3_CoV20-8 containing SARS-CoV-2 (SARS-CoV-2 variant P.1 20J/501Y.V3) (with 8.77*10^3 copies E target/ml sample (determined with dPCR) and 6.60*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 182 workflows that reported an overall conclusion 181/182 (99.5%) correctly identified this sample as SARS-CoV-2 positive and 1/181 (0.5%) workflows reported incorrectly a negative result.*



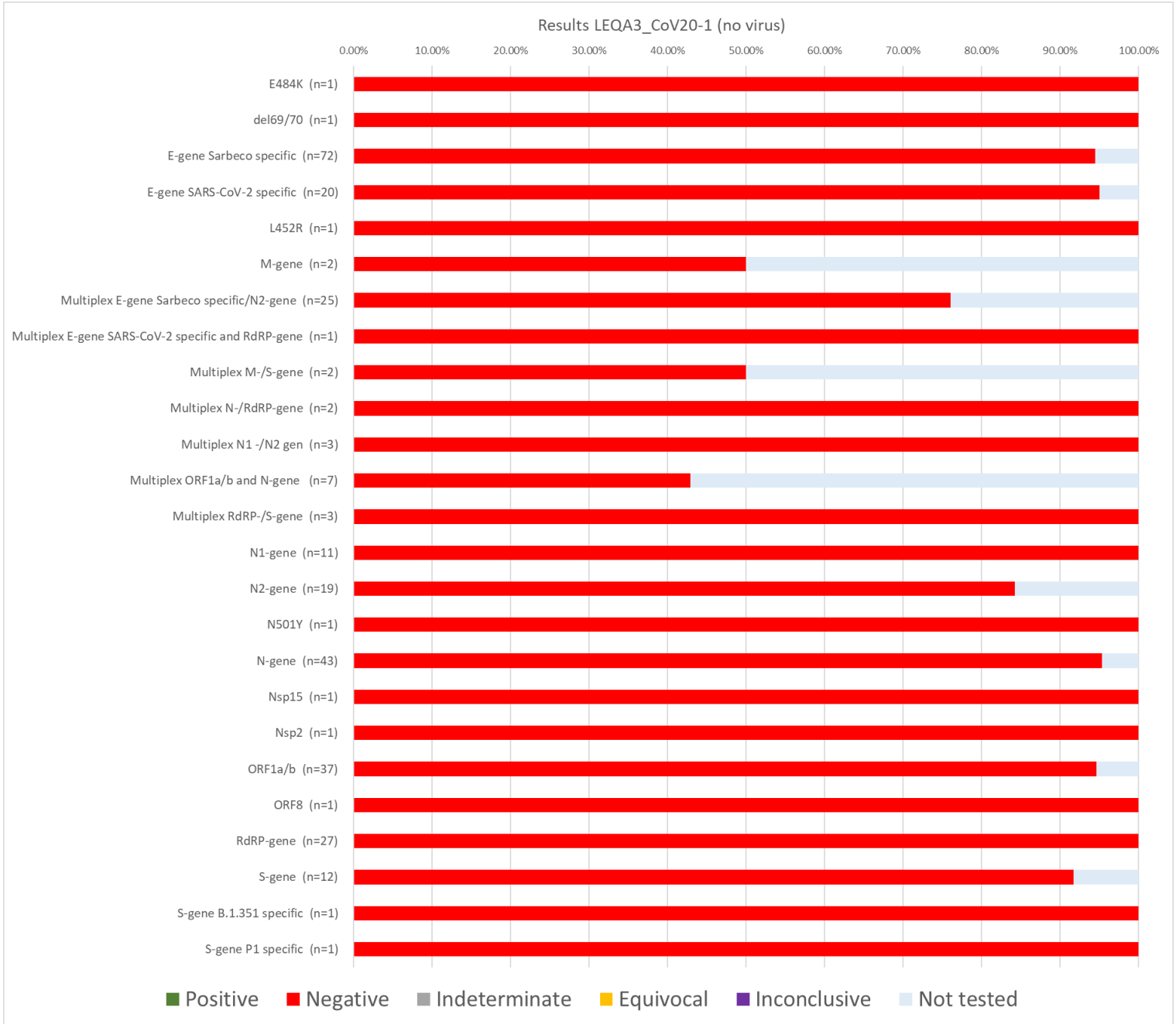
*Supplemental Figure 10: Results obtained for LEQA3_CoV20-9 containing SARS-CoV-2 (with 1.28*10^3 copies E target/ml sample (determined with dPCR) and 1.73*10^3 copies RdRP target/ml sample (determined with dPCR)) combined from all workflows. All laboratories participating in testing of the LEQA were 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 197 workflows that reported an overall conclusion 173/197 (87.8%) correctly identified this sample as SARS-CoV-2 positive and 4/197 (2.0%) workflows gave an indeterminate, equivocal or inconclusive overall conclusion. 20/197 (10.2%) workflows reported incorrectly a negative result.*



Supplemental Figure 12: Results obtained for LEQA3_CoV20-10 containing SARS-CoV-2 (SARS-CoV-2 variant B1.1.7 20B/501Y.V1) 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 196 workflows that reported an overall conclusion 195/196 (99.5%) identified this sample as SARS-CoV-2 positive 1/196 (0.5%) workflows reported incorrectly a negative result.

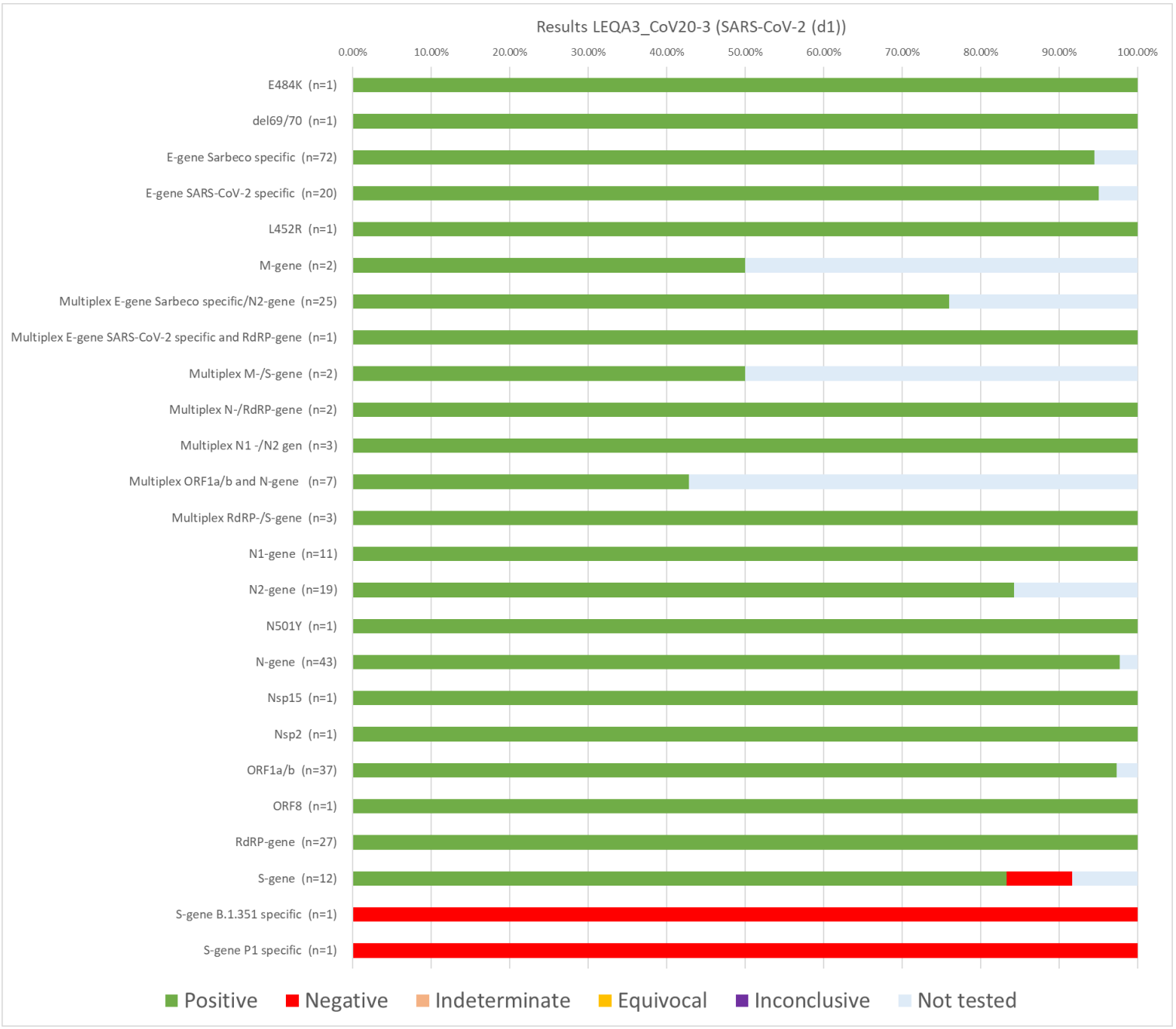
6.3 Results obtained per target gene per sample

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 13-22 these are shown together as one target gene.

