

Summary of updates to concentrations reported with runcontrols and EQA panels SARS-CoV-2

2021-05-26 correction RdRP concentration LEQA1_CoV20-5 and LEQA1_CoV20-7

2021-07-05 correction added sensitivity series #1

2021-30-08 correction added verification panel #2

Introduction

Updates are made to the number of copies of SARS-CoV-2 RNA/ml runcontrol and panel specimens due to errors in calculation formula's. The minimal amount of SARS-CoV-2 RNA that a test can detect is therefore sometimes overestimated or sometimes underestimated when the previously reported absolute quantities have been used. These updates however do not affect the quality of the NAAT 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; see [LEQA1](#) and [LEQA2](#) reports.

Runcontrol 1 and runcontrol 2 are two independent preparations of the same SARS-CoV-2 isolate (hCoV-19/Netherlands/NoordBrabant_10003/2020). Therefore, these preparations have been dPCR-ed separately and this dPCR data has been used for panels prepared from either runcontrol 1 or runcontrol 2.

In the below description of concentrations only the new correct concentrations are shown for clarity. Where possible the concentration was recalculated taking into account all concentration and dilution factors from original specimen to raw dPCR output. If not possible, the dPCR was performed anew to determine the copy number. What has been done is indicated with each update below.

Check the documents you have received before whether concentrations are correct and revise where appropriate. As said, to guide you with this a brief description of the modifications precedes the detailed data.

Runcontrol 1

Modification: digital copies positive strand RdRp-gene RNA/ml determined anew and digital copies positive strand E-gene RNA/ml added.

Runcontrol SARS-CoV-2 (extraction required, positive control material): 1 tube containing 1ml SARS-CoV-2 sample from a 1:100 dilution derived from stock (hCoV-19/Netherlands/Noord_Braband_0117R/2020 renamed hCoV-19/Netherlands/NoordBrabant_10003/2020) in MEM with Hanks' salts; heat inactivated at 60°C for 2 hours. The runcontrol contains SARS-CoV-2 with 5.62×10^5 TCID₅₀/ml; **1.40×10^8** digital copies of RdRp-gene positive strand RNA/ml; **1.08×10^8** digital copies of E-gene positive strand RNA/ml; Ct 16.2 in E-gen PCR after extraction of 200 µl using a MagNApure 96 and elution in 50 µl, after which 5 µl of the elution is used in the PCR reaction.

Runcontrol 2

Modification: digital copies positive strand RdRp-gene and E-gene RNA/ml recalculated.

Runcontrol SARS-CoV-2 (extraction required, positive control material): 1 tube containing 1ml SARS-CoV-2 sample from a 1:100 dilution derived from stock (hCoV-19/Netherlands/NoordBrabant_10003/2020) in MEM with Hanks' salts; heat inactivated at 60°C for 2 hours. The runcontrol contains SARS-CoV-2 with 5.62×10^5 TCID₅₀/ml; 1.73×10^8 digital copies of RdRp-gene positive strand RNA/ml; 1.28×10^8 digital copies of E-gene positive strand RNA/ml; Ct 16.86 in RdRp-gene qRT-PCR and Ct 16.97 in E-gene qRT-PCR after extraction of 200 µl using a MagNApure 96 and elution in 50 µl, after which 5 µl of the elution is used in the PCR reaction.

UK variant runcontrol

Modification: none

Runcontrol SARS-CoV-2 B.1.1.7 VOC-20201201(UK) variant (extraction required, positive control material): 1 tube containing 1ml SARS-CoV-2 B.1.1.7 VOC-20201201(UK) variant from a 1:100 dilution derived from stock virus hCoV-19/Netherlands/NH-RIVM-20432/2020 cultured on VERO-E6 cells; in MEM with Hanks' salts and heat inactivated at 60°C for 2 hours. The runcontrol contains SARS-CoV-2 with a 2.37×10^5 TCID₅₀/ml; 2.32×10^7 digital copies of RdRp-gene positive strand RNA/ml; 3.39×10^7 digital copies of E-gene positive strand RNA/ml; Ct 19.90 in RdRp-gene qRT-PCR and Ct 18.74 in E-gene qRT-PCR after extraction of 200 µl using a MagNApure 96 and elution in 50 µl, after which 5 µl of the elution is used in the qRT-PCR reaction.

Variant reference strain panel

Modification: digital copies positive strand RdRp-gene and E-gene RNA/ml recalculated and TCID₅₀/ml corrected to 1:100 diluted stock virus.

