



SOP_QM_MethodVal_A04_1.0_VR_EN	
<b>Revalidation Report</b> <b>Detection of SARS-Cov2 RNA by RT-PCR</b>	Validation ID: APG20_021 Version: 1.0

**Review / Approval**

	Name	Date (DD.MM.YYYY)	Signature
Author 5.1.2e	5.1.2e		
Technical Review/Approval 5.1.2e			
QM/QA Review 5.1.2e			
Approval external reviewer (Life Codexx, LC)			
Approval external reviewer (GelLaMed)			
Approval external reviewer (GelLaMed)			
Approval Management 5.1.2e			

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## 1 Introduction


The aim of this revalidation was to validate the modifications of Eurofins in-house RT-PCR assay (internal validation ID: APG20\_020) for qualitative detection of the novel coronavirus (SARS-CoV-2) RNA from human respiratory samples due to the change of the design of probe for IPC specific assay.

## 2 Scope and limitations of revalidation

The revalidation was intended to check if the following changes of the validated SARS-CoV2 in-house assay (APG\_2020-020) have any impact on the performance of the validated in-house SARS-CoV2 assay:

- Change 1: For the in-house SARS-CoV2 assay a new probe was designed for IPC assay, as performance of IPC assay was not completely satisfactory after changing the manufacturer of the probe. Probe from Eurofins Genomics Europe Synthesis GmbH shall be used.

Since all other assay parameters were unchanged only those criteria were evaluated, which probably may be affected by potentially different probe quality. Assuming that performance of a multiplex PCR assay is more influenced by change of single primer design than by change of single probes design, accuracy of the two in-house assays variants (old" and "new" probe) and the commercially available RIDAGene kit (R BioPharm) was determined by analysis of 324 patient samples and 24 positive and negative controls.

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### 3 Summary

The following table concludes the results of the setup experiments:


<u>Validation Parameter</u>	<u>Results</u>	<u>Acceptance criteria</u>	<u>Status</u>
Accuracy	98,7%	As close to 100% as possible	passed

### 4 Definitions and abbreviations

Term / Abbreviation	Definition
APG	Applied Genomics
DNA	deoxyribonucleic acid
cDNA	complementary DNA
dPCR	digital PCR
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 @eurofins.com
		5.1.2e @eurofins.com
		5.1.2e @eurofins.com
5.1.2e	5.1.2e	5.1.2e @eurofins.com
		5.1.2e @eurofins.de
		5.1.2e @gelamed.de
		5.1.2e @gelamed.de

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## 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 (N1-gene, 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.. 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. Especially the performance of this new designed probe and the putative influence to the performance of the corona specific assays was monitored

#### 6.1.1 RNA Extraction

The RNA extraction was performed on the KingFisher extraction system by using the PurePrep Pathogens Kit from Molg3n (Utrecht, Netherlands; Art.No.OE00290096). Positive and negative extraction controls (included within the kit) were included in every extraction run.

#### 6.1.2 RT-PCR

RNA extracts from anonymized patient samples which were already tested with a commercial kit will be analysed with Eurofins in-house RT-PCR assay with the probe used in prior validation and with the new designed probe In parallel reference RNA used as extraction control and as PCR control will be analysed in concentrations used in routine analyses ( 1000 copies per RNA extraction and 1000 copies per RT PCR). The RT-PCRs will be performed in a reaction volume of 12 µl with *2x qPCRBio Probe 1-Step Go Mix*. The PCR primers/probe sequences are displayed in 5.2.2.1.

The real time qPCR setup and program are described in tables 3 and 4. The analysis was performed with the Lightcycler480II instrument in 384-well format.