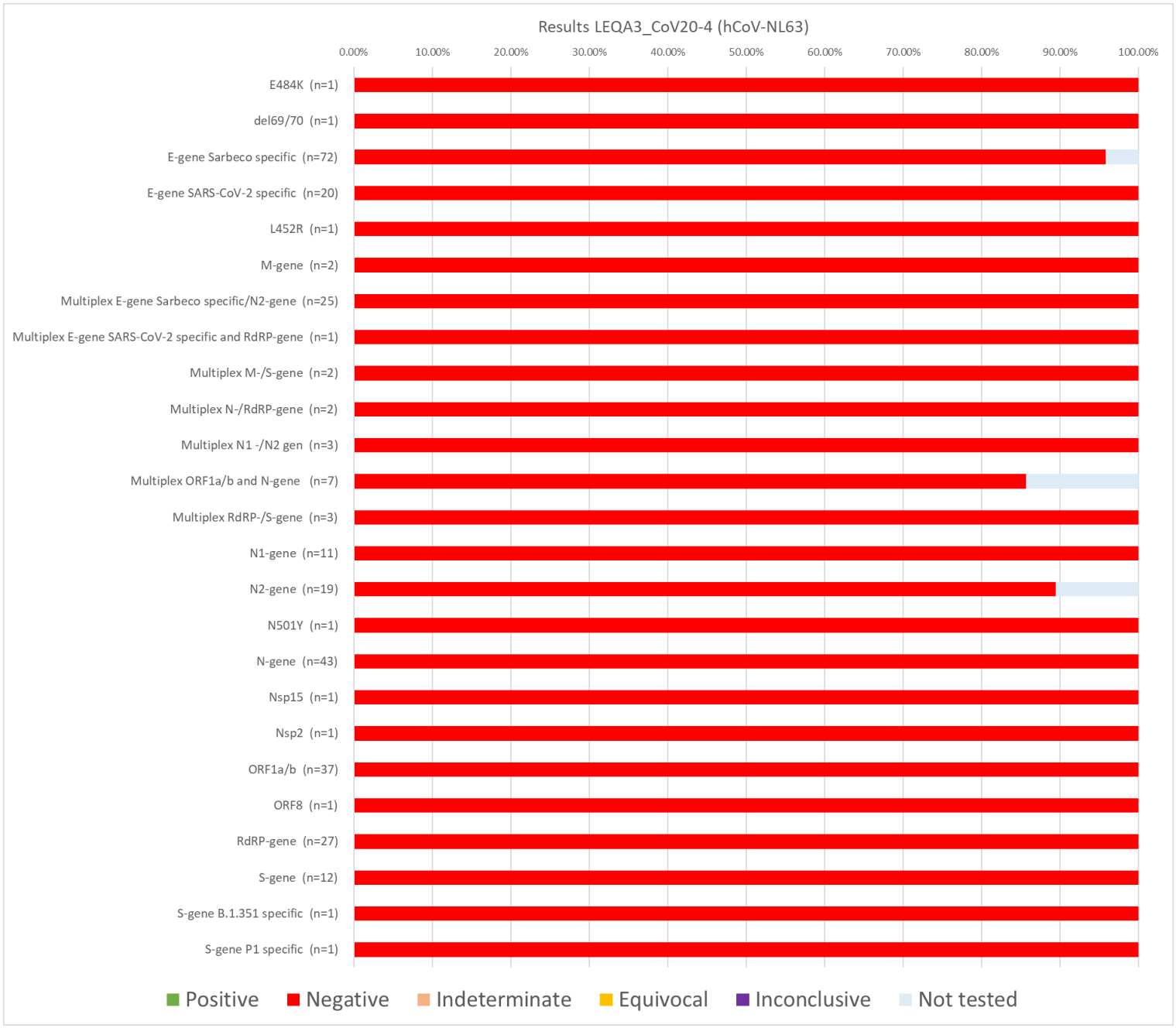


Supplemental Figure 13: The percentages of the various scores obtained for LEQA3_CoV20-1 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample

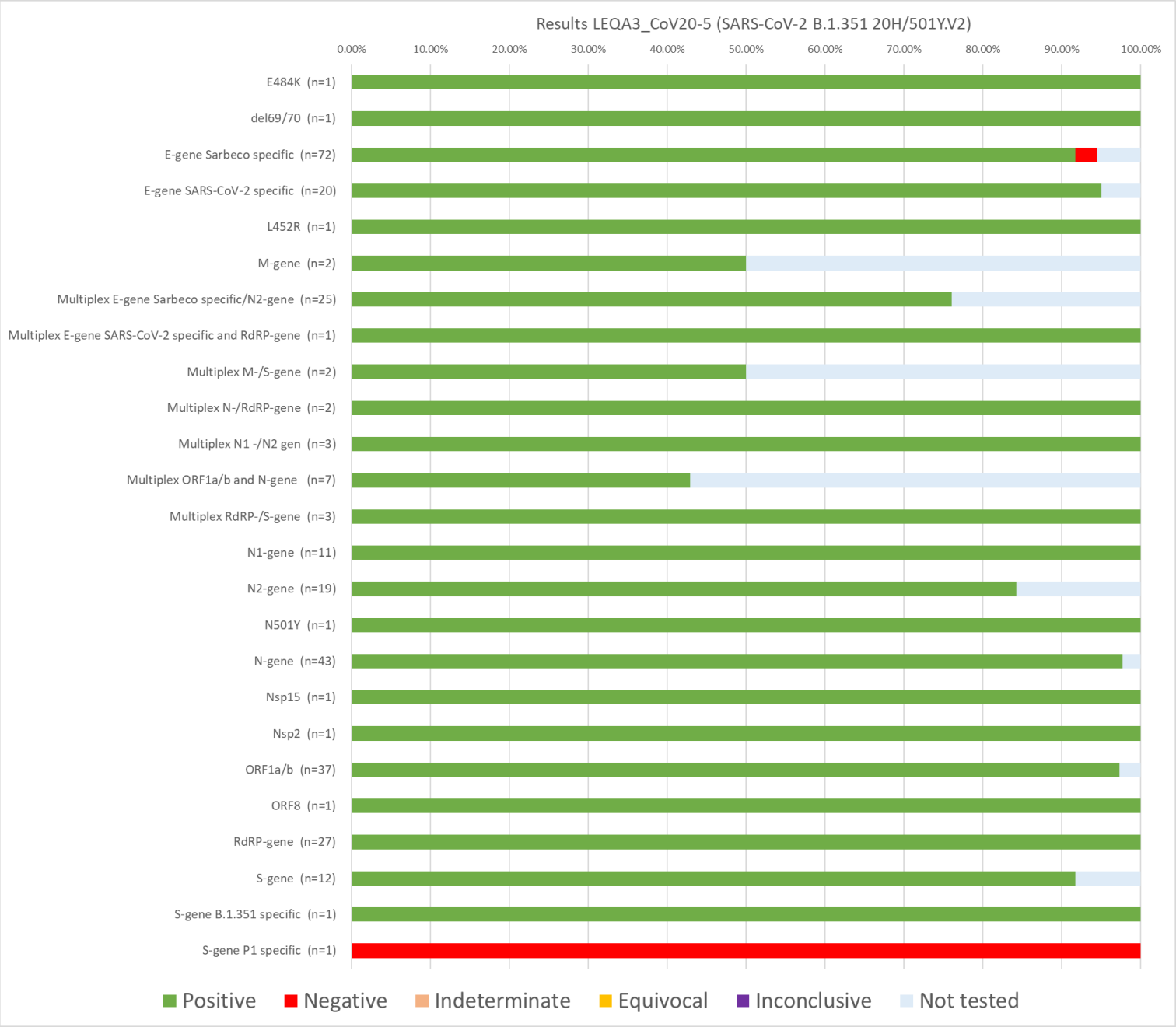
Supplemental Figure 14: The percentages of the various scores obtained for LEQA3_CoV20-2 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



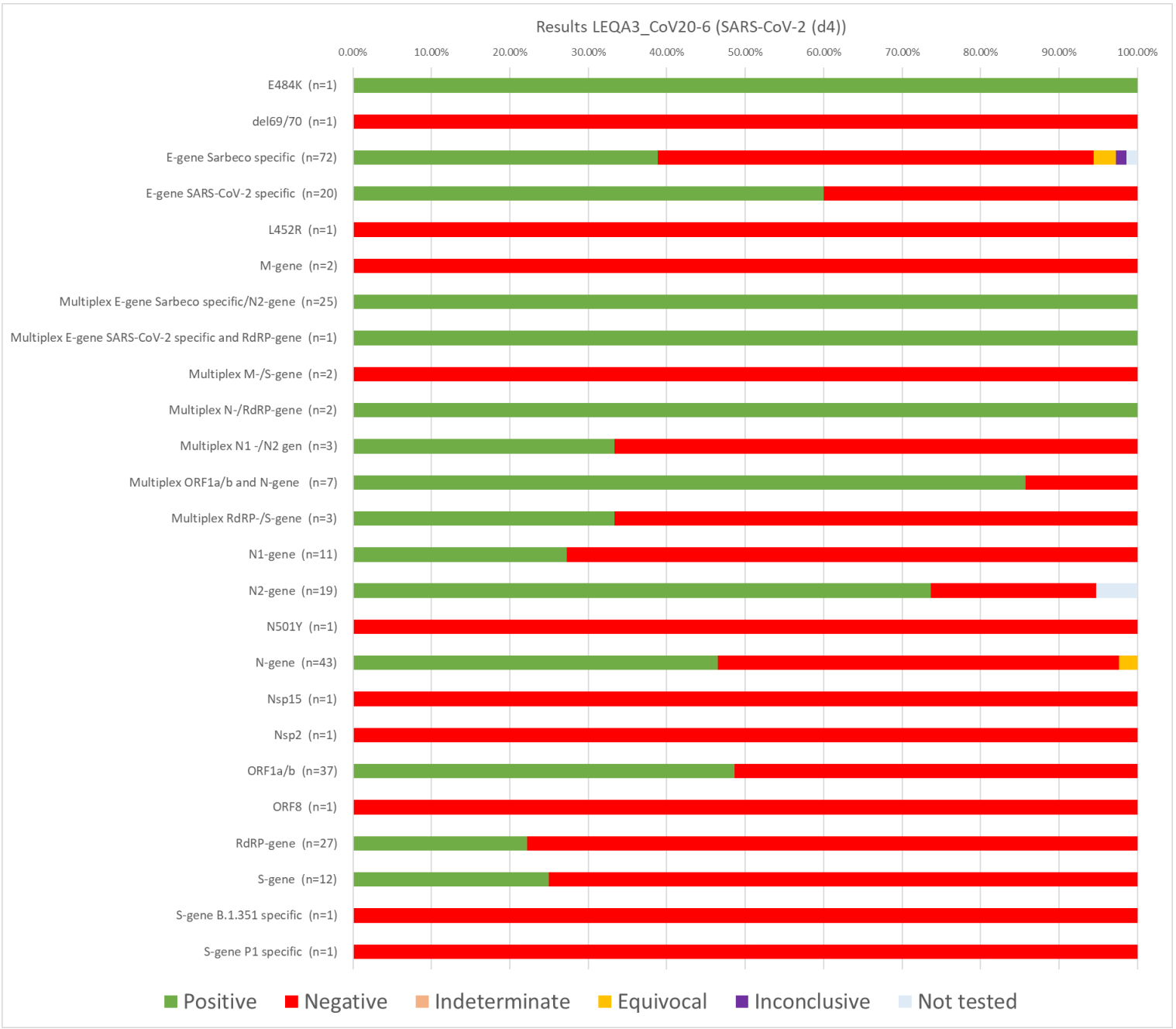
Supplemental Figure 15: The percentages of the various scores obtained for LEQA3_CoV20-3 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



