Supersedes: E_APG_2020-003_Detection of SARS-Cov2 RNA by RT-PCR

Review / Approval

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1 Introduction

At the end of December 2019, i[redacted] 5.1.2a a large number of pneumonia with unclear causes occurred. In early January, [redacted] 5.1.2a authorities identified a novel coronavirus (SARS-CoV-2) as the cause of these diseases. The disease caused by SARS-CoV-2 was given the official name COVID-19 ("Corona Virus disease 2019") and is transmissible from person to person.

The aim of this validation was to develop and approve a qualitative multiplex RT-PCR qPCR assay for qualitative detection of the novel coronavirus (SARS-CoV-2) RNA from human respiratory samples, which can be used as a Laboratory Developed Test (LDT).

2 Scope and limitations of validation

The validation was performed on human respiratory samples only. The experimental design of the validation is based on the publication from Rabenau et al.
Commercially available synthetic RNA containing the SARS-CoV-2 sequence (from Twist Bioscience) was used as reference material and the LOD measurements was done on the basis of the copy number concentration provided by the supplier.

3 Summary

The following table concludes the results of the setup experiments:

Validation Parameter	Results	Acceptance criteria	Status
Specificity	100%	As close to 100% as possible	n.a.(since no specific criterion was defined in the validation plan
Sensitivity	86% (≤Cp38), 95% (≤Cp33)	As close to 100% as possible	passed
Linearity	R2 = 0,9966	>0,98	passed
LOD	40 copies RNA/RT-PCR	As low as possible	n.a.(since no specific criterion was defined in the validation plan
Accuracy	93,7 % (≤Cp38), 97,3% (≤Cp33)	As close to 100% as possible	n.a.(since no specific criterion was defined in the validation plan
Matrix effect	No effect detected	Cp <1,0	passed

4 Definitions and abbreviations

Term / Abbreviation	Definition
APG	Applied Genomics
DNA	deoxyribonucleic acid
cDNA	complementary DNA
IPC	Internal positive control
LOD	Limit of Detection
LOQ	Limit of Quantification
NTC	No Template Control
RT-PCR	real-time PCR
RNA	ribonucleic acid
qPCR	quantitative Polymerase Chain Reaction

5 Responsibilities

Name	Function	Contact details
5.1.2e	5.1.2e	5.1.2e@eurofins.com
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5.1.2e	5.1.2e	5.1.2e@eurofins.com
5.1.2e	5.1.2e	5.1.2e@eurofins.com
5.1.2e	5.1.2e	5.1.2e@eurofins.de
5.1.2e	5.1.2e	5.1.2e@gelamed.de
5.1.2e	5.1.2e	5.1.2e@gelamed.de

6 Method

6.1 Description of the procedure

The detection was performed in one-step real-time RT-PCR format, i.e. reverse transcription (RT) and the subsequent PCR took place in a reaction vessel. The isolated RNA was transcribed into cDNA. The specific gene fragments for SARS-CoV-2 (E gene) were subsequently amplified by real-time PCR. The amplified target sequences were treated with hydrolysis probes, which are connected at one end to a quencher and at marked at the other end with a reporter fluorescent dye (fluorophore). In the presence of a target sequence, the probes hybridize with amplicons. During the extension, the Taq polymerase separates the reporter from quencher. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR device is detected. The fluorescence signal increases with the amount of amplicons formed. The assays contained an Internal Control RNA or Plasmid (ICR/ICP) and corresponding primers/probe to facilitate sample preparation and/or control potential PCR inhibition.

6.1.1 RNA Extraction

The RNA extraction was performed on the KingFisher extraction system by using the NucleoMag® VET Kit from Macherey Nagel (Düren, Germany; Ref. 744200). Positive and negative extraction controls (included within the kit) were included in every extraction run.

6.1.2 RT-PCR

The sample RNA extracts and the reference RNA (Twist Synthetic SARS-CoV2 RNA Control) were used as template and were transcribed in cDNA and amplified by quantitative polymerase chain reaction (qPCR) in a two-step reaction (see table 1). The PCR reaction was run with as triplex PCR consisting of two SARS-CoV2 specific assays (SARS-CoV E-gene and for 2019-nCoV_N1 assay) and one control assay ("mycoplasma assay"). The reference RNA was set into RT-PCR in a LOG10 dilution series (V1-V5 with V1 = 1,00 +E05 RNA copies/µl). Additionally, a reference plasmid was be set into RT-PCR in a LOG10 dilution series (V1-V10 with V1 = 1,00 +E09 DNA copies/µl). The RT-PCRs were performed in a reaction volume of 12 µl with *2x qPCRBio Probe 1-Step Go Mix (Lot-No. PB092618-032-5).* The PCR primers/probe sequences are displayed in 6.2.2.1

The real time qPCR setup and program are described in tables 1 and 2. The analysis was performed with the Lightcycler480II instrument in 384-well format.

Table 1: Mastermix for single step RT-PCR (reverse transcription+qPCR)

Component	Volume (µl)
2x qPCRBIO Probe 1-Step Go Mix	6,000
Promega H_2O	0,696
pMS164_IC (10^5 cop./µL)	0,03
ic_fw1 (100µM)	0,048
ic_bw1 (100µM)	0,048
ic_p1r (HEX) (100µM)	0,029
2019-nCoV_N1-F (100µM)	0,048
2019-nCoV_N1-R (100µM)	0,048
2019-nCoV_N1-P (100µM)	0,029
E_Sarbeco_F1 (100µM)	0,048
E_Sarbeco_R2 (100µM)	0,048
E_Sarbeco_P (100µM)	0,029
20x Rtase Go	0,900
Eluat (RNA)	4,000

Table 2: Cycling conditions for RT-PCR

Step	°C	Time	No. of Cycles
pre-Incubation	50	2 min	1
Initial denaturation	95	10 min	1
Denaturation	95	15 sec	45
Annealing/Elongation	60	1 min	45
Cooling	4	∞	-

6.2 Equipment and Material

6.2.1 Equipment

Description	Supplier
Eppendorf Research® plus (single channel pipette) (100-1000 µl/ 20-200 µl/ 10-100 µl/ 2-20 µl/ 0,5-10 µl/ 0,1-2,5 µl) (or comparable instrument)	Eppendorf AG, Hamburg, Germany
PIPETMAN® Classic (P2, P10, P20, P100, P200, P1000) (or comparable instrument)	Gilson S.A.S., Villiers-le-Bel, France
Centrifuge MiniSpin® plus (or comparable instrument)	Eppendorf AG, Hamburg, Germany
Centrifuge 5810 R (or comparable instrument)	Eppendorf AG, Hamburg, Germany
Vortex-shaker (or comparable instrument)	VWR International GmbH, Darmstadt, Germany
LightCycler® 480 Instrument II	Roche Diagnostics GmbH, Mannheim, Germany
NanoDrop® Lite	Thermo Scientific, Schwerte, Deutschland
Trinean Dropsense96®, Photometer	Trinean, Gentbrugge, Belgium

Note 1: All equipment used in this validation was qualified for the application, released and functional. Test and measuring equipment was calibrated, calibrations were valid.

6.2.2 Materials

6.2.2.1 Primer/Probes

Primer and probe sequences for the SARS-CoV E-gene and for 2019-nCoV_N1 assay were taken from the WHO recommendations. The probe for the E-gene gene and the N1-assay are FAM-labelled and the probe for the mycoplasma target is HEX-labelled, in order to be able to perform duplex PCR reactions.

2019-nCoV_N1 assay:

2019-nCoV_N1-F: 5`-GAC CCC AAA ATC AGC GAA AT-3`
2019-nCoV_N1-R: 5`-TCT GGT TAC TGC CAG TTG AAT CTG-3`
2019-nCoV_N1-P: 5`- FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3`

5`>GACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGA<3`

Amplicon size: 72 bp

E-gene assay:

E_Sarbeco_F1: 5`-ACAGGTACGTTAATAGTTAATAGCGT -3`
E_Sarbeco_R2: 5`- ATATTGCAGCAGTACGCACACA-3`
E_Sarbeco_P1: 5`-FAM- ACACTAGCCATCCTTACTGCGCTTCG-BHQ1-3´

5`>ACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTGCTAGTCACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT<3`

Amplicon size: 113 bp

Mycoplasma assay:

ic_fwd1: 5`- GAATTGTGTGCCCCATCTGTT-3`
ic_bw1: 5`- TCCGGTGTCTGGCTCTGATCTT -3`
ic_p1r: 5`- BBQ- GATCCTGCAGCAGCAACGAAGTATCCTCT -HEX -3´

5`>GGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCAATTGGGTACCGAGCTCGCGGCCGCAAGCCCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAATCTACCATGGCTGATCCTGCAGCAGCAACGAAGTATCCTCTCCTGAAATTATTAGGCAGCACTTGGCCAACCACCCCGCCGCGACCCATACCAAAGCCGTCGCCTTGGGCACCGAAGAAACACAGACGACTATCCAGCGACCAAGATCAGAGCCAGACACCGGAAACCCCTGCCACACCACTAAGTTGTTGCACAGAGACTCAGTGGACAGTGCTCCAATCCTCACTGCATTTAACAGCTCACACAAAGGACGGATTAACTGTA <3` Amplicon size: 199 bp

6.2.2.2 Sample Material

Any additional samples used for this validation study are listed in the following table

Description	Supplier	Validation Parameter
Clinical routine samples, tested in-house with VIASURE SARS-CoV-2 CE IVD kit or ViroReal® Kit SARS-CoV-2 & SARS according to *SOP_APG_Sars-CoV-2*	Eurofins Genomics Europe Applied Genomics GmbH, Ebersberg, Germany	- Accuracy - Precision - Specificity - Sensitivity - Matrix effect
Samples from INSTAND EQA scheme (340) virus genome detection of SARS-CoV-2 - April 2020	INSTAND e.V., Düsseldorf, Germany	- Accuracy - Specificity - Sensitivity