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Table 3: Mastermix for single step RT-PCR (reverse transcription+qPCR)

Component	Volume (µl)
2x qPCRBIO Probe 1-Step Go Mix	6.000
Promega H <sub>2</sub> O	0.696
RNA_IC_AmpTec (10 <sup>6</sup> cop./µL)	0.03
ic_fw1 (100µM)	0.048
ic_bw1 (100µM)	0.048
ic_p1r / IPCV3 (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 4: 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	∞	-

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## 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
STILLA, Naica system (Prism and Geode)	STILLA Technologies, Villejuif, Frankreich
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 are taken from the WHO recommendations. The probe for the N1-assay is FAM-labelled, whereas the probe for the E-gene is labelled with Atto647N (new design). Both probes for the HPV target are HEX-labelled, in order to be able to perform triplex 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`

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5'>GACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCA  
GA<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'>ACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTGCTAGTCACACTAGCC  
ATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT<3'

Amplicon size: 113 bp

Mycoplasma assay:

ic\_fwd1: 5'-GAATTGTGTGCCCCATCTGTT-3'  
ic\_bw1: 5'-TCCGGTGTCTGGCTCTGATCTT-3'  
ic\_probe: 5'-HEX-GATCCTGCAGCAGCAACGAAGTATCCTCT-BHQ1-3' ("old probe")  
IPCv3: 5'-HEX-AGGCAGCACTTGGCCAACCACC-BHQ1-3' ("new probe")

5'>GGGGCTGGCTTAACCTATGCGGCATCAGAGCAGATTGTAAGTGCACCAATTGGGTACCGAGCTCG  
CGGCCGCAAGCCCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAATCTACC  
ATGGCTGATCCTGCAGCAGCAACGAAGTATCCTCTCCTGAAATTATTAGGCAGCACTTGGCCAACCACC  
CCGCGACCCATACCAAAGCCGTGCGCTTGGGCACCGAAGAAACACAGACGACTATCCAGCGACC  
AAGATCA  
GAGCCAGACACCGGAAACCCCTGCCACACCACTAAGTTGTTGCACAGAGACTCAGTGGACAGTGTCTCAAT  
CCTCACTGCATTTAACAGCTCACACAAAGGACGGATTAAGTGA <3' Amplicon size: 199 bp

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### 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 with R BioPharm RIDAgene Kit	Eurofins Genomics Europe Applied Genomics GmbH, Ebersberg, Germany	- Accuracy

### 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	-30°C to -15°C (12 month)	PCR Biosystems Limited, London, UK (Cat. No.: PB25.43)

**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)	- Accuracy
Synthetic RNA used as internal positive control, named "ssRNA MYCOPOSKO"	AmpTec GmbH, Hamburg, Germany	- Accuracy

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## 7 Results

### 7.1 Accuracy

#### 7.1.1 Methodology and samples:

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

- RT-PCR on 78 samples, positive tested with R BioPharm kit

	Cp15 to 20	CP20 to 25	CP 25 to 30	Cp 30 to 34	34 to 38	total
Number of positive samples	21	18	17	12	10	78

- RT-PCR on 246 samples, negative tested with R BioPharm kit

The accuracy was tested by comparison of the sample results obtained by the test assay with those obtained with the validated in-house assay and the in-house assay with the new designed probe.


#### 7.1.2 Test and Results:

##### 7.1.2.1. Corona specific Assays

The results for the accuracy testing can be extracted from table 1 to 3. In addition to the samples that were tested positive with the R-BioPharm Kit 24 samples were tested positive with one or both variants of the in-house assay. In total 64 % of all positive samples were detected with all three assays, 22 % with the assay with the new probe, 7 % only with the R BioPharm Kit, 6 % with the assay with the new probe, and with the R-BioPharm Kit. Almost all samples which were not detected with all three assay showed CP values above 33.

Table 1: Categories of results


Category	Description	# of samples	% of samples	% of positive samples
A	Positive with all	65	20%	64%
B	Negative with all	222	69%	
C	Positive with new probe only	22	7%	22%
D	Positive with R Biopharm only	7	2%	7%
E	Positive with new probe and with R BioPharm	6	2%	6%
F	Positive with new probe and old Probe	1	0,31%	1%

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G	Positive with old probe only	1	0,31%	1%
	Total samples	324	100%	
	Positive samples	102	31%	100%

Table 2: Results for the accuracy testing from patient samples (hyphens indicate that no increase of fluorescence was determined by the instrument)