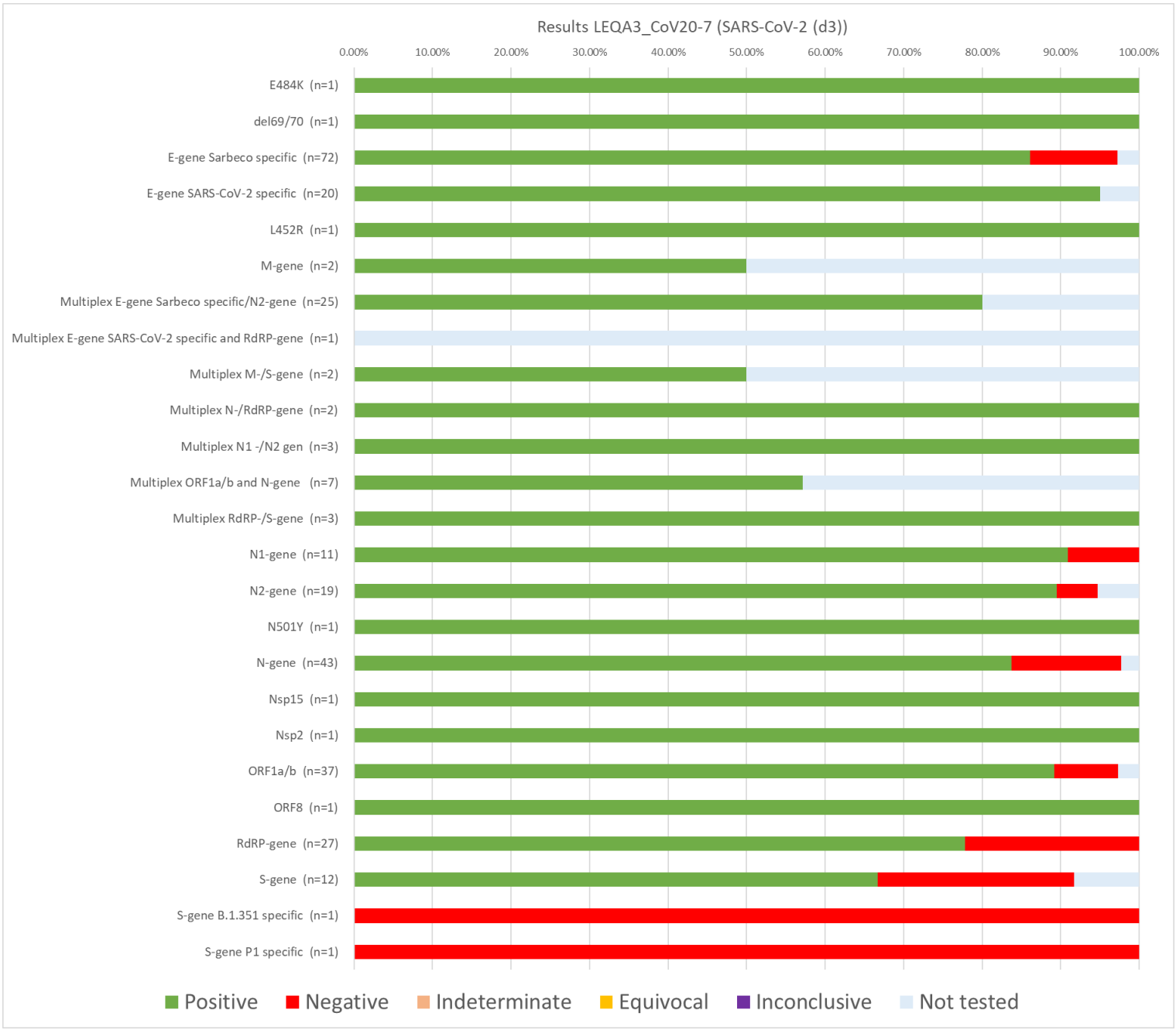
Supplemental Figure 16: The percentages of the various scores obtained for LEQA3_CoV20-4 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample. This sample was not deemed a core sample from the LEQA panel.



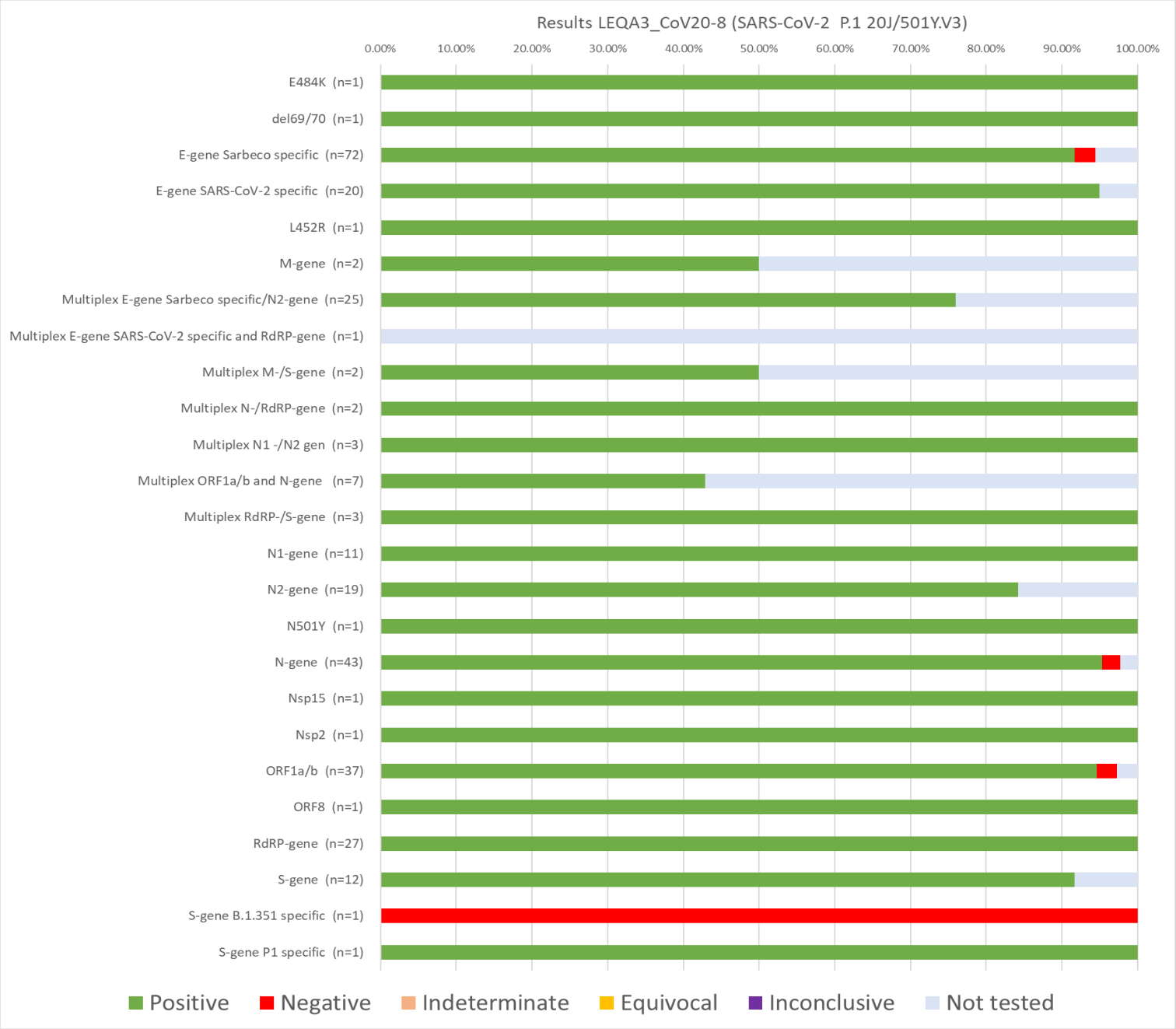
Supplemental Figure 17: The percentages of the various scores obtained for LEQA3_CoV20-5 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



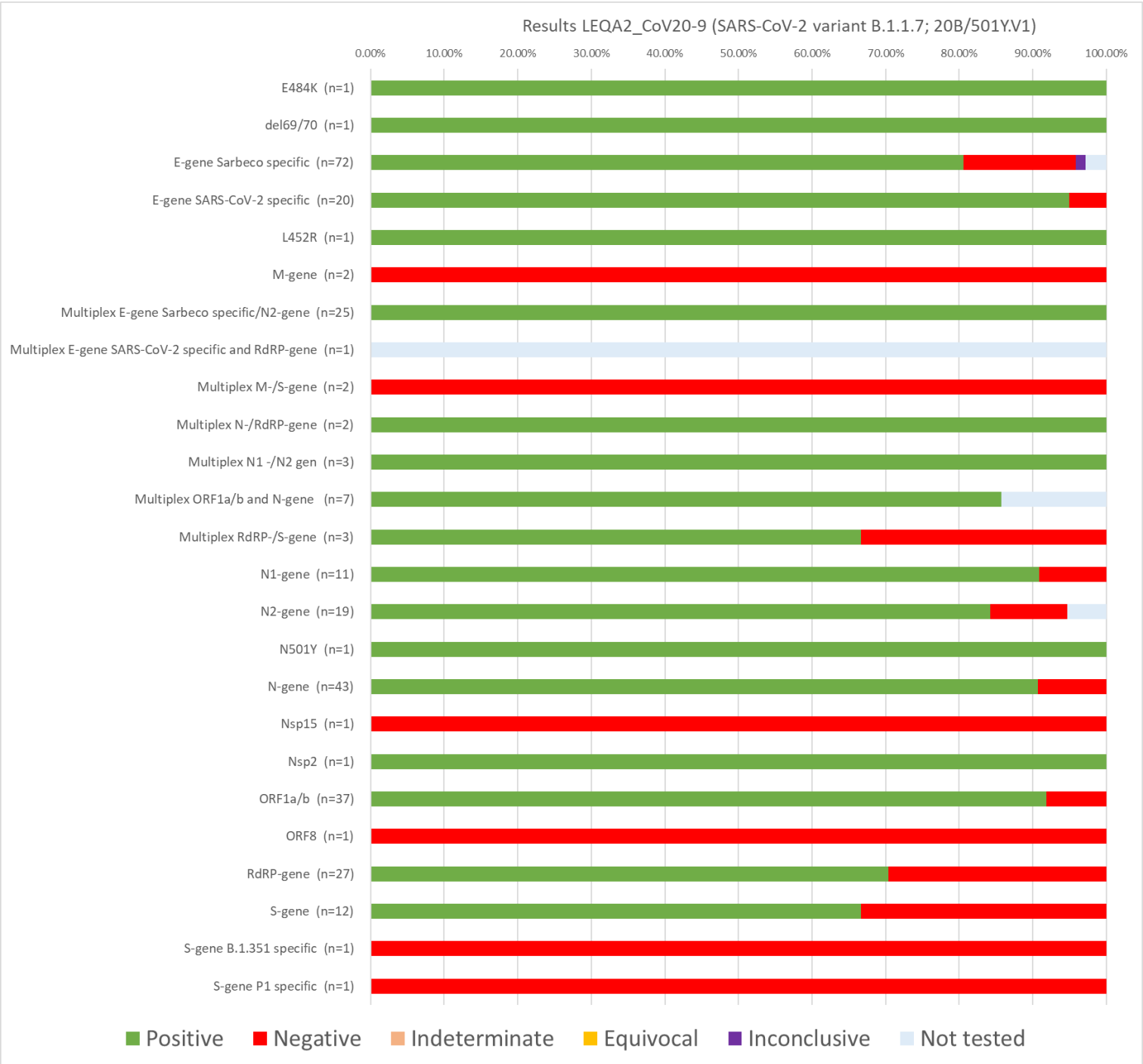
Supplemental Figure 18: The percentages of the various scores obtained for LEQA3_CoV20-6 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample



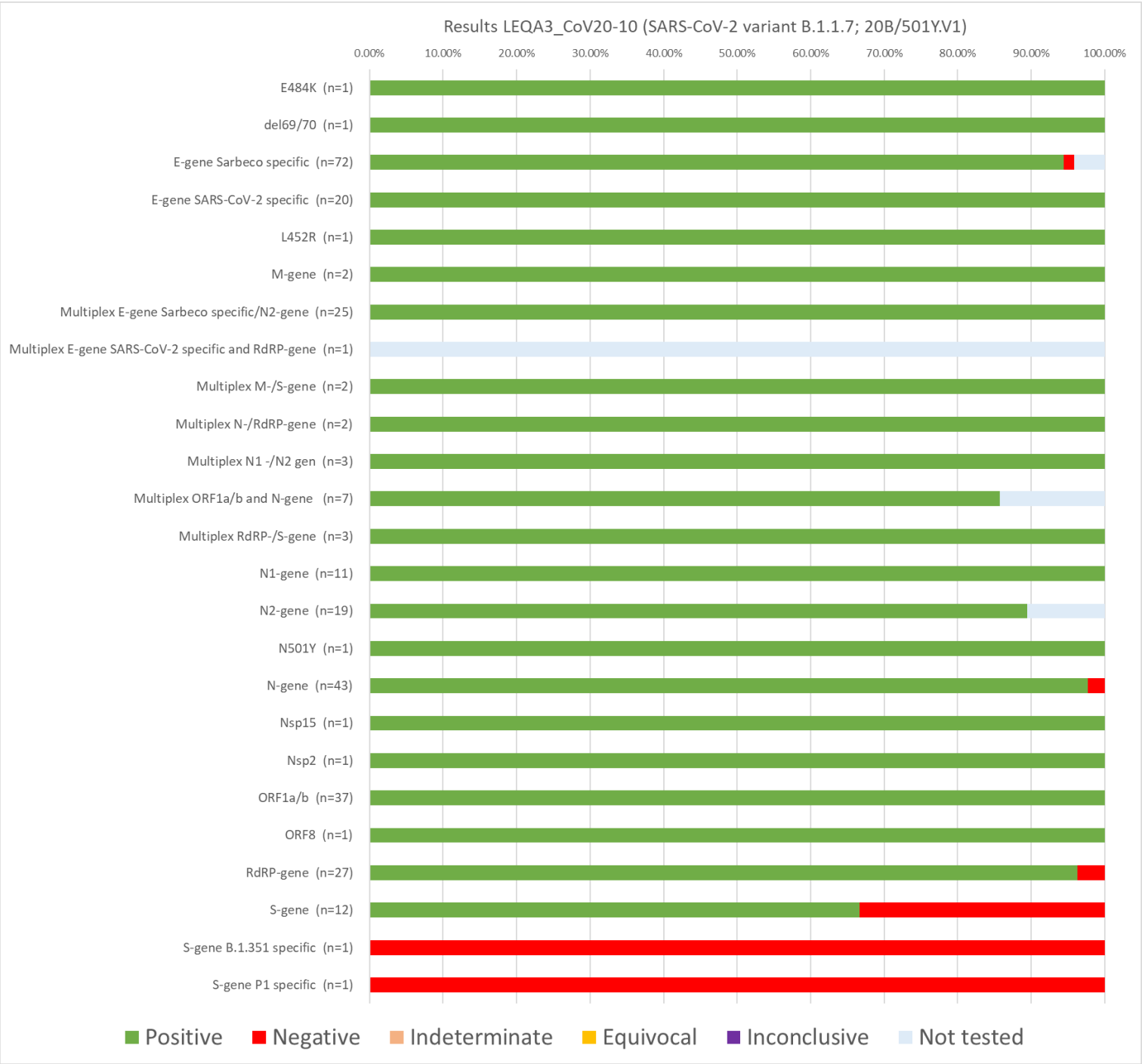
Supplemental Figure 19: The percentages of the various scores obtained for LEQA3_CoV20-7 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample.



Supplemental Figure 20: The percentages of the various scores obtained for LEQA3_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 21: The percentages of the various scores obtained for LEQA3_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.



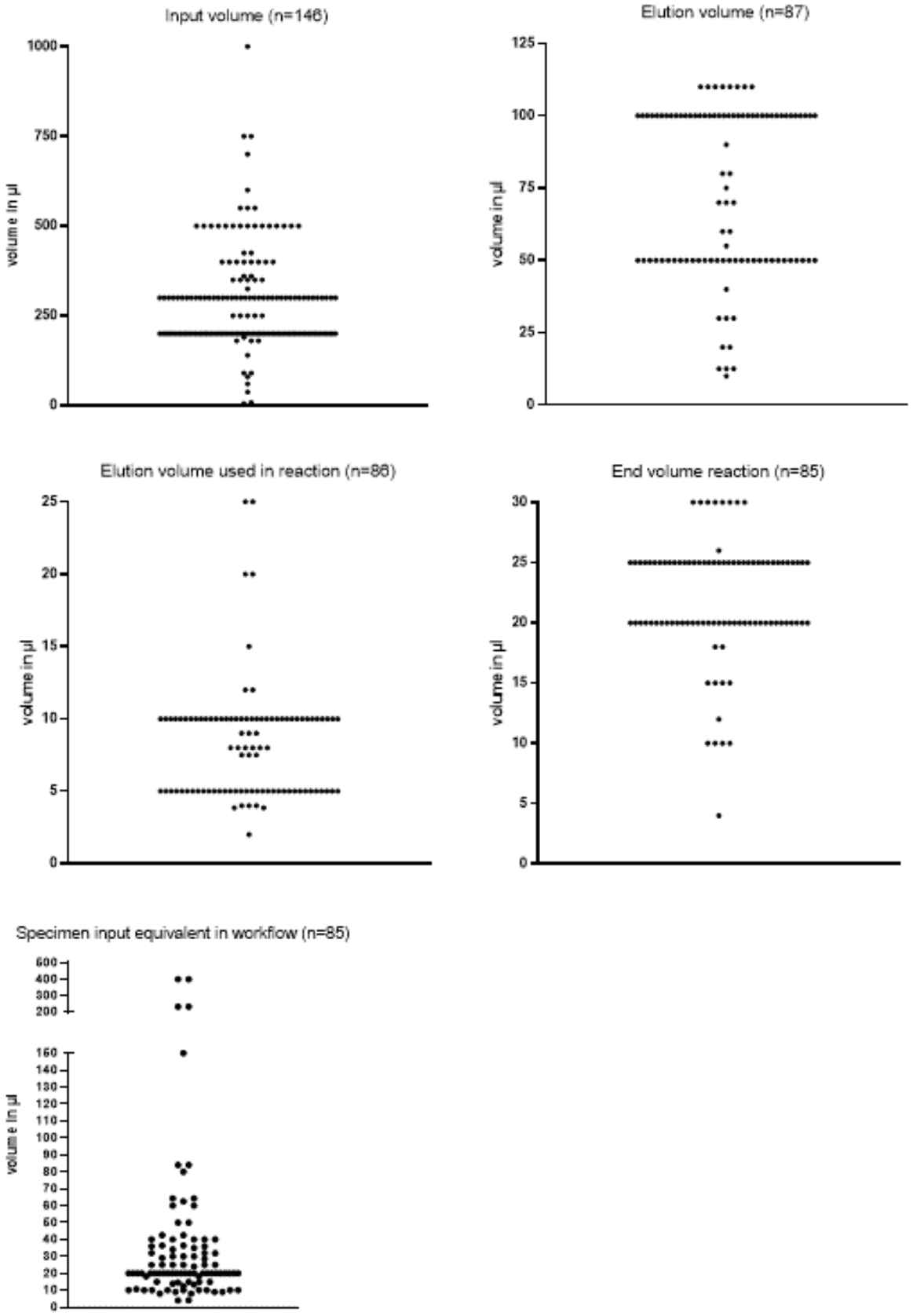
Supplemental Figure 22: The percentages of the various scores obtained for LEQA3_CoV20-10 using the various target genes implemented in the workflows reported. The percentages shown are combined from target genes reported as either target gene 1, 2, 3 or 4 in the various workflows. The results are depicted in relation to SARS-CoV-2 presence in the sample. This sample was not deemed a core sample from the LEQA panel.