6.2.3 Reagent and kits

Description	Storage	Supplier
Nuclease-Free Water	Room temperature	Promega GmbH, Mannheim, Germany
qPCRBIO Probe 1-Step Go No-ROX (*Lot-No. PB092618-032-5)*	-30°C – 15°C (12 month)	PCR Biosystems Limited, London, UK (Cat. No.: PB25.43)
VIASURE SARS-CoV-2 CE IVD kit (Lot-No. VC020C-028)	2°C to 40°C	CerTest BIOTEC, San Mateo de Gállego Zaragoza, Spain (Cat. No.: VS-NCO213L)
RIDA®GENE SARS-CoV-2 RUO (Lot-No. 24140N)	-20°C	R-Biopharm, Darmstadt, Germany (Cat. No.: PG6815RUO)
ViroReal® Kit SARS-CoV-2 & SARS (Lot-No. 2004010KSAR)	-15°C to 25°C	Ingenetix, Wien, Austria (Cat. No.: DHUV02353

Note 2: All materials, kits and reagents as outlined in 6.2.2 and 6.2.3 used were approved for use. Expiration dates were not exceeded. Kits and reagents were stored according to the manufacturer's instructions in monitored facilities.

6.3 Reference Materials

The reference materials to be used in this validation are listed in the next table. All concentrations of the various standards are based on dilutions of the respective primary standard (PRI).

Reference material	Supplier	Validation Parameter
Synthetic RNA control for variant of the SARS-CoV-2 virus: MT007544.1 (GenBank IDs).	Twist Bioscience, San Francisco, USA (Catalog No 102019)	- Linearity - LOD - Accuracy - Precision

7 Results

7.1 Specificity/Sensitivity

7.1.1 Methodology and samples:

The evaluation of sensitivity and specificity was based on the following sample size:

- RT-PCR on 12 positive samples in triplicates
- RT- PCR on 16 low positive samples in triplicates
- RT-PCR on 38 negative samples in triplicates

Due to practical reason all samples were analyzed in three repetitions, but only repetition 1 was used for evaluation of specificity and sensitivity. The sample material was taken from routine analysis plates *BL20:RT0166* and GE20_RT0021 (internal plate number). The samples on this plate had already been analyzed with the RIDA®GENE SARS-CoV-2 RUO kit and were divided in positive (i.e. Cp value < 28), low positive (Cp value >28) and negative (no signal) for comparison with the validation results. The Cp threshold of 28 was specified internally based on the instructions of RIDA®GENE SARS-CoV-2 RUO kit, which recommend considering only those Cp values as valid, which lie between Cp19 and Cp31. All Cp values between Cp28 and Cp31 therefore belong to the lower third of the recommended Cp range.

The results of the validation experiments, however, were categorized based on the LOD results (see paragraph **7.2**), i.e. all signals with a Cp below or within the LOD range (= Cp 35,5 ±1,4) were considered as "positive", whereas signals above the upper LOD limit of Cp 38 were considered as "invalid". Samples with no signal were counted as "negative".

Positive controls (plasmid and reference RNA) and NTCs were analyzed together with the sample on every RT-PCR plate.

Moreover 14 samples (5 positive, 3 low positive and 6 negative) from the INSTAND ring trial 304 (April 2020) were analyzed with the test assay in triplicates as well as with the RIDA®GENE SARS-CoV-2 RUO kit and the ViroReal® Kit SARS-CoV-2 & SARS kit (in single reactions, two repetitions).

Additionally a batch of 345 patient samples was analyzed with the test assay and the results were compared to the results obtained with the two IVD kits RIDA GENE SARS-CoVG-2RUO kit (R-Biopharm) and ViroReal® Kit SARS-CoV-2 & SARS (Ingenetix).

7.1.2 Test and Results:

The following table shows the results (obtained with RIDA®GENE SARS-CoV-2 RUO kit kit) and categorization of the routine samples, taken as reference for the validation as well as the sample results obtained with the test assay in triplicates derived from one repetition (qPCR plate name: PGX20_P0238_PCRBIO-Mix_Val_5784_20200411_JFA). The data of each repetition is shown in attachment 1-3.

Table 3: Comparison of sample results derived from routine testing and from validation experiments. The table displays the sample number (**green** = tested positive; **orange** = tested low positive; **red** = tested negative), the Cp values detected with the RIDA®GENE SARS-CoV-2 RUO kit, the Cp values of the test assay, mean Cp value of triplicates, standard deviation of triplicates and the categorized test results (**green** = tested positive; **orange** = tested low positive; **red** = tested negative) with the threshold between positive and invalid at Cp38.

Routine testing results		Validation results (Rep 1)					
Sample number	**Cp**	**Cp 1**	**Cp 2**	**Cp 3**	**ØCp**	**SD**	**pos./neg.**
74126793	-	-	-	-	-	-	neg
74126838	-	42,64	-	-	42,64	-	invalid
74694383	-	-	-	-	-	-	neg
77599153	-	-	-	-	-	-	neg
95584900	-	-	-	-	-	-	neg
95584906	32,28	40,70	39,3	39,71	39,90	0,72	invalid
95585041	30,33	34,95	34,48	34,84	34,76	0,25	pos
95585042	-	-	-	-	-	-	neg
95585043	25,72	29,23	28,82	29,16	29,07	0,22	pos
95585050	-	-	-	-	-	-	neg
95585051	-	-	-	-	-	-	neg
95585052	29,61	33,62	33,86	33,54	33,67	0,17	pos
95585059	-	-	-	-	-	-	neg
95585063	32,66	35,83	36,75	36,41	36,33	0,47	pos
95585064	23,7	26,90	27,03	26,9	26,94	0,08	pos
95585067		-	-	-	-	-	neg
95585070	21,16	24,83	24,53	24,46	24,61	0,20	pos
95585071	-	-	-	-	-	-	neg
95585072	-	-	-	-	-	-	neg
95585073	31,86	37,12	36,7	37,29	37,04	0,30	pos
95585074	29,83	34,00	33,39	33,82	33,74	0,31	pos
95585076	-	-	-	-	-	-	neg
95585077	-	-	-	-	-	-	neg

95585079	-	-	-	-	-	-	neg
95585081	-	-	-	-	-	-	neg
95585086	25,85	29,70	29,64	29,58	29,64	0,06	pos
95585124	19,33	21,66	21,68	21,71	21,68	0,03	pos
95585130	-	-	-	-	-	-	neg
95585131	-	-	-	-	-	-	neg
95585134	-	-	-	-	-	-	neg
95585135	-	-	-	-	-	-	neg
95585136	-	-	-	-	-	-	neg
95585137	-	-	-	-	-	-	neg
95585140	-	-	-	-	-	-	neg
95585142	-	-	-	-	-	-	neg
95585144	-	-	-	-	-	-	neg
95585145	-	-	-	-	-	-	neg
95585146	15,66	19,47	18,88	18,8	19,05	0,37	pos
95585148	-	-	-	-	-	-	neg
95585149	-	-	-	-	-	-	neg
95585151	-	-	-	-	-	-	neg
95585152	-	-	-	-	-	-	neg
95585153	30,88	35,09	34,48	33,74	34,44	0,68	pos
95585157	-	-	-	-	-	-	neg
95585158	-	-	-	-	-	-	neg
95585159	-	-	-	-	-	-	neg
95585167	34,43	36,87	-	37,24	37,06	0,26	pos
95585169	27,71	-	41,17	45,77	43,47	3,25	invalid
95585173	-	-	-	-	-	-	neg
95585175	-	-	-	-	-	-	neg
95585180	25,6	28,29	28,39	28,29	28,32	0,06	pos
95585181	30,54	34,09	33,85	33,38	33,77	0,36	pos
95585182	-	-	-	-	-	-	neg
95585183	-	-	-	-	-	-	neg
95585184	-	-	-	-	-	-	neg
GB00006277	21,61	21,57	21,57	21,7	21,61	0,08	pos
GB00007041	33,71	33,53	33,53	33,83	33,63	0,17	pos
GB00008062	35,13	-	-	-	-	-	neg
GB00008664	29,6	30,6	30,13	30,13	30,29	0,27	pos
GB00009192	24,9	24,83	25,02	24,87	24,91	0,10	pos
GB00009193	34,83	-	-	-	-	-	neg
GB00009244	35,2	-	-	-	-	-	neg
GB00009472	26,3	26,85	26,85	26,66	26,79	0,11	pos
GB00020911	35,13	-	-	36,93	36,93	-	pos
GB00020945	33,82	35,96	35,74	37,81	36,50	1,14	pos

GB00020956	16,94	16,81	16,69	16,42	16,64	0,20	pos

As shown in table 3 the test assay detected 23 out of 28 positive samples (consisting of 12 positive and 16 low positive samples) as "positive", which corresponds to a sensitivity of (15*100)/17 = 82%. Two samples (No. 95584906 and 95585169) obtained specific signals in the test assay (see fig. 1), but the signals were above the LOD range and thus considered as "invalid". Three low positive samples (No. GB00008062, GB00009193, GB00009244) obtained no signal in the test assay and were determined as "negative".