Variant panel: 8 tubes containing 1ml of 8 different SARS-CoV-2 strains (Table 1). Each strain is diluted 1:100 derived from the stock virus, cultured on VERO-E6 cells; in MEM with Hanks' salts and heat inactivated at 60°C for 2 hours. Each sample is tested in E-gene and RdRp-gene qRT-PCR after extraction of 200 µl using a MagNApure 96 and elution in 50 µl, after which 5 µl of the elution is used in the qRT-PCR reaction.

Table 1. Information about the 8 different strains used for the SARS-CoV-2 variant panel.

SARS-CoV-2 strain name	qRT-PCR Ct-value		dPCR copies/ml		TCID ₅₀ /ml
	E-gene	RdRp-gene	E-gene	RdRp-gene	
hCoV-19/Netherlands/NH-RIVM-10159/2021 B.1.351; 20H/501Y.V2; South Africa	17.29	17.56	1.15E+08	1.14E+08	1.78E+05
hCoV-19/Netherlands/NH-RIVM-10915/2021 P.1; 20J/501Y.V3; Brasil	18.84	18.97	8.77E+07	6.60E+07	7.50E+02
hCoV-19/Netherlands/NB-RIVM-10706/2021 P.2; 20B; Brasil	17.89	17.62	1.42E+08	2.21E+08	1.78E+03
hCoV-19/Aruba/AW-RIVM-11401/2021 B.1.429; 20C/S.452R; California CAL.20.C	17.97	17.57	1.56E+08	1.64E+08	5.62E+02
hCoV-19/Netherlands/NB-RIVM-20274/2020 B.1.258.21 20A AZC-variant	18.27	18.14	1.31E+08	1.24E+08	2.37E+04
hCoV-19/Netherlands/GE-RIVM-20637/2020 B.1.1.7; 20I/501Y.V1; UK-VOC 202012/01	19.33	20.00	5.82E+07	3.14E+07	5.62E+03
hCoV-19/Netherlands/GE-RIVM-20300/2020; B.1.177; 20E.EU1	18.81	18.51	9.64E+07	9.87E+07	1.33E+04
hCoV-19/Netherlands/GE-RIVM-20949/2020 C.2.1; 20D	16.91	16.80	3.13E+08	3.05E+08	1.33E+04

Sensitivity series #1 based on runcontrol 1

Modification: digital copies positive strand RdRp-gene RNA/ml determined anew

Table 2. Sensitivity panel #1, comprised of a dilution series of inactivated SARS-CoV-2 stock and tested in real-time RT-PCR using Fast-Virus Mastermix (Thermo Fisher) after extraction of 200 µl on a MagNA Pure 96 with total nucleic acid kit small volume (Roche), elution in 50 µl and 5 µl extraction per reaction. The dilution series numbering was randomized when preparing the panel. The PCRs are performed in triplicate; after the Ct value, the number of positive replicates obtained is given between brackets.

Panel coding	Dilution runcontrol	Number of dPCR copies RdRP target per ml panel specimen	Ct, average (amount of positive tests of 3 replicates)		
			E-gene Ct	RdRP gene Sarbeco probe Ct	RdRP-gene SARS-CoV-2 probe Ct
Sen. Serie-03	10-4	1.40*10 ⁶	23.12 (3)	29.61 (3)	25.53 (3)
Sen. Serie-07	10-5	1.40*10 ⁵	28.01 (3)	33.76 (3)	30.33 (3)
Sen. Serie-01	10-6	1.40*10 ⁴	30.72 (3)	37.09 (3)	32.76 (3)
Sen. Serie-05	10-7	1.40*10 ³	33.47 (3)	38.00 (2)	35.19 (3)
Sen. Serie-04	10-8	1.40*10 ²	33.95 (2)	40.95 (1)	35.15 (2)
Sen. Serie-06	10-9	1.40*10 ¹	Neg	Neg	Neg
Sen. Serie-02	10-10	1.40	Neg	Neg	Neg

Sensitivity series #2 based on runcontrol 1

Modification: digital copies positive strand RdRp-gene RNA/ml determined anew

Table 2. Sensitivity panel #2, comprised of a dilution series of inactivated SARS-CoV-2 stock and tested in real-time RT-PCR using Fast-Virus Mastermix (Thermo Fisher) after extraction of 200 µl on a MagNA Pure 96 with total nucleic acid kit small volume (Roche), elution in 50 µl and 5 µl extraction per reaction. The dilution series numbering was randomized when preparing the panel. The PCRs are performed in triplicate; after the Ct value, the number of positive replicates obtained is given between brackets.