6.4 Used volumes, equipment, kits and reagents

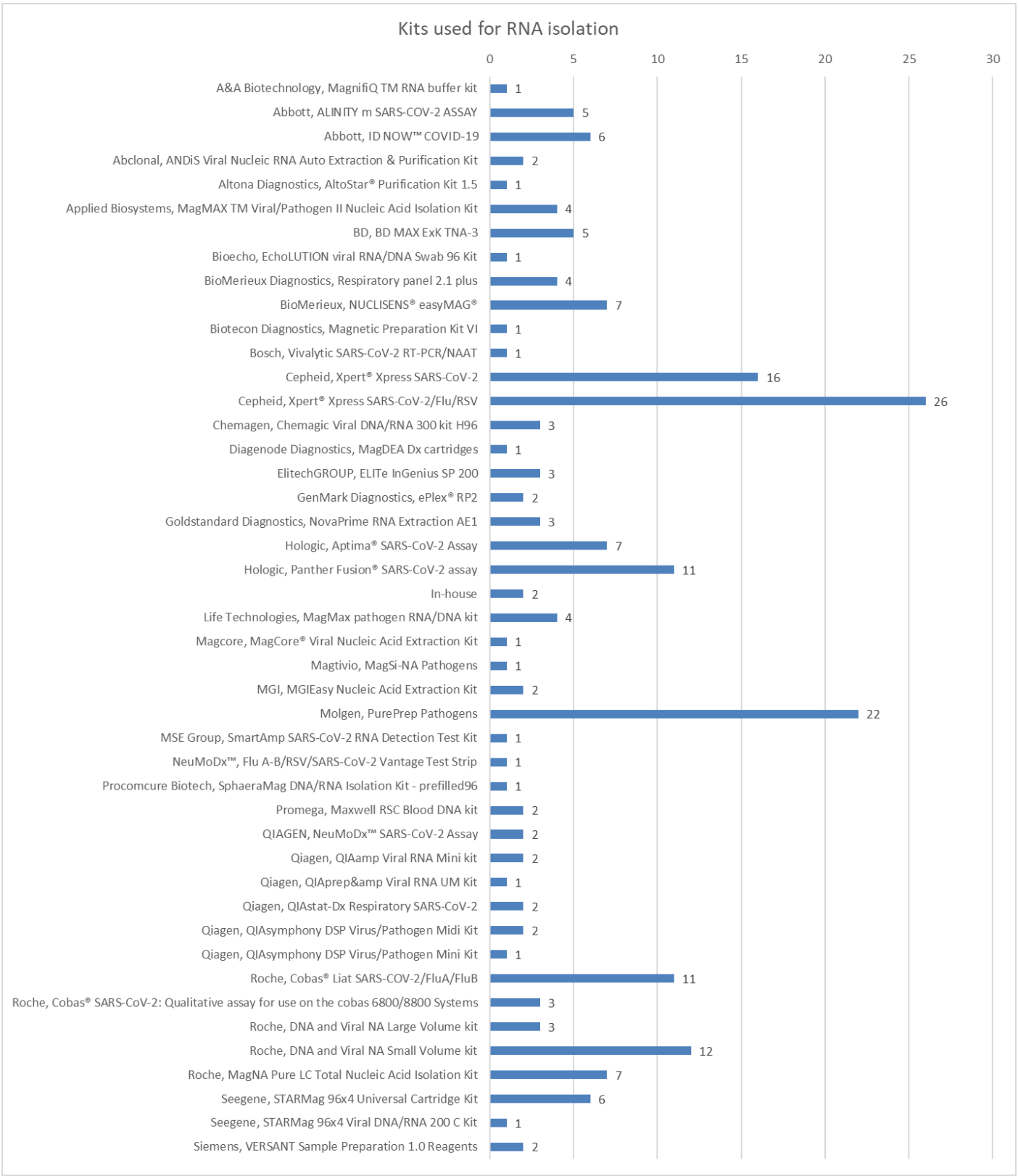
Because the sensitivity of a workflow is partly defined by the sample equivalent input volume in the RT-qPCR/other NAAT, a subset of questions revolved around the volumes used for testing of clinical samples for each specific workflow: volume specimen in nucleic acid extraction; elution volume; volume RNA/total NA in RT-qPCR reaction or other NAAT; end volume of RT-qPCR reaction or other NAAT. Supplemental Figure 23 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 (Supplemental Figure 23).

Unfortunately the specimen input equivalent volume in the PCR could only be calculated for 85/202 workflows and therefore this factor is not included in Supplemental Figure 29. For the 85 workflows for which it was calculated the specimen equivalent volume was median 20 µl (range 4 µl – 400 µl). For the 163 workflows for which specimen input volume in extraction was reported the median volume was 300 µl (range 5 µl – 1000 µl). The median reaction volume reported for 86 workflows was 25 µl (range 4 µl – 30 µl).

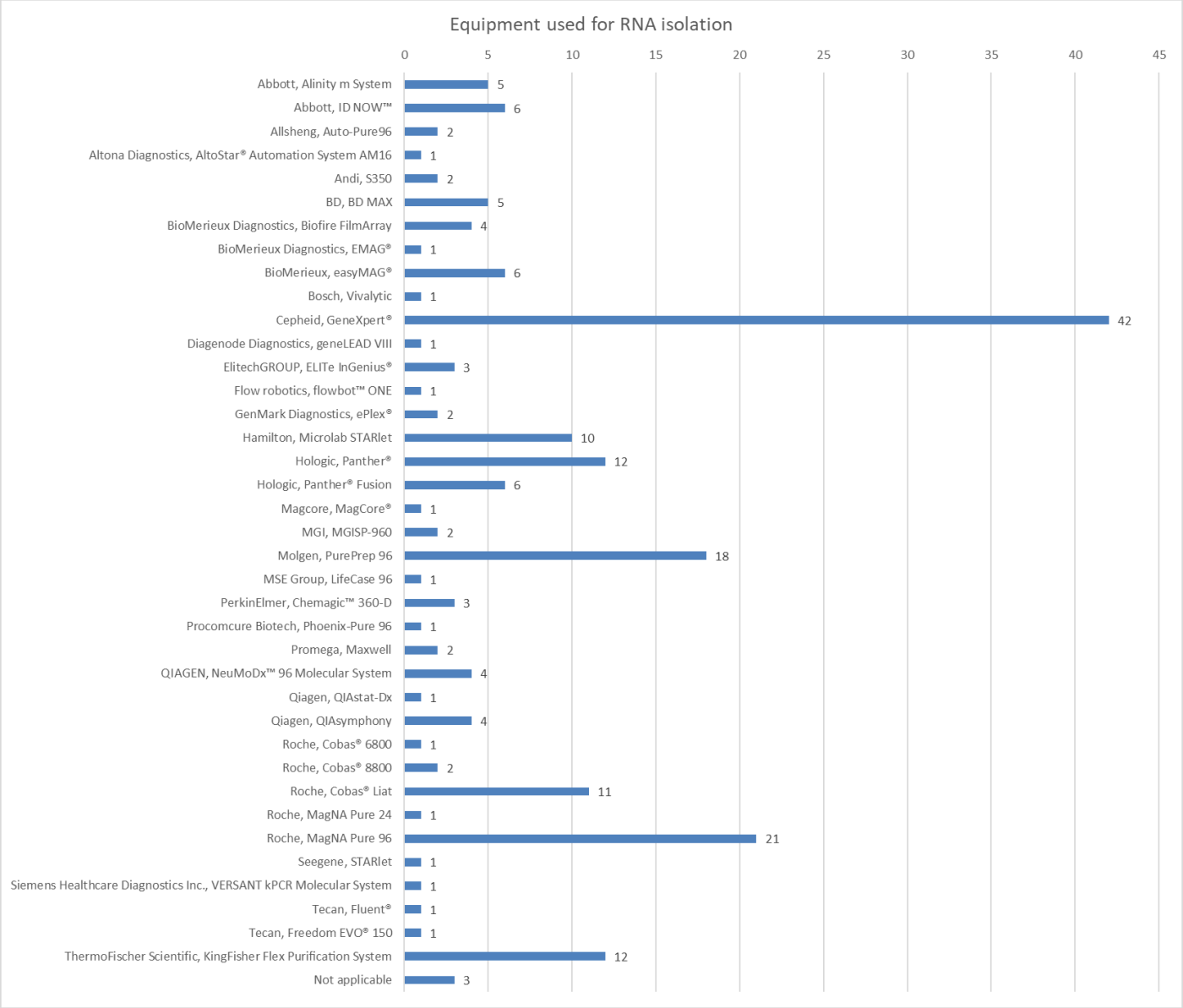
Other factors that may determine the performance of the workflows are the used kits, equipment and/or separate enzymes used for extraction and amplification implemented in SARS-CoV-2 diagnostics for the Dutch population. Therefore for each workflow these details were inventoried. Supplemental Figure 24 shows the kits used for RNA/total NA isolation, Supplemental Figure 25 shows the RNA isolation equipment, Supplemental Figure 26 shows the kits used for the RT-PCR or other NAAT reaction, Supplemental Figure 27 shows the separate enzymes used for the in-house RT-PCR or other NAAT reaction and Supplemental Figure 28 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.



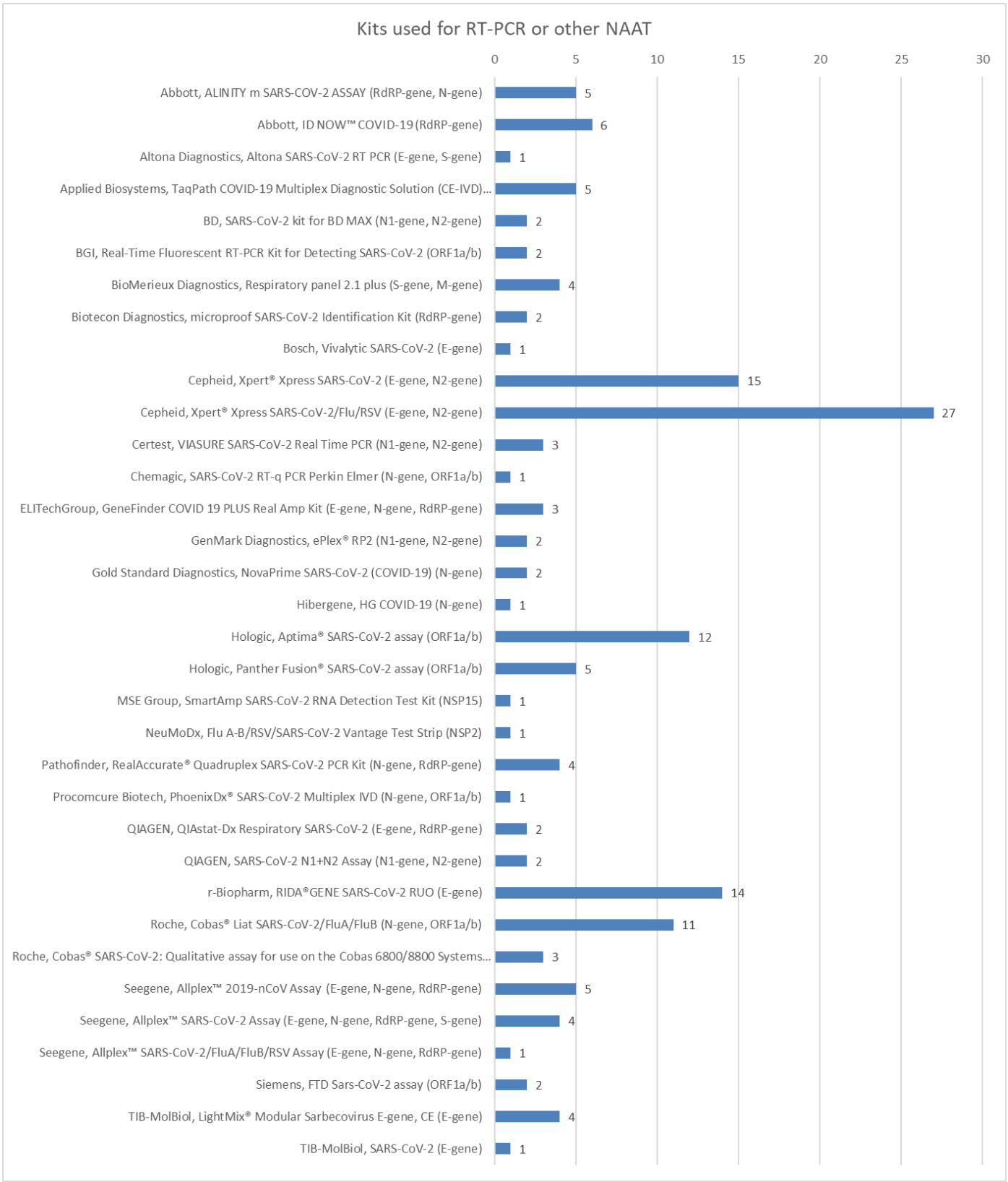
Supplemental Figure 23: Volumes used in the RT-PCRs or other NAATs described for the workflows reported. Most commonly used volumes: 200 µl input volume; 100 µl elution volume; 5 µl elution input volume in reaction; 20 µl end volume of reaction; 20 µl specimen input equivalent.