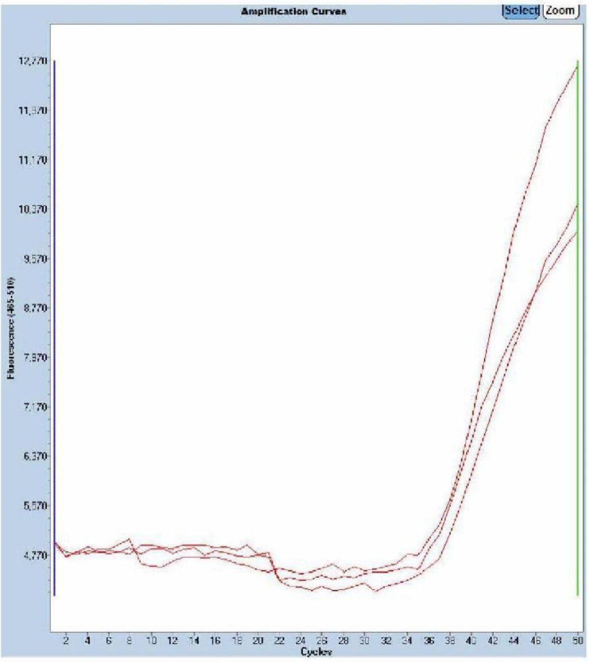

Figure 1: Amplification curves from triplicate measurement of sample No. 95584906 (FAM channel) illustrating the signal intensity of an "invalid" flagged sample . X-Axis = cycler number (Cp value); Y-axis = fluorescence value

37 of the 38 negative samples showed no signal in the test assay and one sample obtained a very late signal (No. 74126838 with Cp = 42,64), which was considered as "invalid". Since the last five cycles of a LightCycler run exhibit a higher uncertainty of measurement and the experimental setup consisted of 45 qPCR cycles, the invalid sample was excluded from statistical analysis. That means, that 37 of 37 negative samples showed no signal, which corresponds to a specificity of 100%.

The results of the parallel analysis of the INSTAND ring trial 304 samples (sample plate BL20_RT0359) are summarized in table 4:

Table 4: Comparison of results on the ring trial samples derived from the RIDA®GENE SARS-CoV-2 RUO kit, the ViroReal® Kit SARS-CoV-2 & SARS kit and the test assay. The table displays the sample ID, the Cp values detected with the IVD kits on two consecutive repetitions (Cp1, Cp2) and the rating of the results (green = positive; orange = low positive; red = negative) as described in paragraph 7.1.1. Regarding the test assay the table displays the Cp values of triplicates, the mean Cp values of each triplicate and the rating of the results (based on the LOD range).

	RIDA®GENE SARS-CoV-2 RUO				ViroReal® Kit SARS-CoV-2 & SARS				Test Assay				
	Repetition 1		Repetition 2		Repetition 1		Repetition 2						
SampleID	Cp1	SARS-CoV-2 rating	Cp2	SARS-CoV-2 rating	Cp 1	SARS-CoV-2 rating	Cp 2	SARS-CoV-2 rating	Cp1	Cp2	Cp3	Ø Cp	SARS-CoV-2 rating
NTC	-	negative	-	negative	-	negative	-	negative	-	-	-	-	negative
Positive Control	24,84	positive	24,63	positive	27,38	positive	24,89	positive	25,37	25,55	25,54	25,49	positive
Ring340059A	22,29	positive	21,9	positive	20,93	positive	19,79	positive	24,06	24,09	23,93	24,03	positive
Ring340059B	21,79	positive	21,95	positive	20,46	positive	19,95	positive	23,85	23,94	23,8	23,86	positive
Ring340060A	-	negative	-	negative	-	negative	-	negative	-	-	-	-	negative
Ring340060B	-	negative	-	negative	-	negative	-	negative	-	-	-	-	negative
Ring340061A	31,97	low positive	30,57	low positive	30,8	low positive	29,98	low positive	32,87	33,34	33,08	33,10	positive
Ring340061B	31,97	low positive	31,71	low positive	30,96	low positive	29,71	low positive	33,02	32,87	33,32	33,07	positive
Ring340062A	-	negative	-	negative	36,85	low positive	-	negative	-	-	-	-	negative
Ring340062B	-	negative	-	negative	-	negative	-	negative	-	-	-	-	negative
Ring340063A	24,73	positive	24,78	positive	23,75	positive	22,43	positive	26,79	26,72	26,78	26,76	positive
Ring340063B	25,63	positive	24,7	positive	24,13	positive	23,18	positive	26,85	26,87	26,88	26,87	positive
Ring340064A	27,73	positive	29,38	low positive	27,91	positive	27,22	positive	30,59	30,65	30,5	30,58	positive
Ring340064B	28,48	low positive	28,24	low positive	27,8	positive	26,49	positive	30,04	30,14	29,91	30,03	positive
Ring340065A	-	negative	-	negative	-	negative	36,05	low positive	-	-	-	-	negative
Ring340065B	-	negative	-	negative	-	negative	-	negative	-	-	-	-	negative

Since the criteria evaluation is based on the comparison between the test assay and the RIDA®GENE SARS-CoV-2 RUO kit, the data derived from the ViroReal® Kit SARS-CoV-2 & SARS kit shown in table 4 will not be taken into account for specificity and sensitivity calculation. By doing so the ring trial samples showed up to consist of 5 positive, 3 low positive and 6 negative samples, based on the results gained by the RIDA®GENE SARS-CoV-2 RUO kit.

On 16.04.2020 INSTAND e.V., in agreement with the Joint Diagnostic Commission of the German Association for the Control of Viral Diseases (DVV e.V.) and the Society for Virology (GfV e.V.), has decided to uncover the characteristics of three of the seven above-mentioned samples during the ongoing interlaboratory comparison for orientation purposes and to notify an interim evaluation. The following samples were uncovered:

- Sample 340059 (internal sample IDs Ring340059A and Ring340059B): positive
- Sample 340064 (internal sample IDs Ring340064A and Ring340064B): positive
- Sample 340060 (internal sample IDs Ring340060A and Ring340060B): negative

Although sample ID "Ring340064A" is detected as positive due to a Cp of 27,73 it is considered as low positive since the mean Cp of both measurement is 28,55 and thereby above defined threshold of Cp28.
As shown in table 4 the test assay detected all eight positive samples as "positive" too, which corresponds to 100% concordance and 100% sensitivity respectively.
Also all six negative samples were identified as "negative" by the test assay, which results in a specificity of 100%.

If one combines the data from table 3 and table 4, the following results are obtained:

- 31 out of 36 positive samples were identified as "positive" by the test assay, which corresponds to a sensitivity of 86 %, one sample was identified as "invalid" due to late Cp; the other 4 "positive" samples which were not identified as positive showed Cp values between 34,83 and 35,2
- 43 out of 43 negative samples were identified as "negative" by the test assay, which corresponds to a specificity of 100 %

The comparison test of 345 samples obtained the following results (data displays in attachment 4):

		Number of Results	Percentage rate
Plate Nr. BL20_RT0281	**Identical with all assays:**	84	92,3%
	Only ViroReal pos	5	5,5%
	Only PCRBio pos:	2	2,2%
	Total:	91	100,0%
Plate Nr. Cov20_RT0161	**Identical with all assays:**	87	95,6%
	Only R Biopharm pos:	1	1,1%
	Only PCRBio pos:	3	3,3%
	Total:	91	100,0%
Plate Nr. Cov20_RT0163	**Identical with all assays:**	90	98,9%
	Only ViroReal pos:	1	1,1%
	Only PCRBio pos:	0	0,0%
	Total:	91	100,0%
Plate Nr. Cov20_RT0164	**Identical with all assays:**	67	94,5%
	Only ViroReal pos:	4	4,4%
	Only PCRBio pos:	1	1,1%
	Total:	72	100,0%
Total ViroReal:	**Identical Results**	241	94,9%
	Only ViroReal pos	10	3,9%
	Only PCRBio pos	3	1,2%
	Total:	254	100,0%
Total all	**Identical Results:**	328	95,1%
	Only ViroReal or R BioPharm pos:	11	3,2%
	Only PCRBio pos:	6	1,7%
	Total:	345	100,0%

The results show a concordance between 94.5% and 98.9% with an overall concordance of 95.1%.

If all samples with a Cp ≤ 33 were excluded from the comparison test (attachment 4), the results concordance increases from 95.1% to 100% as shown in the following table:

		Number of Results	Percentage rate
Plate No. BL20_RT0281	**Identical with all assays:**	82	100,0%
	Only ViroReal pos	0	0,0%
	Only PCRBio pos:	0	0,0%
	Total:	82	100,0%
Plate No. Cov20_RT0161	**Identical with all assays:**	82	100,0%
	Only R Biopharm pos:	0	0,0%
	Only PCRBio pos:	0	0,0%
	Total:	82	100,0%
Plate No. Cov20_RT0163	**Identical with all assays:**	86	100,0%
	Only ViroReal pos:	0	0,0%
	Only PCRBio pos:	0	0,0%
	Total:	86	100,0%
Plate No. Cov20_RT0164	**Identical with all assays:**	62	100,0%
	Only ViroReal pos:	0	0,0%
	Only PCRBio pos:	0	0,0%
	Total:	62	100,0%
Total ViroReal:	**Identical Results**	230	100,0%
	Only ViroReal pos	0	0,0%
	Only PCRBio pos	0	0,0%
	Total:	230	100,0%
Total all	**Identical Results:**	312	100,0%
	Only ViroReal or R BioPharm pos:	0	0,0%
	Only PCRBio pos:	0	0,0%
	Total:	312	100,0%

7.2 Limit of Detection

7.2.1 Methodology and samples:

The LOD of the duplex assay was determined by performing RT-PCRs on a 10-fold dilution series of synthetic reference RNA (1,00 E+05 copies/µl down to 1,00 E+01 copies/µl) in triplicates and in three consecutive repetitions.