Panel coding	Dilution stock virus	Number of dPCR copies RdRP target per ml panel specimen	Ct, average (amount of positive tests of 3 replicates)	
			E-gene (Sarbeco)	RdRP-gene (SARS-CoV-2)
Sen. Serie-04	10-4	1.40*10 ⁶	24.68 (3)	25.03 (3)
Sen. Serie-02	10-5	1.40*10 ⁵	28.26 (3)	28.71 (3)
Sen. Serie-06	10-6	1.40*10 ⁴	30.82 (3)	31.43 (3)
Sen. Serie-07	10-7	1.40*10 ³	33.11 (3)	34.69 (3)
Sen. Serie-01	10-8	1.40*10 ²	35.16 (1)	37.38 (3)
Sen. Serie-05	10-9	1.40*10 ¹	Neg	Neg
Sen. Serie-03	10-10	1.40	Neg	Neg

Sensitivity series #3 based on runcontrol 2

Modification: digital copies positive strand RdRp-gene and E-gene RNA/ml recalculated

Table 3. Composition of sensitivity panel #3, comprised of a dilution series of inactivated SARS-CoV-2 stock, and tested in real-time RT-PCR using Fast-Virus Mastermix (Thermo Fisher) after extraction of 200 µl on a MagNA Pure 96 with total nucleic acid kit small volume (Roche), elution in 50 µl and 5 µl extraction per reaction. The dilution series numbering was randomized prior to panel distribution. The SARS-CoV-2 PCRs are performed in fourfold; after the Ct value the number of positive results obtained is given between brackets.

Panel coding	Dilution stock virus	Number of dPCR copies E target per ml panel specimen	Number of dPCR copies RdRP target per ml panel specimen	Ct, average (number of positive tests of 4 replicates)	
				E-gene (Sarbeco)	RdRP-gene (SARS-CoV-2)
Sen.Series3-07	10-4	1.28*10 ⁶	1.73*10 ⁶	25.82 (4)	25.69 (4)
Sen.Series3-01	10-5	1.28*10 ⁵	1.73*10 ⁵	29.28 (4)	29.32 (4)
Sen.Series3-05	10-6	1.28*10 ⁴	1.73*10 ⁴	33.21 (4)	34.31 (4)
Sen.Series3-04	10-7	1.28*10 ³	1.73*10 ³	34.21 (4)	36.10 (4)
Sen.Series3-03	10-8	1.28*10 ²	1.73*10 ²	Neg	37.31 (3)
Sen.Series3-02	10-9	1.28*10 ¹	1.73*10 ¹	Neg	Neg
Sen.Series3-06	10-10	1.28	1.73	Neg	Neg

Saliva panel #1 based on runcontrol 1

Modification: digital copies positive strand RdRp-gene RNA/ml determined anew

Table 4. Saliva sensitivity panel #1, composed of dilution series of inactivated SARS-CoV-2 stock in SARS-CoV-2 negative donor saliva. Tested after thawing in real-time RT-PCR with Fast-Virus Master Mix after extraction of 200 µl on MagNAPure 96 with total nucleic acid kit small volume, elution in 50 µl and 5 µl extract per reaction. The SARS-CoV-2 PCRs were performed in 3-fold; after the average Ct value between brackets the number of times positive is shown. The series is offered randomized.

Panel coding	Dilution stock virus	Number of dPCR copies RdRP target per ml panel specimen	E-gene Sarbeco Ct	RdRP-gene SARS-CoV-2 Ct
Sen.SALIVA_CoV20-01	10-4	1.40*10 ⁶	25.29 (3)	25.71 (3)
Sen.SALIVA_CoV20-03	10-5	1.40*10 ⁵	28.26 (3)	28.90 (3)
Sen.SALIVA_CoV20-05	10-6	1.40*10 ⁴	30.82 (3)	30.95 (3)
Sen.SALIVA_CoV20-02	10-7	1.40*10 ³	31.70 (3)	31.92 (3)
Sen.SALIVA_CoV20-06	10-8	1.40*10 ²	31.94 (1)	neg
Sen.SALIVA_CoV20-04	No virus	-	neg	neg

Saliva panel #2 based on runcontrol 2

Table 5. Saliva sensitivity panel #2, composed of dilution series of inactivated SARS-CoV-2 stock in SARS-CoV-2 negative donor saliva. Tested after thawing in real-time RT-PCR with Fast-Virus Master Mix after extraction of 200 µl on MagNApure 96 with total nucleic acid kit small volume, elution in 50 µl and 5 µl extract per reaction. The SARS-CoV-2 PCRs were performed in 3-fold; after the average Ct value between brackets the number of times positive is shown. The series is offered randomized.