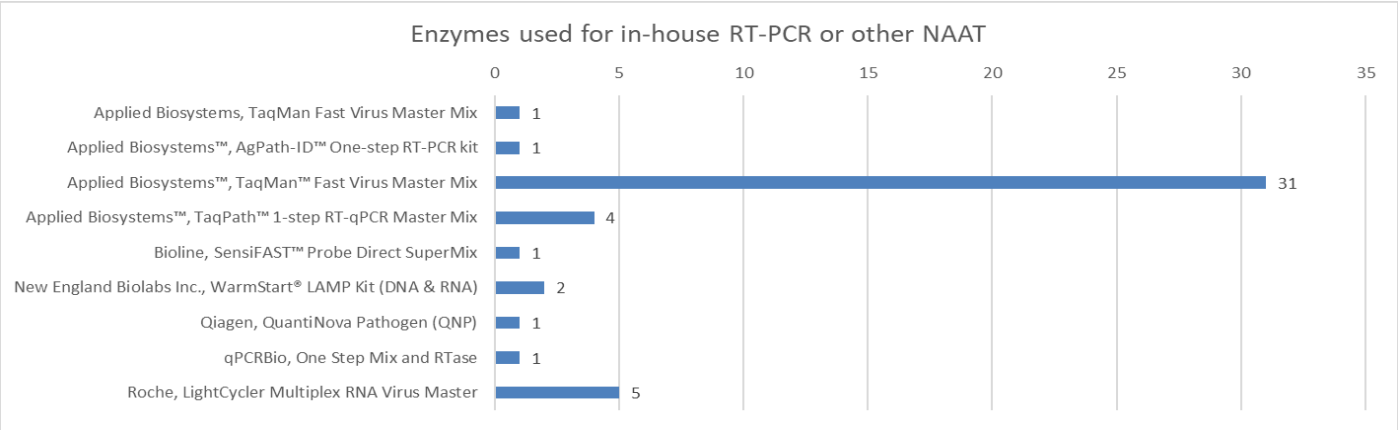
Supplemental Figure 24: The RNA isolation kits used by workflows testing for SARS-CoV-2 together with the number of workflows per kit (n=202)



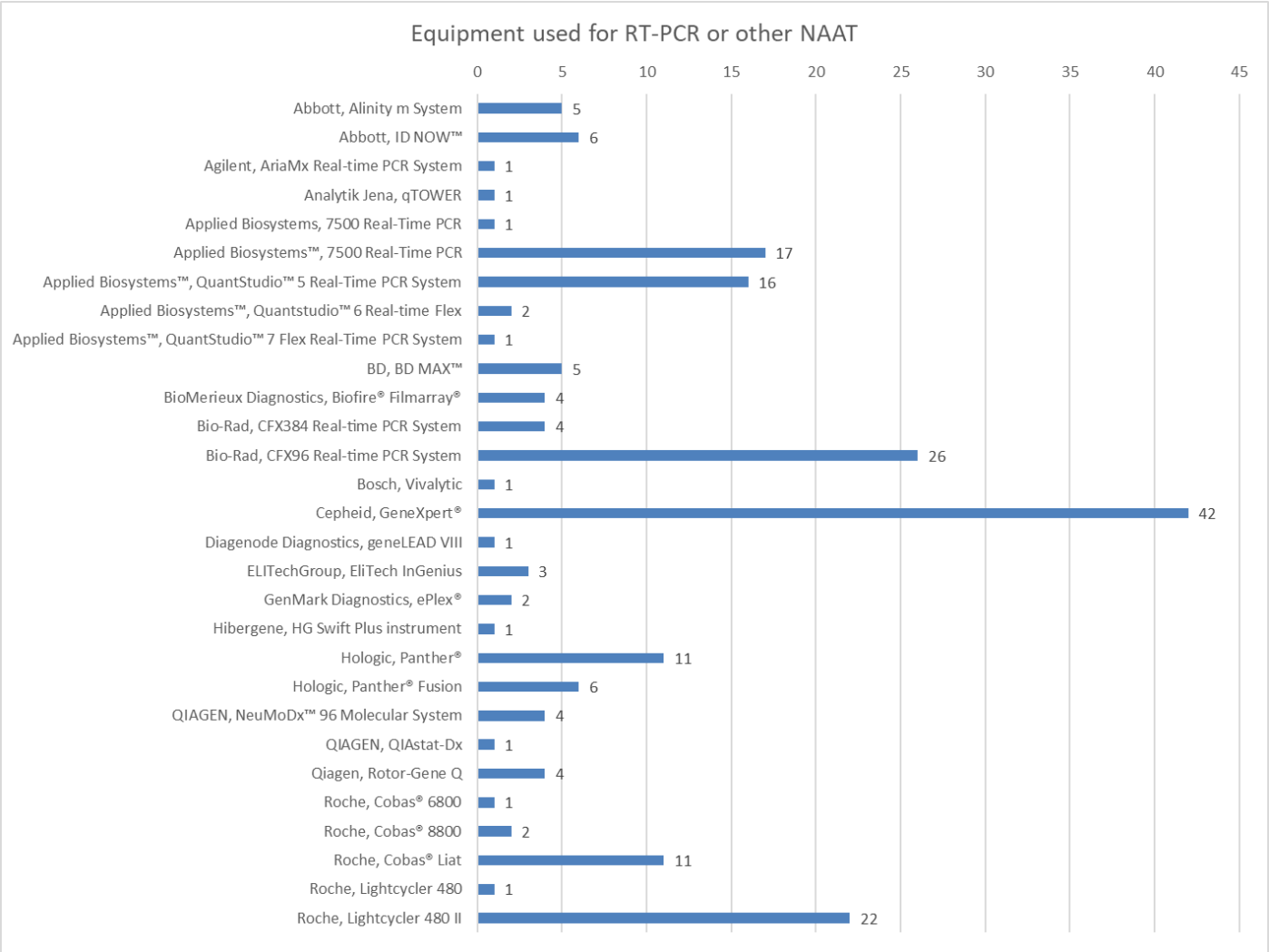
Supplemental Figure 25: The RNA isolation equipment used by workflows testing for SARS-CoV-2 together with the number of workflows per machine (n=202)



Supplemental Figure 26: The RT-qPCR or other NAAT kits used by workflows testing for SARS-CoV-2 together with the number of workflows per kit (n=155). Not all workflows use 'ready to use' kits for their RT-qPCR or other NAAT, so the total N is not equal to the number of workflows tested. For each kit the used target genes are listed. Workflows using separate enzymes and primers and probe are listed in Supplemental Figure 27.