7.2.2 Test and Results:

Table 5: Results of the LOD evaluation of each repetition and the combined data of all three repetitions. The tables display the dilution steps of the reference RNA, the RNA copy number concentration inserted into RT-PCR, the decadic logarithm of the concentration, the Cp values of each triplicate, the mean Cp value of the triplicates and the standard deviation of the triplicates. The combined table displays the mean Cp values of each repetition and the mean Cp and SD of all three repetitions. The green coloured dilution step indicates the evaluated LOD (= mean Cp). Hyphens indicate, that no signal were detected.

	Dilution Step	copies/µl	LOG (conc.)	Cp1	Cp2	Cp3	ØCp	SD
Rep. 1	V1	100000	5	22,41	22,25	22,15	22,3	0,1
	V2	10000	4	25,47	24,79	25,31	25,2	0,4
	V3	1000	3	28,35	27,74	28,41	28,2	0,4
	V4	100	2	31,97	31,76	31,66	31,8	0,2
	V5	10	1	36,2	36,6	37,59	36,8	0,7
	NTC	-	-	-	-	-	-	-

	Dilution Step	copies/µl	LOG (conc.)	Cp1	Cp2	Cp3	ØCp	SD
Rep. 2	V1	100000	5	20,99	20,59	20,39	20,7	0,3
	V2	10000	4	25,24	23,89	24,62	24,6	0,7
	V3	1000	3	26,75	27,41	26,87	27,0	0,4
	V4	100	2	30,39	31,46	29,9	30,6	0,8
	V5	10	1	33,5	34,62	33,99	34,0	0,6
	NTC	-	-	-	-	-	-	-

	Dilution Step	copies/µl	LOG (conc.)	Cp1	Cp2	Cp3	ØCp	SD
Rep.3	V1	100000	5	20,52	21,39	21,41	21,1	0,5
	V2	10000	4	24,56	24,68	24,86	24,7	0,2
	V3	1000	3	27,91	28,07	28,33	28,1	0,2
	V4	100	2	31,91	31,65	31,76	31,8	0,1
	V5	10	1	35,62	36,23	35,13	35,7	0,6
	NTC	-	-	-	-	-	-	-

	Dilution Step	copies/µl	LOG (conc.)	Rep. 1	Rep. 2	Rep. 3	ØCp	SD
Combined	V1	100000	5	22,3	20,7	21,1	21,3	0,8
	V2	10000	4	25,2	24,6	24,7	24,8	0,3
	V3	1000	3	28,2	27,0	28,1	27,8	0,7
	V4	100	2	31,8	30,6	31,8	31,4	0,7
	V5	10	1	36,8	34,0	35,7	35,5	1,4
	NTC	-	-	-	-	-	-	-

All five dilution steps of the reference RNA were able to be detected with the test assay and were clearly distinguishable from the NTC, with dilution step V5 having a mean Cp of 35,5 with SD = 1,4 Cps. Therefore, the maximum LOD range is 35,5+1,4 = 36,9 Cp. Based on this results, all sample results <Cp 38 will be treated as positive and in turn all sample results >Cp38 will be treated as "invalid". Since the last dilution step has a RNA concentration of 10 copies/µl and 4µl of each dilution step were set into RT-PCR, the LOD corresponds to 40 copies of RNA per RT-PCR reaction.

7.3 Linearity

7.3.1 Methodology and samples:

The linearity of the duplex assay was be determined by performing RT-PCR on a 10-fold dilution series of reference RNA (1,00 E+05 copies/µl down to 1,00 E+01 copies/µl) in triplicates on three consecutive repetitions.

7.3.2 Test and Results:

Based on the combined results of the RNA dilution series shown in table 4 the regression curve and R^2 was calculated:

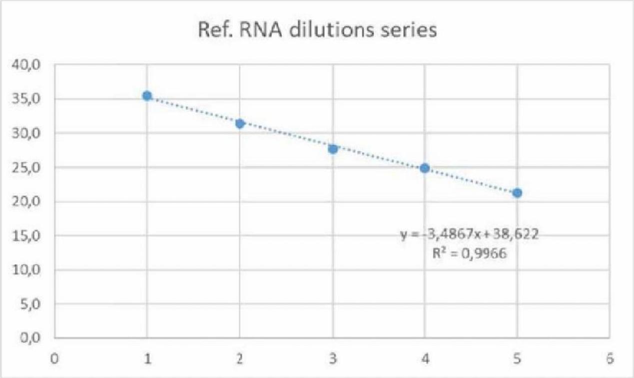

Figure 2: regression curve of the reference RNA dilution series. The figure displays the regression equation and R^2. X-axis: decadic logarithm of the RNA copy number concentration; Y-Axis: mean Cp values (derived from all three repetitions).

The regression curve of the five RNA dilutions obtained the following regression equation:

$y = 3,4867x + 38,622$

The R^2 was calculated with 0,9966 and thus fulfills the acceptance criteria of $R^2 > 0,98$.

7.4 Accuracy

7.4.1 Methodology and samples:

The accuracy of the test assay was determined by performing following experiments:

- RT-PCR on 12 positive samples in triplicates
- RT-PCR on 16 low positive samples in triplicates
- RT-PCR on 38 negative samples in triplicates

The accuracy was tested by comparison of the sample results obtained by the test assay with those obtained with the RIDA®GENE SARS-CoV-2 RUO kit used for routine analysis.

In addition 14 samples (5 positive, 3 low positive and 6 negative) from the INSTAND ring trial (April 2020) were analyzed with the test assay in triplicates as well as with the RIDA®GENE SARS-CoV-2 RUO kit and the ViroReal® Kit SARS-CoV-2 & SARS kit (in single reactions, two repetitions).

7.4.2 Test and Results:

The results for the accuracy testing can be extracted from table 3 and show that the test assay recognized 11 out of 12 positive samples as "positive" and one positive sample as "invalid" (sample number 95585169) due to a too high Cp value. Among the 16 low positive sample the test assay detected 12 as "positive" and one sample as "invalid" (sample number 95584906) and 3 samples as "negative" . Among the 38 negative samples the test assay detected 37 correctly as "negative" and 1 negative sample as "invalid", which was excluded from statistical analysis due to high uncertainty of measurement (see arguments described in paragraph 7.1.2). Taken together 52 out of 65 samples were identified correctly by the test assay, which corresponds to accuracy of ((11+12+37)x100)/65 = 92,3%.

The data from the ring trial is shown in table 4 and leads to the following results:

- All 5 positive samples were identified as "positive" by the test assay
- All 3 low positive samples were identified as "positive" by the test assay
- All 6 negative samples were identified as "negative" by the test assay

Taking together all 14 ring trial samples were identified in concordance with the RIDA®GENE SARS-CoV-2 RUO kit, which corresponds to an accuracy of 100%.

If one combines the data from table 3 and table 4 the following results are obtained:

- 16 out of 17 positive samples were identified as “positive” by the test assay
- 12 out of 16 low positive samples were identified as “positive” by the test assay
- 37 out of 37 negative samples were identified as “positive” by the test assay

In summary 74 out of 79 samples were identified in concordance with the RIDA®GENE SARS-CoV-2 RUO kit, which corresponds to an accuracy of 93,7%.

7.5 Precision: Repeatability/Intermediate Precision

7.5.1 Methodology and samples:

The repeatability of the test assay was determined by performing following experiments:

- RT-PCR on following 6 positive samples in triplicates: 95585043, 95585064, 95585070, 95585086, 95585124, 95585146
- RT-PCR on following 3 low- positive samples in triplicates: 95585041, 95585052, 95585181

The intermediate precision of the duplex assay was determined by performing following experiments:

- RT-PCR on following 6 positive samples in triplicates (repetition 1) and duplicates (repetition 2+3) on three consecutive repetitions: 95585043, 95585064, 95585070, 95585086, 95585124, 95585146
- RT-PCR on following 3 low- positive samples in triplicates (repetition 1) and duplicates (repetition 2+3) on three consecutive repetitions: 95585041, 95585052, 95585181

7.5.2 Test and Results:

Table 6: Results of the repeatability evaluation on 6 positive (green) and 3 low positive (orange) samples derived from repetition 1 of the intermediate precision test. The table displays the sample numbers, the Cp values of each triplicate, the mean Cp value of the triplicates and the standard deviation of the triplicates.

Repetition 1					
Sample Number	Cp1	Cp2	Cp3	ØCp	SD
95585041	34,95	34,48	34,84	34,76	0,25
95585043	29,23	28,82	29,16	29,07	0,22
95585052	33,62	33,86	33,54	33,67	0,17
95585064	26,9	27,03	26,9	26,94	0,08
95585070	24,83	24,53	24,46	24,61	0,20
95585086	29,7	29,64	29,58	29,64	0,06
95585124	21,66	21,68	21,71	21,68	0,03
95585146	19,47	18,88	18,8	19,05	0,37
95585181	34,09	33,85	33,38	33,77	0,36

Table 7: Results of the intermediate precision evaluation on 6 positive (green) and 3 low positive (orange) samples. Repetition 1 was performed in triplicates (see table 5), whereas repetitions 2 (table 7A) and 3 (table 7B) were performed in duplicates. The tables of the single repetitions display the sample numbers, the Cp values of each duplicate, the mean Cp value of the duplicates and the standard deviation of the duplicates. The table 7C shows the combined data of all three repetitions and displays the sample numbers, the mean Cp values of each repetition, the mean Cp value of the repetitions and the standard deviation of the repetitions.