Panel coding	Dilution stock virus	Number of dPCR copies RdRP target per ml panel specimen	E-gene Sarbeco Ct	RdRP-gene SARS-CoV-2 Ct
Sen.SALIVA_CoV20-04	10-4	1.73*10 ⁶	24.99 (3)	24.89 (3)
Sen.SALIVA_CoV20-01	10-5	1.73*10 ⁵	28.29 (3)	27.82 (3)
Sen.SALIVA_CoV20-06	10-6	1.73*10 ⁴	31.06 (3)	29.57 (3)
Sen.SALIVA_CoV20-05	10-7	1.73*10 ³	33.12 (3)	32.38 (3)
Sen.SALIVA_CoV20-03	10-8	1.73*10 ²	33.32 (2)	Neg
Sen.SALIVA_CoV20-02	No virus	-	Neg	Neg

Pooling panel developed from sensitivity panel #2 based on runcontrol 1

Modification: digital copies positive strand RdRp-gene RNA/ml determined anew

Table 6. Sensitivity panel #2, comprised of a dilution series of inactivated SARS-CoV-2 stock and tested in real-time RT-PCR using Fast-Virus Mastermix (Thermo Fisher) after extraction of 200 µl on a MagNA Pure 96 with total nucleic acid kit small volume (Roche), elution in 50 µl and 5 µl extraction per reaction. The dilution series numbering was randomized when making the panel. The PCRs are performed in triplicate; after the mean Ct value, the number of positive replicates obtained is given between brackets. The pooling panel specimen number in the series of 60 specimens to be pooled is shown.

Panel #	Load	E-gene RT-PCR		RdRP-gene RT-PCR		Pooling panel specimen number
	dPCR RdRp copies/ml	µ Ct	# pos/# tested	µ Ct	# pos/# tested	
Sen. Serie-01	1.40*10 ²	35.16	(1/3)	37.38	(3/3)	47
Sen. Serie-02	1.40*10 ⁵	28.26	(3/3)	28.71	(3/3)	15, 34
Sen. Serie-03	1.40	neg	(0/3)	neg	(0/3)	nvt
Sen. Serie-04	1.40*10 ⁶	24.68	(3/3)	25.03	(3/3)	nvt
Sen. Serie-05	1.40*10 ¹	neg	(0/3)	neg	(0/3)	nvt
Sen. Serie-06	1.40*10 ⁴	30.82	(3/3)	31.43	(3/3)	3
Sen. Serie-07	1.40*10 ³	33.11	(3/3)	34.69	(3/3)	13, 59

Verification panel 1 based on runcontrol 1

Modification: digital copies positive strand RdRp-gene RNA/ml determined anew

Table 7. Verification panel composed of dilution series of inactivated SARS-CoV-2 stock in MEM with Hanks' salts and 10,000 HEp2 cells per ml. Tested after thawing in real-time RT-PCR with Fast-Virus Mastermix after extraction of 200 µl on MagNAPure 96 with total nucleic acid kit small volume, elution in 50 µl and 5 µl extract per reaction. The series is offered randomly.

Panel coding	Dilution stock virus	Number of dPCR copies RdRp target per ml panel specimen	E-gene Ct	RdRP gene Sarbeco probe Ct	RdRP-gene SARS-CoV-2 probe Ct
Ver. Series-02	10-5	1.40*10 ⁵	28.65	35.29	29.88
Ver. Series-04	10-7	1.40*10 ³	33.18	Neg	33.74
Ver. Series-01	10-8	1.40*10 ²	35.74	Neg	33.82
Ver. Series-03	No virus	-	Neg	Neg	Neg

Verification panel 2 based on runcontrol 2

Modification: digital copies positive strand RdRp-gene RNA/ml recalculated

Table 8. Verification panel composed of dilution series of heat inactivated SARS-CoV-2 stock, live influenza A virus, influenza B virus or RSV in MEM with Hanks' salts and 10,000 HEp2 cells per ml. Tested after thawing in real-time RT-PCR with Fast-Virus Master Mix after extraction of 200 µl on MagNApure 96 with total nucleic acid kit small volume, elution in 50 µl and 5 µl extract per reaction. The series is offered randomly. Sequence in the table in order of SARS-CoV-2 concentration.