Name	Cp N Gen (old Probe)	Cp E Gen (old Probe)	Cp N Gen (new Probe)	Cp E Gen (new Probe)	Cp R Biopharm	Category
GB00051850	15,58	15,69	15,78	15,92	12,84	A
GB00041830	16,45	16,57	15,39	16,65	13,33	A
GB00023297	14,56	14,65	14,61	14,58	14,5	A
GB00051562	17,46	17,57	16,92	17,65	14,51	A
GB00029676	14,77	14,9	15,6	15,49	14,69	A
GB00014051	15,94	16,31	16,65	16,37	15,89	A
GB00029677	17,42	17,53	17,47	17,94	16,44	A
GB00022960	18,49	18,61	18,37	18,66	16,44	A
GB00051361	18,53	18,67	19,4	18,82	16,65	A
GB00015788	17,32	17,42	17,76	17,48	16,69	A
GB00029711	19,54	19,67	19,3	19,82	16,7	A
GB00029507	19,63	19,77	19,27	19,79	17,24	A
GB00029682	25,28	25,38	25,31	25,46	17,48	A
GB00014043	19,41	19,52	19,43	19,71	17,55	A
GB00051550	20,62	20,73	20,69	20,83	17,86	A
GB00051517	18,88	19,24	18,48	19,33	18,38	A
GB00023430	19,96	20,46	19,67	20,43	18,51	A
GB00022872	18,9	19,33	18,99	19,37	18,62	A
GB00004874	21	21,42	20,95	21,66	18,98	A
GB00029664	21,87	22,36	21,28	22,81	19,52	A
GB00004287	21,86	22,39	21,44	22,43	19,82	A
GB00041878	22,87	23,44	22,44	23,51	20,39	A
GB00023295	20,78	20,9	21,17	21,22	20,6	A
GB00004868	23,27	23,36	23,28	22,68	20,66	A
GB00041870	18,7	20,53	21,35	22,31	21,24	A
GB00004863	23,58	23,69	24,69	23,98	21,25	A
GB00052809	22,41	22,51	22,43	22,6	21,63	A
GB00004280	23,69	23,83	24,3	24,27	21,68	A
GB00015785	22,48	22,58	21,94	22,64	21,91	A
GB00004883	24,83	24,96	24,61	24,84	21,99	A
GB00029668	24,84	24,96	24,31	24,95	22,23	A
GB00029679	22,73	22,88	22,76	23,56	22,49	A

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GB00004853	23,85	23,95	23,53	23,99	22,87	A
GB00005250	23,77	23,89	23,72	23,75	23,45	A
GB00023481	27,33	27,44	24,5	26,84	23,56	A
GB00004282	25,72	25,84	24,86	25,84	23,89	A
GB00023375	27,34	27,43	27,96	27,5	24,35	A
GB00023343	24,73	24,84	24,46	24,54	24,43	A
GB00051269	27,44	27,56	27,8	28,4	24,79	A
GB00004885	27,66	27,77	27,78	27,73	25,55	A
GB00004882	28,44	28,59	27,7	28,53	25,64	A
GB00023405	27,96	28,23	28,61	28,32	25,72	A
GB00004855	28,95	29,16	28,95	28,9	26,76	A
GB00029688	27,63	27,77	25,94	27,74	27,29	A
GB00041880	27,81	27,92	27,95	27,65	27,43	A
GB00051340	30,47	30,59	30,66	29,92	27,43	A
GB00004850	29,77	29,94	29,8	30,73	27,57	A
GB00041850	27,37	27,48	28,48	27,54	27,71	A
GB00041891	28,42	28,54	28,47	28,44	27,99	A
GB00051561	29,87	30,16	28,82	30,2	28,33	A
GB00023371	32,79	32,46	33,24	32,61	28,71	A
GB00023383	31,24	31,39	31,73	31,23	28,78	A
GB00004290	29,6	29,73	28,98	29,98	29	A
GB00050227	29,6	29,7	29,58	29,33	29,19	A
GB00022848	29,55	29,71	30,51	29,54	30,41	A
GB00029665	38,71	40,7	31,43	41,07	30,89	A
GB00014084	30,74	31	31,18	31,09	31,1	A
GB00024637	30,63	30,81	31,28	31,17	31,21	A
GB00022846	33,09	33,9	32,3	33,63	31,26	A
GB00004852	35,86	37,52	35,19	36,73	31,43	A
GB00098663	31,45	31,69	32,17	31,96	31,53	A
GB00041898	33,73	34,41	33,93	33,96	31,66	A
GB00051545	34,04	34,56	35,11	37,26	32,12	A
GB00014066	37,69	38,91	34,67	39,09	34,26	A
GB00050529	32,48	32,71	32,81	31,73	34,34	A
GB00004068	-	-	-	-	-	B
GB00004079	-	-	-	-	-	B
GB00004099	-	-	-	-	-	B
GB00004101	-	-	-	-	-	B
GB00004102	-	-	-	-	-	B
GB00004104	-	-	-	-	-	B
GB00004105	-	-	-	-	-	B
GB00004107	-	-	-	-	-	B
GB00004182	-	-	-	-	-	B