A.	Repetition 2			
Sample Number	Cp1	Cp2	ØCp	SD
95585041	32,73	33,2	32,97	0,33
95585043	27,72	27,88	27,80	0,11
95585052	32,48	32,66	32,57	0,13
95585064	25,46	25,83	25,65	0,26
95585070	22,62	22,78	22,70	0,11
95585086	28,33	28,54	28,44	0,15
95585124	20,37	20,37	20,37	0,00
95585146	17,42	17,51	17,47	0,06
95585181	32,76	32,63	32,70	0,09
B.	Repetition 3			
Sample Number	Cp1	Cp2	ØCp	SD
95585041	33,69	33,28	33,49	0,29
95585043	27,95	28,09	28,02	0,10
95585052	32,83	32,58	32,71	0,18
95585064	26,09	26,15	26,12	0,04
95585070	23,46	23,25	23,36	0,15

95585086	28,58	27,35	27,97	0,87
95585124	20,8	20,75	20,78	0,04
95585146	17,87	17,83	17,85	0,03
95585181	32,34	32,71	32,53	0,26

C.	**Combined**				
Sample Number	**Rep 1**	**Rep 2**	**Rep 3**	**ØCp**	**SD**
95585041	34,76	32,97	33,49	33,74	0,92
95585043	29,07	27,80	28,02	28,30	0,68
95585052	33,67	32,57	32,71	32,98	0,60
95585064	26,94	25,65	26,12	26,24	0,66
95585070	24,61	22,70	23,36	23,55	0,97
95585086	29,64	28,44	27,97	28,68	0,86
95585124	21,68	20,37	20,78	20,94	0,67
95585146	19,05	17,47	17,85	18,12	0,83
95585181	34,09	33,85	33,38	33,77	0,36

The standard deviation of all 9 sample triplicates of repetition 1 (table 6) is below 0,5 Cps and therefore fulfills the acceptance criteria of repeatability.

The standard deviation of all 9 sample repetitions is below 1,0 Cps (table 7C) and therefore fulfills the acceptance criteria of intermediate precision.

7.6 Matrix Effect

7.6.1 Methodology and samples:

The matrix effect on the duplex assay was determined by performing following experiments:

- RT-PCR on following 3 positive samples in triplicates: 95585043, 95585064, 95585070
- RT-PCR on following 4 low positive samples in triplicates: 95584906, 95585041, 95585052, 95585063
- RT-PCR on following 5 negative samples in triplicates: 95585042, 95585050, 95585051, 95585059, 95585067

The results of the mycoplasma assay (IPC) was used in order to detect differences in Cp values between the three sample groups.

7.6.2 Test and Results:

Table 8: Results of the evaluation of matrix effects on 3 positive (green), 4 low positive (orange) and 5 negative (red) samples from repetition 1. The data was obtained by detecting the internal (mycoplasma) control plasmid in the HEX-channel. The table displays the sample numbers, the Cp values of each triplicate, the mean Cp value of the triplicates and the standard deviation of the triplicates.

IPC signal (HEX channel)					
Sample Number	**Cp1**	**Cp2**	**Cp3**	**ØCp**	**SD**
95584906	29,47	28,87	29,5	29,28	0,36
95585041	28,89	28,74	29,27	28,97	0,27
95585042	28,67	28,85	28,73	28,75	0,09
95585043	28,45	28,53	28,83	28,60	0,20
95585050	29,32	28,91	29,27	29,17	0,22
95585051	28,62	28,74	28,92	28,76	0,15
95585052	28,76	28,52	28,52	28,60	0,14
95585059	28,81	29,41	28,93	29,05	0,32
95585063	28,71	28,81	28,74	28,75	0,05
95585064	27,89	27,96	27,84	27,90	0,06
95585067	29,27	28,89	28,81	28,99	0,25
95585070	27,3	26,75	26,77	26,94	0,31
NTC	29,89	29,48	28,81	29,39	0,55

As shown in table 8 the standard deviation of all 12 samples is below 0,5 Cps for each sample. The standard deviation calculated over of all 12*3 = 36 Cp values/data points and the NTCs was calculated with 0,67 Cps (with a mean Cp value of 28,7) and thereby lies within the defined accepted range of SD < 1,0. This again indicates that there is no significant difference between Cp values and therefore no evidence of possible inhibitory effects.

8 Deviations

In order to gain more data points and increase the statistical power an additional batch of 345 patient samples were analyzed with the test assay and the results were compared with the results obtained by the two IVD kits RIDA GENE SARS-CoVG-2RUO kit (R-Biopharm) and ViroReal® Kit SARS-CoV-2 & SARS (Ingenetix).

9 Discussion

In summary, the described experiments demonstrate that the test results of the evaluated SARS-CoV-2 assay fulfills the acceptance criteria of linearity and shows no indication of matrix inhibition and 100% specificity, which makes the assay suitable for quantitative and semi-quantitative measurement of SARS-CoV19 target sequences in patient samples. The LOD was determined with 40 copies/µl RNA per RT-PCR reaction with a mean Cp value of 35,5 and a standard deviation of 1,4 Cps, which again results in a LOD range of Cp34-37.

Within the scope of this validation Cp38 was applied as threshold in order to categorize the sample results as positive (Cp <38) or invalid (Cp>38). Taking into account that one Cp difference corresponds to a double or half amount of template, the current threshold of Cp38 categorizes all samples with less than ca. 20 copies RNA per RT-PCR as "invalid".

Since the Cp values of the test assay show up ca. 2-3 Cps later than the corresponding IVD kit values, some of the low positive samples were categorized as "invalid" in the test sample (based on the set LOD threshold of Cp38), which results in a sensitivity of 82% and a accuracy of 92,3%. Nevertheless these "invalid" samples obtained specific and (from NTC) distinguishable amplification curves (see fig. 1), which indicates the presence of target molecules. Assuming the reported invalid samples would be considered as positive, the sensitivity would be 89,3% and the accuracy would be 95,4%. This is in line with the results of the bigger cohort of 345 samples, which showed a concordance of 95% or even 100 % (for samples with a Cp ≤33).

But there is another aspect and/or threshold, which may be applied for correct classification and interpretation of the sample results: The positive control of of the RIDA GENE SARS-CoVG-2RUO kit has a concentration of 10^3 copies/µl (according to the kit manual) with a RT-PCR input volume of 2,5 µl, i.e. the RT-PCR reaction contains $2{,}5 \times 10^3$ copies/reaction. Since the mean Cp value of this positive control is 24,7 (Cp1 = 24,84 and Cp 2 = 24,63; see table 4) and a Cp values increase of 3,3 Cps is equivalent to a 10-fold reduction of target concentration, a Cp of 33 corresponds (i.e. a Cp difference of ca. 8 Cps) to a theoretical target concentration of 10 copies/RT-PCR, which is below the evaluated LOD of 40 copies/RT-PCR. If only those samples are taken into account, which have a Cp ≤ 33, the sample numbers 95585167 (low positive, detected as positive), GB00008062 (low positive, detected as negative), GB00009193 (low positive, detected as negative), GB00009244 (low positive, detected as negative) and GB00020911 (low positive, detected as positive), would be excluded from evaluation. This again would change the sensitivity to 95% (29 out of 31 detected as "positive") and change the accuracy to 97,3% (72 out of 74 samples correctly identified). If all samples with a Cp ≤ 33 are excluded from the comparison test (attachment 4), the results concordance increases from 95,1% to 100% as shown on page 15.

Three possible scenarios may be considered in order to address the observed sensitivity/accuracy:

1. The threshold for categorizing samples as "valid" and "invalid" may be lowered in future analysis. Since the VIASURE SARS-CoV-2 CE IVD kit defines the valid Cp range between Cp19 and Cp31 and the test assay exhibits a shift of ca. 2 Cp values, the limit of quantification for the test assay could be set to Cp31+2 = Cp33 for instance.
2. All Cp values may be counted as valid as long as they are below Cp40 (for analytical reasons) and/or distinguishable from the NTC.
3. The working range will be guided by the mean Cp value of the positive control. For example if the positive control has a concentration of 4×10^3 copies/RT-PCR the threshold could be set to Cp which reflects the LOD, i.e. 40 copies/RT-PCR.

10 Records

The analysis and documentation of the results are stored in a separate validation folder.

Location: [redacted] 5.1.2h

Storage: 10 years, department APG

11 References and Related Documents

<u>SOPs:</u>

- SOP_APG_DNA-QuantiFluor_1.0
- SOP_APG_LightCycler480II_1.0
- SOP_APG_Methodenentwicklung_1.0
- SOP_APG_SARS-CoV-2_1.0

<u>Analysis / Method Specification:</u>

<u>N.A.</u>

<u>References:</u>

- EMA guideline for bioanalytical method validation, 21 July 2011, Effective 1 February 2012. EMEA/CHMP/EWP/192217/2009
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12 Attachments

Attachment 1: Comparison of sample results derived from routine testing and from validation experiments (repetition 1). The table displays the sample number (green = tested positive; orange = tested low positive; red = tested negative), the Cp values detected with the IVD kit, the Cp values of the triplicates, mean Cp value of triplicates and standard deviation of triplicates. Results are shown for the SARS-CoV-2 specific signal (FAM channel) and IPC specific signals (HEX channel).