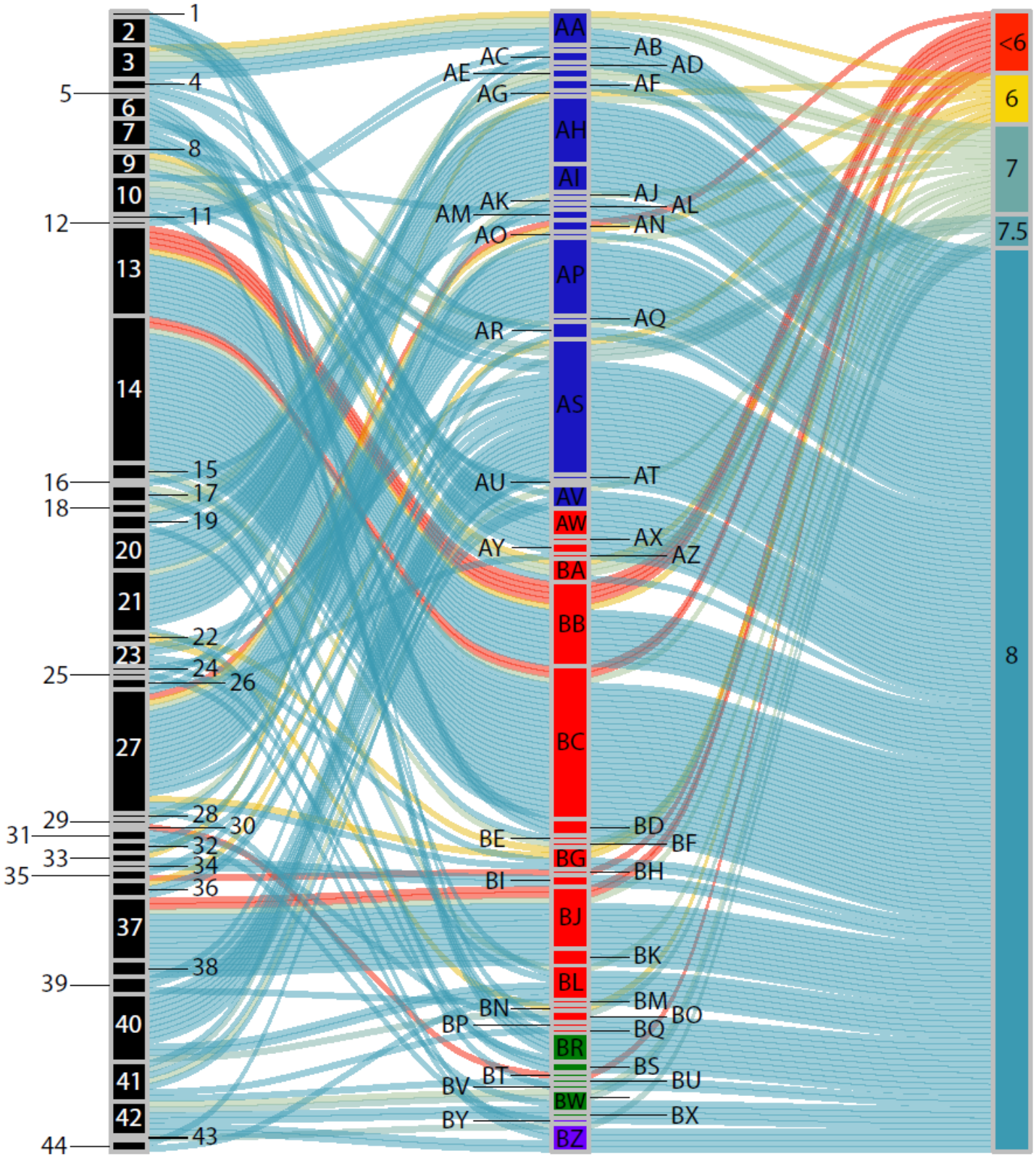


Supplemental Figure 28: The enzymes used for performing RT-PCR or other NAAT by workflows testing for SARS-CoV-2 together with the number of workflows per enzyme (n=47). Not all workflows use separate enzymes for their RT-PCR or other NAAT, so the total N is not equal to the number of workflows tested. In total 34/47 of the above mentioned workflows use 1 target gene to test for SARS-CoV-2 presence (E-gene Sarbeco specific (n=27); N-gene (n=3); ORF1a/b (n=2); RdRP-gene (n=1); S-gene (n=1)). 10/47 workflows use 2 target genes to test for SARS-CoV-2 presence (E-gene Sarbeco specific + N1-gene (n=6); E-gene Sarbeco specific + N-gene (n=2); E-gene Sarbeco specific + RdRP-gene (n=1); E-gene SARS-CoV-2 specific + RdRP-gene (n=1)) 1/47 workflows use 3 target genes to test for SARS-CoV-2 presence (E-gene Sarbeco specific + N-gene + S-gene (n=1)). 6/47 workflows use 4 target genes to test for SARS-CoV-2 presence (del69/70 + E484K + L452R + N501Y (n=1); E-gene Sarbeco specific + N-gene + RdRP-gene + S-gene (n=4); E-gene SARS-CoV-2 specific + RdRP-gene + S-gene B.1.351 specific + S-gene P1 specific (n=1)).Workflows using complete 'ready to use' kits for SARS-CoV-2 detection are listed in Supplemental Figure 26



Supplemental Figure 27: The RT-PCR or other NAAT equipment used by workflows testing for SARS-CoV-2 together with the number of workflows per machine (n=202)

6.5 Effect of RNA isolation and NAAT reaction on score obtained by workflow



Supplemental Figure 29: A flow diagram showing all workflows reported to have tested the LEQA3 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 Supplemental Figure 26. Color of trails per workflow are based on the grade obtained for LEQA3 All workflows receiving grades below 6 are grouped in <6.

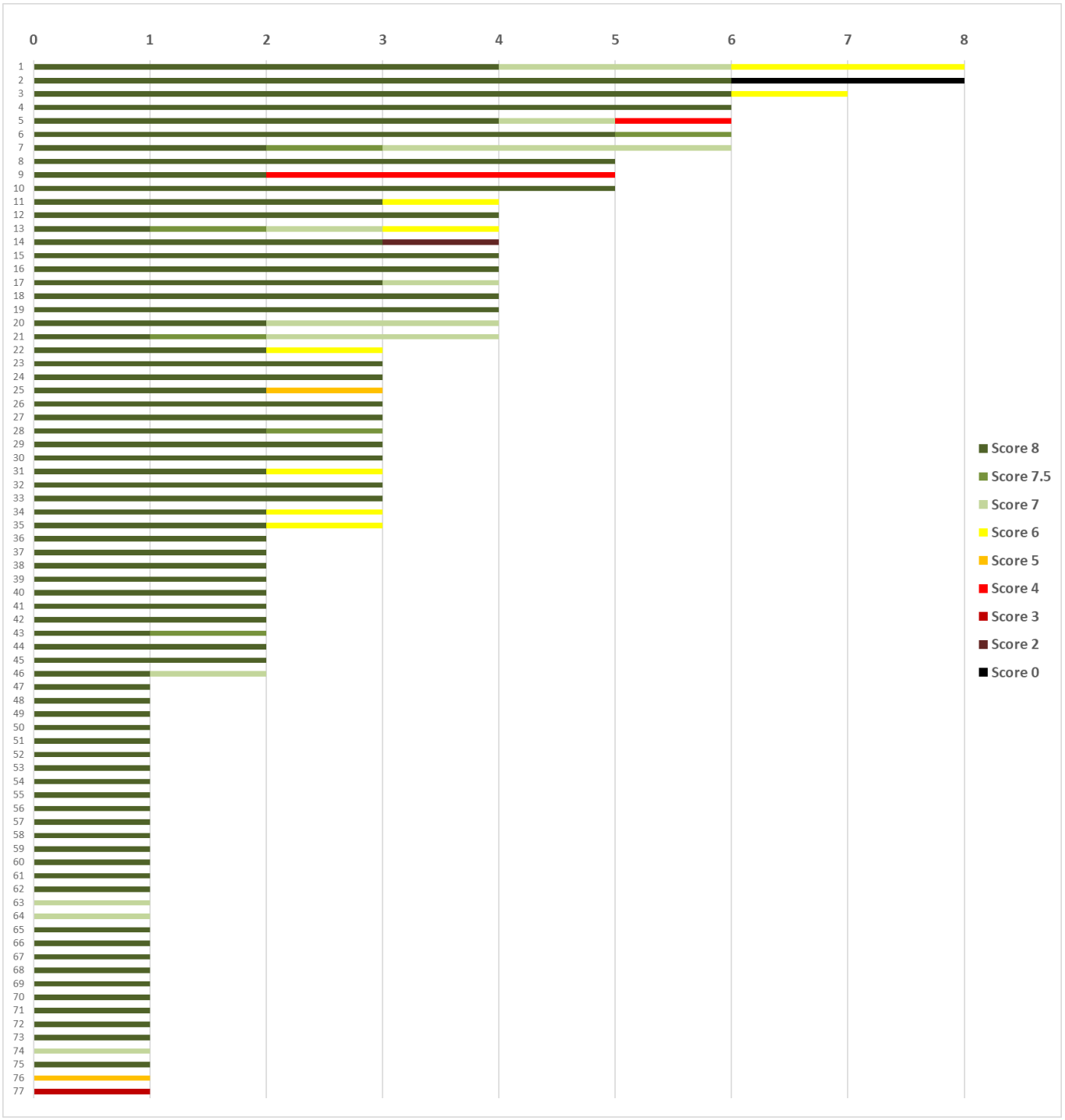
RNA isolation method	
1	A&A Biotechnology, MagnifiQ TM RNA buffer kit
2	Abbott, ALINITY m SARS-COV-2 ASSAY
3	Abbott, ID NOW™ COVID-19
4	Abclonal, ANDiS Viral Nucleic RNA Auto Extraction & Purification Kit
5	Altona Diagnostics, AltoStar® Purification Kit 1.5
6	Applied Biosystems, MagMAX TM Viral/Pathogen II Nucleic Acid Isolation Kit
7	BD, BD MAX™ ExK™ TNA-3
8	Bioecho, EchoLUTION viral RNA/DNA Swab 96 Kit
9	BioMerieux Diagnostics, Respiratory panel 2.1 plus
10	BioMerieux, NUCLISENS® easyMAG®
11	Biotecon Diagnostics, Magnetic Preparation Kit VI
12	Bosch, Vivalytic SARS-CoV-2 RT-PCR/NAAT
13	Cepheid, Xpert® Xpress SARS-CoV-2
14	Cepheid, Xpert® Xpress SARS-CoV-2/Flu/RSV
15	Chemagen, Chemagic Viral DNA/RNA 300 kit H96
16	Diagenode Diagnostics, MagDEA Dx cartridges
17	ElitechGROUP, ELITe InGenius® SP 200
18	GenMark Diagnostics, ePlex® RP2
19	Goldstandard Diagnostics, NovaPrime RNA Extraction AE1
20	Hologic, Aptima® SARS-CoV-2 Assay
21	Hologic, Panther Fusion® SARS-CoV-2 assay
22	In-house
23	Life Technologies, MagMax pathogen RNA/DNA kit
24	Magcore, MagCore® Viral Nucleic Acid Extraction Kit
25	Magtivio, MagSi-NA Pathogens
26	MGI, MGIEasy Nucleic Acid Extraction Kit
27	Molgen, PurePrep Pathogens
28	MSE Group, SmartAmp SARS-CoV-2 RNA Detection Test Kit
29	NeuMoDx™, Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip
30	Procomcure Biotech, SphaeraMag DNA/RNA Isolation Kit - prefilled96
31	Promega, Maxwell RSC Blood DNA kit
32	QIAGEN, NeuMoDx™ SARS-CoV-2 Assay
33	Qiagen, QIAamp Viral RNA Mini kit
34	Qiagen, QIAprep& Viral RNA UM Kit
35	Qiagen, QIAstat-Dx Respiratory SARS-CoV-2
36	Qiagen, QIAsymphony DSP Virus/Pathogen Midi Kit
37	Roche, Cobas® Liat SARS-COV-2/FluA/FluB
38	Roche, Cobas® SARS-CoV-2: Qualitative assay for use on the cobas 6800/8800 Systems
39	Roche, DNA and Viral NA Large Volume kit
40	Roche, DNA and Viral NA Small Volume kit
41	Roche, MagNA Pure LC Total Nucleic Acid Isolation Kit
42	Seegene, STARMag 96x4 Universal Cartridge Kit
43	Seegene, STARMag 96x4 Viral DNA/RNA 200 C Kit
44	Siemens, VERSANT Sample Preparation 1.0 Reagents