Panel coding	SARS-CoV-2 RdRp-gene copies/ml panel specimen	Virus 1	Virus 2	Specific Target (influenza or RSV) Ct		SARS-CoV-2 E-gene Ct	SARS-CoV-2 RdRp-gene Ct
Ver. Series.2-02	8.65*10 ⁴	SARS-CoV-2	Influenza B-Yamagata	27.65		28.86	29.20
Ver. Series.2-03	8.65*10 ³	SARS-CoV-2	Influenza B-Victoria	24.80		31.45	31.83
Ver. Series.2-05	8.65*10 ²	SARS-CoV-2	Influenza A(H3N2) 3C.3a	27.78		34.15	33.63
Ver. Series.2-01	1.73*10 ²	SARS-CoV-2	RSV-A	22.97		Neg	34.11
Ver. Series.2-04	no SARS-CoV-2 virus	Influenza A(H1N1)pdm09	RSV-B	29.88	24.35	Neg	Neg

LEQA1 based on runcontrol 2, expected results

Modification: digital copies positive strand RdRp-gene and E-gene RNA/ml recalculated

Table 9. LEQA1 composition and Ct values in real-time RT-PCR with Fast-Virus Mastermix after extraction of 200 µl on MagNApure 96 with total nucleic acid kit small volume, elution in 50 µl and 5 µl extract per RT-PCR reaction. The SARS-CoV-2 RT-PCRs were performed in 4-fold; after the average of the Ct value the number of times found positive is given between brackets.

Panel coding	Virus ¹	Number of copies E target/ml specimen, determined with dPCR	Number of copies RdRP target/ml specimen, determined with dPCR	Target specific Ct ³	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
LEQA1_CoV20-3	hCoV-229E	-	-	17.22 (4)	Neg	Neg	Negative
LEQA1_CoV20-4	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	SARS-CoV-1	-	-	n/a	28.57 (4)	Neg	Negative ⁵

LEQA2 based on runcontrol 2, expected results

Modification: digital copies positive strand RdRp-gene and E-gene RNA/ml recalculated

Table 10. LEQA2 composition and Ct values in real-time RT-PCR with Fast-Virus Mastermix after extraction of 200 µl on MagNAPure 96 with total nucleic acid kit small volume, elution in 50 µl and 5 µl extract per RT-PCR reaction. The RT-PCRs were performed in 4-fold or 2-fold (LEQA2_CoV20-9); after the average of the Ct value the number of times found positive of tested is given between brackets.

Panel coding	Virus ¹	Number of copies E target/ml specimen, determined with dPCR	Number of copies RdRP target/ml specimen, determined with dPCR	Target specific Ct	E-gene (Sarbeco) Ct	RdRP-gene (SARS-CoV-2) Ct	Conclusion SARS-CoV-2
LEQA2_CoV20-1	SARS-CoV-1	-	-	NA	28.57 (4/4)	Neg	Negative
LEQA2_CoV20-2	SARS-CoV-2 (d1)	1.28*10 ⁵	1.73*10 ⁵	NA	28.52 (4/4)	28.37 (4/4)	POSITIVE
LEQA2_CoV20-3	hCoV-NL63	-	-	28.10 (4/4)	Neg	Neg	Negative
ELQA2_CoV20-4	hCoV-229E	-	-	17.22 (4/4)	Neg	Neg	Negative
LEQA2_CoV20-5	SARS-CoV-2 (d4)	1.28*10 ²	1.73*10 ²	NA	36.95 (2/4)	35.59 (2/4)	Weakly POSITIVE
LEQA2_CoV20-6	SARS-CoV-2 (d3)	1.28*10 ³	1.73*10 ³	NA	34.80 (4/4)	34.88 (4/4)	POSITIVE
LEQA2_CoV20-7	hCoV-OC43	-	-	27.77 (4/4)	Neg	Neg	Negative
LEQA2_CoV20-8	SARS-CoV-2 (d3)	1.28*10 ³	1.73*10 ³	NA	34.68 (4/4)	34.74 (4/4)	POSITIVE
LEQA2_CoV20-9	SARS-CoV-2 UK variant	3.39*10 ⁵	2.32*10 ⁵	NA	26.89 (2/2)	27.33 (2/2)	POSITIVE
LEQA2_CoV20-10	No virus	-	-	Neg	Neg	Neg	Negative