PGX20_P0238_PCRBIO-Mix_Val_5784_20200411_JFA

REPETITION 1

	FAM					HEX				
Sample Number	**Cp1**	**Cp2**	**Cp3**	**ØCp**	**SD**	**Cp1**	**Cp2**	**Cp3**	**ØCp**	**SD**
74126793	-	-	-	-	-	28,75	28,91	28,5	28,7	0,21
74126838	42,64	-	-	42,64	-	28,6	28,72	28,86	28,7	0,13
74694383	-	-	-	-	-	28,5	28,77	29,31	28,9	0,41
77599153	-	-	-	-	-	28,81	28,83	28,59	28,7	0,13
95584900	-	-	-	-	-	29,36	28,86	28,74	29,0	0,33
95584906	40,7	39,3	39,71	39,90	0,72	29,47	28,87	29,5	29,28	0,36
95585041	34,95	34,48	34,84	34,76	0,25	28,89	28,74	29,27	28,97	0,27
95585042	-	-	-	-	-	28,67	28,85	28,73	28,75	0,09
95585043	29,23	28,82	29,16	29,07	0,22	28,45	28,53	28,83	28,60	0,20
95585050	-	-	-	-	-	29,32	28,91	29,27	29,17	0,22
95585051	-	-	-	-	-	28,62	28,74	28,92	28,76	0,15
95585052	33,62	33,86	33,54	33,67	0,17	28,76	28,52	28,52	28,60	0,14
95585059	-	-	-	-	-	28,81	29,41	28,93	29,05	0,32
95585063	35,83	36,75	36,41	36,33	0,47	28,71	28,81	28,74	28,75	0,05
95585064	26,9	27,03	26,9	26,94	0,08	27,89	27,96	27,84	27,90	0,06
95585067	-	-	-	-	-	29,27	28,89	28,81	28,99	0,25
95585070	24,83	24,53	24,46	24,61	0,20	27,3	26,75	26,77	26,94	0,31
95585071	-	-	-	-	-	28,88	28,88	28,69	28,82	0,11
95585072	-	-	-	-	-	28,81	28,74	28,72	28,76	0,05
95585073	37,12	36,7	37,29	37,04	0,30	29,32	28,85	28,89	29,02	0,26
95585074	34	33,39	33,82	33,74	0,31	28,89	28,96	28,92	28,92	0,04
95585076	-	-	-	-	-	28,83	29,32	28,83	28,99	0,28
95585077	-	-	-	-	-	29,42	29,23	29,24	29,30	0,11
95585079	-	-	-	-	-	28,91	29,26	29,27	29,15	0,21
95585081	-	-	-	-	-	29,28	29,41	29,53	29,41	0,13
95585086	29,7	29,64	29,58	29,64	0,06	28,73	28,76	28,6	28,70	0,09
95585124	21,66	21,68	21,71	21,68	0,03	24,4	24,69	24,44	24,51	0,16
95585130	-	-	-	-	-	28,87	28,6	28,86	28,78	0,15
95585131	-	-	-	-	-	28,99	29,34	28,73	29,02	0,31
95585134	-	-	-	-	-	28,81	28,79	28,68	28,76	0,07
95585135	-	-	-	-	-	28,62	29,25	28,39	28,75	0,45

95585136	-	-	-	-	-	29,37	29,23	28,75	29,12	0,33
95585137	-	-	-	-	-	28,88	29,28	29,4	29,19	0,27
95585140	-	-	-	-	-	28,72	28,9	28,92	28,85	0,11
95585142	-	-	-	-	-	28,83	28,9	28,96	28,90	0,07
95585144	-	-	-	-	-	28,96	28,81	28,84	28,87	0,08
95585145	-	-	-	-	-	28,59	28,73	28,81	28,71	0,11
95585146	19,47	18,88	18,8	19,05	0,37	22,54	21,71	21,28	21,84	0,64
95585148	-	-	-	-	-	28,81	28,75	28,68	28,75	0,07
95585149	-	-	-	-	-	28,56	28,73	28,39	28,56	0,17
95585151	-	-	-	-	-	28,98	28,86	28,87	28,90	0,07
95585152	-	-	-	-	-	28,65	28,6	28,5	28,58	0,08
95585153	35,09	34,48	33,74	34,44	0,68	28,75	28,55	28,53	28,61	0,12
95585157	-	-	-	-	-	28,78	28,54	28,79	28,70	0,14
95585158	-	-	-	-	-	28,71	28,48	28,76	28,65	0,15
95585159	-	-	-	-	-	28,57	28,71	28,79	28,69	0,11
95585167	36,87	-	37,24	37,06	0,26	28,82	28,65	28,62	28,70	0,11
95585169	-	41,17	45,77	43,47	3,25	30,94	30,6	30,87	30,80	0,18
95585173	-	-	-	-	-	28,83	29	28,38	28,74	0,32
95585175	-	-	-	-	-	28,57	28,67	28,77	28,67	0,10
95585180	28,29	28,39	28,29	28,32	0,06	27,86	28,48	28,2	28,18	0,31
95585181	34,09	33,85	33,38	33,77	0,36	28,72	28,83	28,77	28,77	0,06
95585182	-	-	-	-	-	28,68	28,88	28,77	28,78	0,10
95585183	-	-	-	-	-	28,94	28,68	29,22	28,95	0,27
95585184	-	-	-	-	-	28,7	28,74	28,54	28,66	0,11
POS_EX	-	-	-	-	-	29,56	29,54	29,33	29,48	0,13
POS_EX	-	-	-	-	-	29,36	28,94	29,4	29,23	0,25

Attachment 2: Comparison of sample results derived from routine testing and from validation experiments (repetition 2). The table displays the sample number (green = tested positive; orange = tested low positive; red = tested negative), the Cp values detected with the IVD kit, the Cp values of the triplicates, mean Cp value of triplicates and standard deviation of triplicates. Results are shown for the SARS-CoV-2 specific signal (FAM channel) and IPC specific signals (HEX channel).

PGX20_P0239_SARS_Cov_PCRbio_Val2_5784_20200412_CPO

REPETITION 2 (Sample Duplicates)

	FAM				HEX			
Sample Number	**Cp1**	**Cp2**	**ØCp**	**SD**	**Cp1**	**Cp2**	**ØCp**	**SD**
74126793	-	-	-	-	27,8	27,93	27,9	0,09
74126838	-	-	-	-	27,68	26,73	27,2	0,67
74694383	-	-	-	-	27,73	27,72	27,7	0,01
77599153	-	-	-	-	27,62	27,52	27,6	0,07
95584900	-	-	-	-	28,35	28,38	28,4	0,02
95584906	36,43	38,08	37,26	1,17	27,97	28,4	28,19	0,30
95585041	32,73	33,2	32,97	0,33	27,8	27,94	27,87	0,10
95585042	-	-	-	-	28,37	27,98	28,18	0,28
95585043	27,72	27,88	27,80	0,11	27,58	27,6	27,59	0,01
95585050	-	-	-	-	27,69	27,98	27,84	0,21
95585051	-	-	-	-	28,7	27,81	28,26	0,63
95585052	32,48	32,66	32,57	0,13	27,98	27,72	27,85	0,18
95585059	-	-	-	-	27,81	28,41	28,11	0,42
95585063	35,33	34,69	35,01	0,45	28,34	27,59	27,97	0,53
95585064	25,46	25,83	25,65	0,26	26,78	26,79	26,79	0,01
95585067	-	-	-	-	28,5	28,4	28,45	0,07
95585070	22,62	22,78	22,70	0,11	24,7	24,79	24,75	0,06
95585071	-	-	-	-	27,78	27,4	27,59	0,27
95585072	-	-	-	-	27,85	27,57	27,71	0,20
95585073	34,86	34,22	34,54	0,45	27,9	27,7	27,80	0,14
95585074	31,85	32,22	32,04	0,26	27,39	27,84	27,62	0,32
95585076	-	-	-	-	28,65	27,81	28,23	0,59
95585077	-	-	-	-	27,77	27,71	27,74	0,04
95585079	-	-	-	-	27,79	27,8	27,80	0,01
95585081	-	-	-	-	28,37	27,81	28,09	0,40
95585086	28,33	28,54	28,44	0,15	27,52	27,63	27,58	0,08
95585124	20,37	20,37	20,37	0,00	22	22,42	22,21	0,30
95585130	-	-	-	-	28,35	27,52	27,94	0,59
95585131	-	-	-	-	28,39	28,64	28,52	0,18
95585134	-	-	-	-	27,52	27,38	27,45	0,10
95585135	-	-	-	-	27,56	27,65	27,61	0,06
95585136	-	-	-	-	27,53	27,53	27,53	0,00
95585137	-	-	-	-	28,37	27,53	27,95	0,59

95585140	-	-	-	-	27,81	27,96	27,89	0,11
95585142	-	-	-	-	28,51	28,8	28,66	0,21
95585144	-	-	-	-	28,53	27,55	28,04	0,69
95585145	-	-	-	-	26,76	27,57	27,17	0,57
95585146	17,42	17,51	17,47	0,06	19,18	19,42	19,30	0,17
95585148	-	-	-	-	27,54	27,87	27,71	0,23
95585149	-	-	-	-	28,39	27,7	28,05	0,49
95585151	-	-	-	-	27,7	27,54	27,62	0,11
95585152	-	-	-	-	27,71	26,9	27,31	0,57
95585153	32,79	32,28	32,54	0,36	27,69	27,58	27,64	0,08
95585157	-	-	-	-	27,66	28,39	28,03	0,52
95585158	-	-	-	-	27,62	27,95	27,79	0,23
95585159	-	-	-	-	27,77	27,74	27,76	0,02
95585167	34,73	33,81	34,27	0,65	27,5	27,49	27,50	0,01
95585169	34,91	36,48	35,70	1,11	28,77	29,34	29,06	0,40
95585173	-	-	-	-	27,44	27,51	27,48	0,05
95585175	-	-	-	-	27,38	27,72	27,55	0,24
95585180	26,65	27,55	27,10	0,64	27,37	27,67	27,52	0,21
95585181	32,76	32,63	32,70	0,09	28,58	28,39	28,49	0,13
95585182	38,65	-	-	-	26,92	27,55	27,24	0,45
95585183	-	-	-	-	27,41	27,91	27,66	0,35
95585184	-	-	-	-	26,99	27,53	27,26	0,38
POS_EX	-	-	-	-	27,68	26,8	27,24	0,62
POS_EX	-	-	-	-	27,45	27,61	27,53	0,11

Attachment 3: Comparison of sample results derived from routine testing and from validation experiments (repetition 3). The table displays the sample number (green = tested positive; orange = tested low positive; red = tested negative), the Cp values detected with the IVD kit, the Cp values of the duplicates, mean Cp value of duplicates and standard deviation of duplicates. Results are shown for the SARS-CoV-2 specific signal (FAM channel) and IPC specific signals (HEX channel).