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GB00004261	-	-	-	-	-	B
GB00004283	-	-	-	-	-	B
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GB00008365	-	-	-	-	-	B
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GB00013987	-	-	-	-	-	B
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GB00014025	-	-	-	-	-	B
GB00014042	-	-	-	-	-	B
GB00014044	-	-	-	-	-	B
GB00014047	-	-	-	-	-	B
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GB00014049	-	-	-	-	-	B
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GB00020759	-	-	-	-	-	B
GB00021089	-	-	-	-	-	B

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GB00021097	-	-	-	-	-	B
GB00021197	-	-	-	-	-	B
GB00021198	-	-	-	-	-	B
GB00021212	-	-	-	-	-	B
GB00022812	-	-	-	-	-	B
GB00022821	-	-	-	-	-	B
GB00022825	-	-	-	-	-	B
GB00022827	-	-	-	-	-	B
GB00022854	-	-	-	-	-	B
GB00023159	-	-	-	-	-	B
GB00023161	-	-	-	-	-	B
GB00023180	-	-	-	-	-	B
GB00023298	-	-	-	-	-	B
GB00023305	-	-	-	-	-	B
GB00023306	-	-	-	-	-	B
GB00023308	-	-	-	-	-	B
GB00023377	-	-	-	-	-	B
GB00023378	-	-	-	-	-	B
GB00023381	-	-	-	-	-	B
GB00023382	-	-	-	-	-	B
GB00023401	-	-	-	-	-	B
GB00023407	-	-	-	-	-	B
GB00023410	-	-	-	-	-	B
GB00023414	-	-	-	-	-	B
GB00023420	-	-	-	-	-	B
GB00023421	-	-	-	-	-	B
GB00023423	-	-	-	-	-	B
GB00023437	-	-	-	-	-	B
GB00023455	-	-	-	-	-	B
GB00023457	-	-	-	-	-	B
GB00023475	-	-	-	-	-	B
GB00023483	-	-	-	-	-	B
GB00023484	-	-	-	-	-	B
GB00023487	-	-	-	-	-	B
GB00024638	-	-	-	-	-	B
GB00024639	-	-	-	-	-	B
GB00024640	-	-	-	-	-	B
GB00024642	-	-	-	-	-	B
GB00026610	-	-	-	-	-	B
GB00026669	-	-	-	-	-	B
GB00026686	-	-	-	-	-	B
GB00026700	-	-	-	-	-	B