PCR/NAAT method	
AA	Abbott, ID NOW™ COVID-19
AB	BGI, Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2
AC	Biotecon Diagnostics, microproof SARS-CoV-2 Identification Kit
AD	Bosch, Vivalytic SARS-CoV-2 RT-PCR/NAAT
AE	GenMark Diagnostics, ePlex® RP2
AF	Gold Standard Diagnostics, NovaPrime SARS-CoV-2 (COVID-19)
AG	Hibergene, HG COVID-19
AH	Hologic, Aptima® SARS-CoV-2 Assay
AH	Hologic, Aptima® SARS-CoV-2 Assay
AI	Hologic, Panther Fusion® SARS-CoV-2 assay
AJ	MSE Group, SmartAmp SARS-CoV-2 RNA Detection Test Kit
AK	NeuMoDx, Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip
AL	N-gene, Jernigan, 2020
AM	N-gene, Lu *et al.*, 2020
AN	ORF1a/b (LAMP assay)
AO	Qiagen, QIAstat-Dx Respiratory SARS-CoV-2
AP	r-Biopharm, RIDAGENE SARS-CoV-2 RUO
AQ	RdRp-gene (unknown)
AR	Sarbeco E-gene (unknown)
AS	Sarbeco E-gene, Corman *et al.*, 2020
AT	S-gene (unknown)
AU	Siemens, FTD Sars-CoV-2 assay
AV	TIB-MolBiol, LightMix® Modular Sarbecovirus E-gene, CE
AW	Abbott, ALINITY m SARS-COV-2 ASSAY
AX	Altona, Altostar SARS-CoV-2 RT PCR
AY	BD, SARS-CoV-2 kit for BD MAX
AZ	BGI, Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2
BA	BioMerieux Diagnostics, Respiratory panel 2.1 plus
BB	Cepheid, Xpert® Xpress SARS-CoV-2
BC	Cepheid, Xpert® Xpress SARS-CoV-2/Flu/RSV
BD	Certest, VIASURE SARS-CoV-2 Real Time PCR
BE	Chemagic, SARS-CoV-2 RT-q PCR Perkin Elmer
BF	ELITechGroup, GeneFinder ® COVID 19 PLUS Real Amp Kit
BG	PathoFinder, RealAccurate® Quadruplex SARS-CoV-2 PCR Kit
BH	Qiagen, QIAstat-Dx Respiratory SARS-CoV-2
BI	Qiagen, SARS-CoV-2 N1+N2 Assay Kit
BJ	Roche, Cobas® Liat SARS-CoV-2/FluA/FluB
BK	Roche, Cobas® SARS-CoV-2: Qualitative assay for use on the cobas 6800/8800 Systems
BL	Sarbeco E-gene, Corman et al. 2020; N1-gene, Lu et al. 2020
BM	Sarbeco E-gene, Corman et al. 2020; N-gene, Corman et al. 2020
BN	Sarbeco E-gene, Corman et al. 2020; N-gene, Lu et al. 2020
BO	Sarbeco E-gene, Corman et al. 2020; RdRP-gene, Corman et al. 2020 (adapted)
BP	Siemens, FTD Sars-CoV-2 assay
BQ	TIB-MolBiol, SARS-CoV-2
BR	Applied Biosystems, TaqPath™ COVID-19 Multiplex Diagnostic Solution (CE-IVD)
BS	ELITechGroup, GeneFinder™ COVID 19 PLUS Real Amp Kit
BT	Procomcure Biotech, PhoenixDx® SARS-CoV-2 Multiplex IVD
BU	Sarbeco E-gene, Corman et al. 2020; N1-gene, Lu et al. 2020; N2-gene, Lu et al. 2020
BV	Sarbeco E-gene, Corman et al. 2020; N1-gene, Lu et al. 2020; S-gene (unknown)
BW	Seegene, Allplex™ 2019-nCoV Assay
BX	Seegene, Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay
BY	del69/70 (unknown); E484K (unknown); N501Y (unknown); L452R (unknown)
BZ	Seegene, Allplex™ SARS-CoV-2 Assay

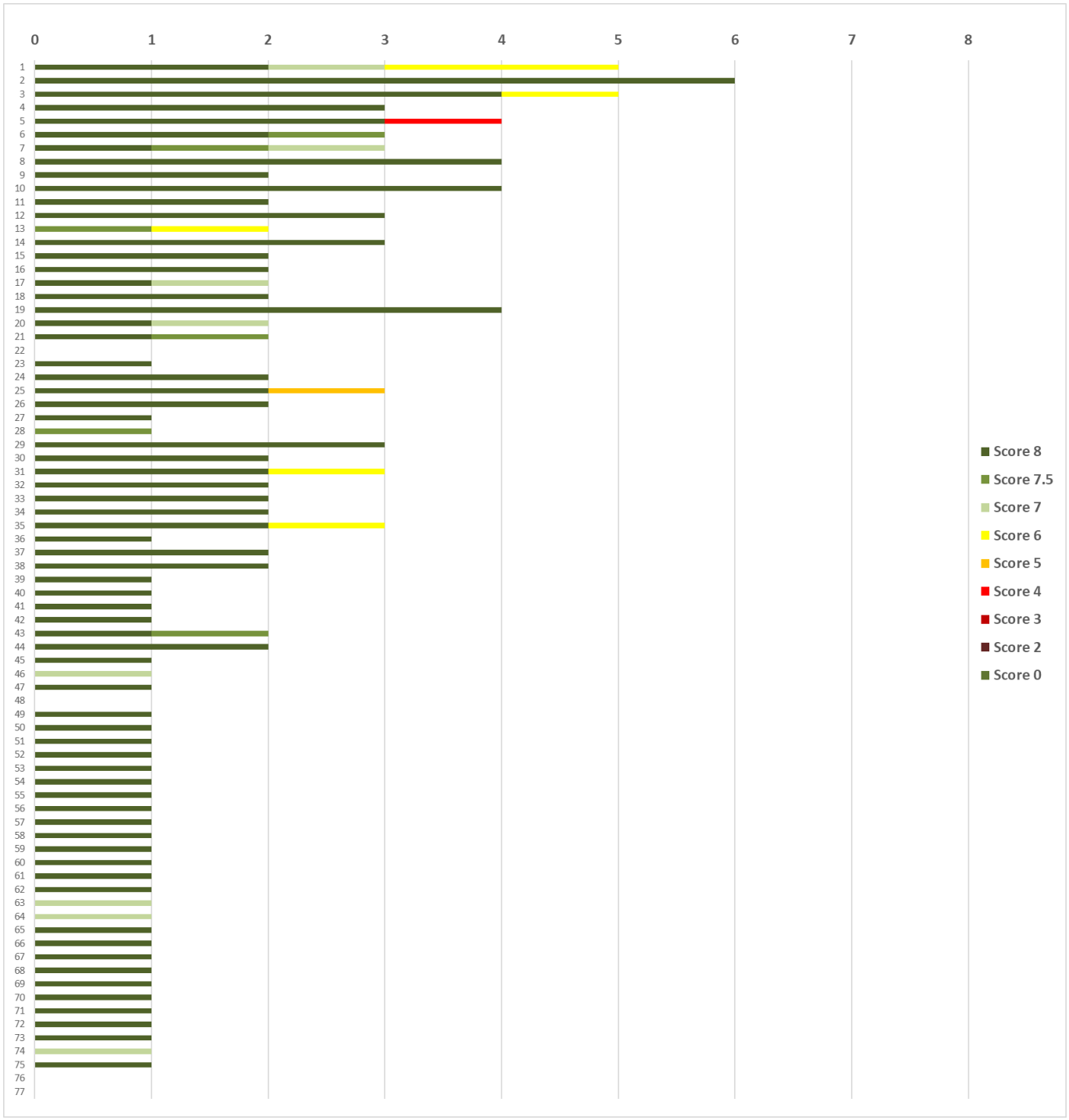
Supplemental figure 29 continued: Legend

6.6 Scores obtained per laboratory

Here all obtained scores per workflow per laboratory are summarized. In total two laboratory reported data for 8 workflows, one laboratory reported data for 7 workflows, four laboratory reported data for 6 workflows, three laboratories reported data for 5 workflows, eleven laboratories reported data for 4 workflows, fourteen laboratories reported data for 3 workflows, eleven laboratories reported data for 2 workflows and thirty-one laboratories reported data for 1 workflow. There are 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 Supplemental Figure 30. Of the 77 laboratories, 72 laboratories reported at least one workflow with fully correct results. When excluding all mPOCT assays from the analysis, one laboratory reported data for 6 workflows, two laboratory reported data for 5 workflows, four laboratories reported data for 4 workflows, nine laboratories reported data for 3 workflows, nineteen laboratories reported data for 2 workflows, thirty-eight laboratories reported data for 1 workflow and four laboratories did not report any other workflows than mPOCT assays. Without mPOCT assays taken into consideration, no laboratories have scores of < 7 for all their reported workflows. This is shown in Supplemental Figure 31. It should be noted that all but one of the 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.



Supplemental Figure 30: Grades obtained per workflow per laboratory (anonymized). For each of the laboratories the number of reported workflows is shown on the X-axis together with their accompanying grades. In total 77 laboratories sent in data of their workflows testing LEQA3. There are 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 Supplemental Figure 31, where all workflows excluding mPOCT assays are listed.



Supplemental Figure 31: Grades obtained per workflow per laboratory excluding all mPOCT assays (anonymized). For each of the laboratories the number of reported workflows is shown on the X-axis together with their accompanying grades. In total 78 laboratories sent in data of their workflows. There are no 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 Supplemental Figure 30, where all workflows including mPOCT assays are listed.

6.7 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 name
Admiraal de Ruyter Ziekenhuis
Alrijne Ziekenhuis
Amsterdam UMC
ArminLabs
Atalmedial
Catharina Ziekenhuis Eindhoven
CBSL Tergooi
Certe
Comicro B.V.
CWZ medische microbiologie
Deventer ziekenhuis
Diagnostiek voor U
Diakonessenhuis Utrecht
Dr. Stein & Kollegen
Erasmus MC, Viroscience
Eurofins Genomics Europe Applied Genomics GmbH
Eurofins NMDL-HVL
Eurofins NMDL-LCPL Klinisch Laboratorium
Fenelab - MasterLab - Nutreco Nederland BV
Fenelab - Nofalab B.V.
Fenelab - Normec Biobeheer
Fenelab - NutriControl B.V.
Fenelab - Nutrilab B.V.
Fenelab - SGS Nederland B.V.
Fenelab - Siliker B.V. - Mérieux NutriSciences Nederland
Fenelab - Triskelion
Franciscus Gasthuis & Vlietland
Gelre Ziekenhuizen Apeldoorn
GGD Amsterdam
Groene Hart Ziekenhuis
Haaglanden Medisch Centrum
How are you diagnostics (HAY)
IJssellandziekenhuis
Ikazia ziekenhuis
inBiome
Isala
Izore
Jeroen Bosch Ziekenhuis
LabMicTA
Labor Dr Wisplinghoff

Laboratory name
Laurentius ziekenhuis
LUMC
Maasstadziekenhuis
Maastricht UMC+
Meander Medisch Centrum
Microbe & Lab
Microvida
Microvida ETZ
Mozand B.V.
Noordwest Ziekenhuisgroep Alkmaar
Novogenia GmbH
Nutrilab B.V.
OLVG Lab B.V.
Pro Health Medical
Radboud UMC
Rijnstate
RLM
Royal GD
Saltro, locatie Hudsondreef
Saltro, locatie Mississippidreef
Sanquin, NSS
St. Antoniusziekenhuis
Star-SHL
Stichting PAMM
Streeklab Haarlem
Synlab Heppignies
Synlab Jena Oncoscreen
Synlab Laboratoire Collard
Synlab MVZ Trier
TLR International Laboratories
UMCG
UMCU
VieCuri MC
Wageningen Bioveterinary Research
Ziekenhuis Gelderse Vallei
Ziekenhuis Rivierenland Tiel
Ziekenhuis St Jansdal
Zuyderland Medisch Centrum