PGX20_P0240_SARS_CoV2-384_Val_PCRBIO-MM_29161_20200412_JFA

REPETITION 3 (Sample Duplicates)

	FAM				HEX			
Sample Number	**Cp1**	**Cp2**	**ØCp**	**SD**	**Cp1**	**Cp2**	**ØCp**	**SD**
74126793	-	-	-	-	28,33	28,15	28,2	0,13
74126838	-	-	-	-	28,39	28,41	28,4	0,01
74694383	-	-	-	-	28,77	28,36	28,6	0,29
77599153	-	-	-	-	28,57	28,54	28,6	0,02
95584900	-	-	-	-	28,62	28,53	28,6	0,06
95584906	35,99	39,05	37,52	2,16	28,87	28,65	28,76	0,16
95585041	33,69	33,28	33,49	0,29	28,4	28,32	28,36	0,06

95585042	-	-	-	-	28,38	28,33	28,36	0,04
95585043	27,95	28,09	28,02	0,10	27,88	28,03	27,96	0,11
95585050	-	-	-	-	28,76	28,3	28,53	0,33
95585051	-	-	-	-	28,61	28,36	28,49	0,18
95585052	32,83	32,58	32,71	0,18	28,42	28,39	28,41	0,02
95585059	-	-	-	-	28,73	28,76	28,75	0,02
95585063	34,4	35,63	35,02	0,87	28,55	28,49	28,52	0,04
95585064	26,09	26,15	26,12	0,04	27,43	27,47	27,45	0,03
95585067	-	-	-	-	28,61	28,29	28,45	0,23
95585070	23,46	23,25	23,36	0,15	25,46	24,98	25,22	0,34
95585071	-	-	-	-	28,65	28,52	28,59	0,09
95585072	-	-	-	-	28,44	27,62	28,03	0,58
95585073	35,79	34,53	35,16	0,89	28,34	28,32	28,33	0,01
95585074	32,36	32,88	32,62	0,37	28,38	28,43	28,41	0,04
95585076	38,3	-	-	-	28,19	28,2	28,20	0,01
95585077	-	-	-	-	28,43	28,4	28,42	0,02
95585079	-	-	-	-	28,57	27,88	28,23	0,49
95585081	-	-	-	-	28,86	28,59	28,73	0,19
95585086	28,58	27,35	27,97	0,87	28,39	26,89	27,64	1,06
95585124	20,8	20,75	20,78	0,04	23,26	23,36	23,31	0,07
95585130	-	-	-	-	28,4	26,85	27,63	1,10
95585131	-	-	-	-	28,2	28,56	28,38	0,25
95585134	-	-	-	-	28,67	28,33	28,50	0,24
95585135	-	-	-	-	28,66	28,51	28,59	0,11
95585136	-	-	-	-	28,67	28,48	28,58	0,13
95585137	-	-	-	-	28,83	28,72	28,78	0,08
95585140	-	-	-	-	28,94	28,63	28,79	0,22
95585142	-	-	-	-	28,5	28,34	28,42	0,11
95585144	-	-	-	-	28,69	28,46	28,58	0,16
95585145	-	-	-	-	28,55	28,47	28,51	0,06
95585146	17,87	17,83	17,85	0,03	20,05	20,12	20,09	0,05
95585148	-	-	-	-	27,97	28,25	28,11	0,20
95585149		37,82	-	-	28,29	28,24	28,27	0,04
95585151	-	-	-	-	28,66	28,82	28,74	0,11
95585152	-	-	-	-	26,92	26,54	26,73	0,27
95585153	32,84	32,99	32,92	0,11	28,28	27,97	28,13	0,22
95585157	-	-	-	-	28,48	28,49	28,49	0,01
95585158	-	-	-	-	28,61	28,43	28,52	0,13
95585159	-	-	-	-	28,57	28,48	28,53	0,06
95585167	36,09	34,9	35,50	0,84	28,16	27,79	27,98	0,26
95585169	36,48	36,07	36,28	0,29	30,14	29,78	29,96	0,25
95585173	-	-	-	-	28,64	28,42	28,53	0,16

95585175	-	-	-	-	28,25	28,33	28,29	0,06
95585180	27,42	27,41	27,42	0,01	27,69	27,9	27,80	0,15
95585181	32,34	32,71	32,53	0,26	28,56	28,51	28,54	0,04
95585182	-	-	-	-	28,71	28,44	28,58	0,19
95585183	-	-	-	-	28,47	28,39	28,43	0,06
95585184	-	-	-	-	28,6	28,32	28,46	0,20
POS_EX	-	-	-	-	29,13	28,53	28,83	0,42
POS_EX	-	-	-	-	28,77	28,78	28,78	0,01

Attachment 4: Comparison of two IVD kits (ViroReal and R-Biopharm) with test assay on 345 patient samples. The table displays the source plate, the IVD kit name, the sample number, the Cp values derived from the IVD kits, the sample number on the PCR plate, the Cp values calculated with the fit point method or calculated with the second derivative method.

Comparative Results				Test Assay Results		
Source Plate	Kit	Sample Number	Cp	Cp Fit Point	Cp sec. Derivative	Comment
BL20_RT0281_20200411	ViroReal	20G48193	-	-	-	-
BL20_RT0281_20200411	ViroReal	20G48377	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M13680	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M13682	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M13683	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M13684	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M13906	36,94	neg	-	late increase, flat curve in ViroReal
BL20_RT0281_20200411	ViroReal	20M13965	35,27	neg	-	late increase, flat curve in ViroReal
BL20_RT0281_20200411	ViroReal	20M13971	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M13972	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M13987	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49163	21,53	26,49	24,84	-
BL20_RT0281_20200411	ViroReal	20M49172	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49182	22,86	29,09	26,91	-
BL20_RT0281_20200411	ViroReal	20M49229	-	-	-	-

BL20_RT0281_20200411	ViroReal	20M49233	33,55	39,9	32,9	-
BL20_RT0281_20200411	ViroReal	20M49302	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49304	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49307	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49308	23,15	28,05	26,13	-
BL20_RT0281_20200411	ViroReal	20M49309	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49315	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49323	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49377	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49383	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49388	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49390	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49395	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49414	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49427	30,95	39,57	34,52	-
BL20_RT0281_20200411	ViroReal	20M49448	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49449	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49451	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49453	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49568	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49579	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49586	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49589	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49590	-	-	-	-

BL20_RT0281_20200411	ViroReal	20M49811	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49822	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49825	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49826	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M49908	25,4	31,13	28,89	-
BL20_RT0281_20200411	ViroReal	20M49909	22,81	28,34	26,47	-
BL20_RT0281_20200411	ViroReal	20M50165	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M50176	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M50783	27,2	31,88	29,51	-
BL20_RT0281_20200411	ViroReal	20M50877	-			-
BL20_RT0281_20200411	ViroReal	20M51004	28,64	34,13	31,26	-
BL20_RT0281_20200411	ViroReal	20M51012	33,27	42,57	37,48	-
BL20_RT0281_20200411	ViroReal	20M51016	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M51238	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M51239	36,59	neg	-	late increase, flat curve in ViroReal
BL20_RT0281_20200411	ViroReal	20M51344	34,78	neg	-	late increase, flat curve in ViroReal
BL20_RT0281_20200411	ViroReal	20M51348	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M51355	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M51359	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52079	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52084	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52089	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52093	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52510	35,83	neg	-	late increase, flat curve in ViroReal

BL20_RT0281_20200411	ViroReal	20M52515	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52519	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52521	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52522	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52523	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52525	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52527	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52530	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52942	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52943	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M52944	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53327	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53333	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53813	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53817	31,75	39,36	35,94	-
BL20_RT0281_20200411	ViroReal	20M53818	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53819	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53822	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53828	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53922	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53926	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M53927		44,94	37,24	-
BL20_RT0281_20200411	ViroReal	20M53936	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M54044	-	-	-	-

BL20_RT0281_20200411	ViroReal	20M54049	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M54062	-	43,21	36,36	-
BL20_RT0281_20200411	ViroReal	20M54065	-	-	-	-
BL20_RT0281_20200411	ViroReal	20M54066	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00006962	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00006964	18,5	21,94	20,21	-
Cov20_RT0161_20200410	R Biopharm	GB00007556	24,99	28,23	26,34	-
Cov20_RT0161_20200410	R Biopharm	GB00007597	32,86	36,83	34	-
Cov20_RT0161_20200410	R Biopharm	GB00008274	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008283	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008285	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008302	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008317	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008325	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008700	27,75	31,22	29,06	-
Cov20_RT0161_20200410	R Biopharm	GB00008710	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008886	25,62	29,9	27,73	-
Cov20_RT0161_20200410	R Biopharm	GB00008893	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008898	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00008936	-	45,77	38,19	-
Cov20_RT0161_20200410	R Biopharm	GB00008978	20,2	23,15	22,04	-
Cov20_RT0161_20200410	R Biopharm	GB00008980	28,32	31,53	29,08	-
Cov20_RT0161_20200410	R Biopharm	GB00008982	-	-	-	-

Cov20_RT0161_20200410	R Biopharm	GB00020696	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020697	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020698	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020699	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020700	-	40,48	34,95	-
Cov20_RT0161_20200410	R Biopharm	GB00020701	38,08	44,89	36,58	-
Cov20_RT0161_20200410	R Biopharm	GB00020702	16,6	20,05	18,57	-
Cov20_RT0161_20200410	R Biopharm	GB00020703	-			-
Cov20_RT0161_20200410	R Biopharm	GB00020704	31,72	34,88	32,11	-
Cov20_RT0161_20200410	R Biopharm	GB00020705	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020707	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020708	35,27	44,26	37,97	-
Cov20_RT0161_20200410	R Biopharm	GB00020710	25,27	28,08	26,22	-
Cov20_RT0161_20200410	R Biopharm	GB00020711	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020712	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020713	23,45	26,69	24,68	-
Cov20_RT0161_20200410	R Biopharm	GB00020714	19,14	22	20,58	-
Cov20_RT0161_20200410	R Biopharm	GB00020715	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020716	31,21	34,34	31,78	-
Cov20_RT0161_20200410	R Biopharm	GB00020717	29,8	32,98	30,31	-
Cov20_RT0161_20200410	R Biopharm	GB00020718	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020719	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020720	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020721	-	-	-	-

Cov20_RT0161_20200410	R Biopharm	GB00020722	33,6	37,89	34,63	-
Cov20_RT0161_20200410	R Biopharm	GB00020723	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020724	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020725	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020726	22,51	25,67	23,84	-
Cov20_RT0161_20200410	R Biopharm	GB00020727	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020728	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020729	28,38	31,29	29,16	-
Cov20_RT0161_20200410	R Biopharm	GB00020731	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020744	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020830	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020840	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020841	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020845	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020848	24,2	27,49	25,52	-
Cov20_RT0161_20200410	R Biopharm	GB00020849	27,8	30,93	28,67	-
Cov20_RT0161_20200410	R Biopharm	GB00020851	19,6	22,84	21,03	-
Cov20_RT0161_20200410	R Biopharm	GB00020852	28,19	30,6	28,54	-
Cov20_RT0161_20200410	R Biopharm	GB00020853	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020855	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020856	26,71	29,18	27,09	-
Cov20_RT0161_20200410	R Biopharm	GB00020857	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020860	-	-	-	-
Cov20_RT0161_20200410	R Biopharm	GB00020861	15,17	18,23	16,77	-

Cov20_RT0161_20200410	R Biopharm	GB00020862	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020863	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020864	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020865	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020866	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020867	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020868	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020869	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020871	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020873	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020876	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020877	20,17	23,28	21,61		-
Cov20_RT0161_20200410	R Biopharm	GB00020878	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020879	-	41,61	36,95		-
Cov20_RT0161_20200410	R Biopharm	GB00020880	26,38	29,63	27,6		-
Cov20_RT0161_20200410	R Biopharm	GB00020881	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020882	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020883	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020884	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020885	36,22	38,54	33,88		-
Cov20_RT0161_20200410	R Biopharm	GB00020886	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020887	-	-	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020888	38,76	neg	-		-
Cov20_RT0161_20200410	R Biopharm	GB00020889	35,54	42,65	36,29		-

Cov20_RT0163_20200411	ViroReal	V026304	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026305	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026306	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026307	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026308	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026309	29,16	30,74	28,38	-
Cov20_RT0163_20200411	ViroReal	V026310	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026311	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026312	25,22	24,96	22,94	-
Cov20_RT0163_20200411	ViroReal	V026313	-	35,78	32,28	-
Cov20_RT0163_20200411	ViroReal	V026314	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026315	28,81	27,98	25,88	-
Cov20_RT0163_20200411	ViroReal	V026316	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026317	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026318	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026319	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026320	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026321	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026322	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026323	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026324	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026325	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026326	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026327	31,96	31,85	29,48	-

Cov20_RT0163_20200411	ViroReal	V026328	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026329	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026330	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026331	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026332	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026333	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026334	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026335	20,6	20,93	19,39	-
Cov20_RT0163_20200411	ViroReal	V026336	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026337	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026338	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026339	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026340	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026341	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026342	41,42	36,39	33,54	-
Cov20_RT0163_20200411	ViroReal	V026343	34,87	35,73	32,8	-
Cov20_RT0163_20200411	ViroReal	V026344	31	31,08	28,69	-
Cov20_RT0163_20200411	ViroReal	V026345	22,87	23,18	21,27	-
Cov20_RT0163_20200411	ViroReal	V026346	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026347	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026348	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026349	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026350	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026351	-	-	-	-

Cov20_RT0163_20200411	ViroReal	V026352	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026353	40,55	37,87	33,5	-
Cov20_RT0163_20200411	ViroReal	V026354	30,36	29,44	26,99	-
Cov20_RT0163_20200411	ViroReal	V026355	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026356	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026357	26,67	26,86	24,8	-
Cov20_RT0163_20200411	ViroReal	V026358	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026359	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026360	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026361	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026362	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026363	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026364	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026365	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026366	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026367	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026368	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026369	30,67	29,97	27,99	-
Cov20_RT0163_20200411	ViroReal	V026370	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026371	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026372	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026373	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026374	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026375	-	-	-	-

Cov20_RT0163_20200411	ViroReal	V026376	26,8	26,88	24,92	-
Cov20_RT0163_20200411	ViroReal	V026377	41,28	38,79	35,81	-
Cov20_RT0163_20200411	ViroReal	V026378	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026379	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026380	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026381	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026382	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026383	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026384	27,93	27,78	26,03	-
Cov20_RT0163_20200411	ViroReal	V026385	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026386	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026387	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026388	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026389	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026390	28	27,3	25,37	-
Cov20_RT0163_20200411	ViroReal	V026391	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026392	24,81	23,96	22,36	-
Cov20_RT0163_20200411	ViroReal	V026393	-	-	-	-
Cov20_RT0163_20200411	ViroReal	V026394	-	-	-	-
Cov20_RT0164_20200411	ViroReal	GB00006225	-	-	-	-
Cov20_RT0164_20200411	ViroReal	GB00006227	17,26	21,6	19,92	-
Cov20_RT0164_20200411	ViroReal	GB00006294	30,59	35,03	31,96	-
Cov20_RT0164_20200411	ViroReal	GB00006902	35,97	neg	-	-
Cov20_RT0164_20200411	ViroReal	GB00007045	22,57	26,68	24,75	-

Cov20_RT0164_20200411	ViroReal	GB00007303	-	-	-	-
Cov20_RT0164_20200411	ViroReal	GB00007367	-	-	-	-
Cov20_RT0164_20200411	ViroReal	GB00008046	19,72	23,91	21,98	-
Cov20_RT0164_20200411	ViroReal	V026395	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026396	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026397	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026398	17,63	21,72	19,93	-
Cov20_RT0164_20200411	ViroReal	V026399	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026400	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026401	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026402	38,37	neg	-	late increase, flat curve in ViroReal
Cov20_RT0164_20200411	ViroReal	V026403	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026404	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026405	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026406	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026407	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026408	32,57	38,62	34,79	-
Cov20_RT0164_20200411	ViroReal	V026409	18,95	23,6	21,72	-
Cov20_RT0164_20200411	ViroReal	V026410	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026411	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026412	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026413	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026414	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026415	-	-	-	-

Cov20_RT0164_20200411	ViroReal	V026416	25,69	30,49	28,11	-
Cov20_RT0164_20200411	ViroReal	V026417	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026418	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026419	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026420	18,26	22,66	20,91	-
Cov20_RT0164_20200411	ViroReal	V026421	35,98	neg	-	late increase, flat curve in ViroReal
Cov20_RT0164_20200411	ViroReal	V026422	34,36	43,21	37,56	-
Cov20_RT0164_20200411	ViroReal	V026423	28,49	33,42	30,57	-
Cov20_RT0164_20200411	ViroReal	V026424	29,16	34,11	31,26	-
Cov20_RT0164_20200411	ViroReal	V026425	21,62	25,84	23,98	-
Cov20_RT0164_20200411	ViroReal	V026426	18,25	23,04	21,11	-
Cov20_RT0164_20200411	ViroReal	V026427	26,22	31,48	28,96	-
Cov20_RT0164_20200411	ViroReal	V026428	26,84	31,82	29,32	-
Cov20_RT0164_20200411	ViroReal	V026429	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026430	23,6	28,39	26,11	-
Cov20_RT0164_20200411	ViroReal	V026431	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026432	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026433	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026434	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026435	31,95	37,28	33,59	-
Cov20_RT0164_20200411	ViroReal	V026436	-	44,68	36,28	-
Cov20_RT0164_20200411	ViroReal	V026437	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026438	27,19	32,26	29,67	-
Cov20_RT0164_20200411	ViroReal	V026439	-	-	-	-

Cov20_RT0164_20200411	ViroReal	V026440	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026441	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026442	26,5	31,17	28,85	-
Cov20_RT0164_20200411	ViroReal	V026443	35,71	neg	-	late increase, flat curve in ViroReal
Cov20_RT0164_20200411	ViroReal	V026444	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026445	23,27	28,38	26,2	-
Cov20_RT0164_20200411	ViroReal	V026446	20,94	25,77	23,62	-
Cov20_RT0164_20200411	ViroReal	V026447	29,76	34,43	31,39	-
Cov20_RT0164_20200411	ViroReal	V026448	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026449	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026450	-	-	-	-
Cov20_RT0164_20200411	ViroReal	V026451	18,44	23,08	21,38	-
Cov20_RT0164_20200411	ViroReal	V026452	17,86	22,63	20,76	-
Cov20_RT0164_20200411	ViroReal	V026453	-	-	-	-