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GB00026709	-	-	-	-	-	B
GB00029467	-	-	-	-	-	B
GB00029509	-	-	-	-	-	B
GB00029663	-	-	-	-	-	B
GB00029673	-	-	-	-	-	B
GB00029678	-	-	-	-	-	B
GB00029680	-	-	-	-	-	B
GB00029689	-	-	-	-	-	B
GB00029690	-	-	-	-	-	B
GB00029691	-	-	-	-	-	B
GB00029693	-	-	-	-	-	B
GB00029703	-	-	-	-	-	B
GB00041809	-	-	-	-	-	B
GB00041813	-	-	-	-	-	B
GB00041816	-	-	-	-	-	B
GB00041817	-	-	-	-	-	B
GB00041822	-	-	-	-	-	B
GB00041829	-	-	-	-	-	B
GB00041854	-	-	-	-	-	B
GB00041860	-	-	-	-	-	B
GB00041867	-	-	-	-	-	B
GB00041868	-	-	-	-	-	B
GB00041874	-	-	-	-	-	B
GB00041877	-	-	-	-	-	B
GB00041884	-	-	-	-	-	B
GB00041892	-	-	-	-	-	B
GB00041894	-	-	-	-	-	B
GB00041895	-	-	-	-	-	B
GB00041901	-	-	-	-	-	B
GB00049976	-	-	-	-	-	B
GB00049977	-	-	-	-	-	B
GB00050126	-	-	-	-	-	B
GB00050131	-	-	-	-	-	B
GB00050132	-	-	-	-	-	B
GB00050133	-	-	-	-	-	B
GB00050195	-	-	-	-	-	B
GB00050201	-	-	-	-	-	B
GB00050211	-	-	-	-	-	B
GB00050226	-	-	-	-	-	B
GB00050381	-	-	-	-	-	B
GB00050382	-	-	-	-	-	B
GB00050384	-	-	-	-	-	B

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GB00050385	-	-	-	-	-	B
GB00050512	-	-	-	-	-	B
GB00050514	-	-	-	-	-	B
GB00050548	-	-	-	-	-	B
GB00050553	-	-	-	-	-	B
GB00050568	-	-	-	-	-	B
GB00050570	-	-	-	-	-	B
GB00050572	-	-	-	-	-	B
GB00050584	-	-	-	-	-	B
GB00050591	-	-	-	-	-	B
GB00050594	-	-	-	-	-	B
GB00050595	-	-	-	-	-	B
GB00050596	-	-	-	-	-	B
GB00050972	-	-	-	-	-	B
GB00051264	-	-	-	-	-	B
GB00051331	-	-	-	-	-	B
GB00051363	-	-	-	-	-	B
GB00051513	-	-	-	-	-	B
GB00051515	-	-	-	-	-	B
GB00051520	-	-	-	-	-	B
GB00051521	-	-	-	-	-	B
GB00051544	-	-	-	-	-	B
GB00051559	-	-	-	-	-	B
GB00051560	-	-	-	-	-	B
GB00051570	-	-	-	-	-	B
GB00051571	-	-	-	-	-	B
GB00051849	-	-	-	-	-	B
GB00052775	-	-	-	-	-	B
GB00052799	-	-	-	-	-	B
GB00052802	-	-	-	-	-	B
GB00052805	-	-	-	-	-	B
GB00052807	-	-	-	-	-	B
GB00052808	-	-	-	-	-	B
GB00052818	-	-	-	-	-	B
GB00098252	-	-	-	-	-	B
GB00098295	-	-	-	-	-	B
GB00098316	-	-	-	-	-	B
GB00098487	-	-	-	-	-	B
GB00098493	-	-	-	-	-	B
GB00098563	-	-	-	-	-	B
GB00098619	-	-	-	-	-	B
GB00098620	-	-	-	-	-	B

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GB00098642	-	-	-	-	-	B
GB00098643	-	-	-	-	-	B
GB00098651	-	-	-	-	-	B
GB00098658	-	-	-	-	-	B
GB00098661	-	-	-	-	-	B
GB00029506	-	-	37,7	40,63	-	C
GB00051518	-	-	-	36,75	-	C
GB00050180	-	-	38,7	-	-	C
GB00024636	-	-	38,7	45,74	-	C
GB00041899	-	-	-	37,04	-	C
GB00023299	-	-	-	31,41	-	C
GB00029672	-	-	37,85	-	-	C
GB00015817	-	-	-	45,32	-	C
GB00098303	-	-	36,19	-	-	C
GB00014077	-	-	-	29,06	-	C
GB00014063	-	-	34,63	-	-	C
GB00041831	-	-	38,58	-	-	C
GB00004871	-	-	37,55	-	-	C
GB00014079	-	-	41,25	-	-	C
GB00050569	-	-	37,85	-	-	C
GB00023279	-	-	34,48	-	-	C
GB00098681	-	-	41,19	-	-	C
GB00003966	-	-	36,31	-	-	C
GB00023412	-	-	33,84	-	-	C
GB00098618	-	-	39,09	-	-	C
GB00098312	-	-	37,67	-	-	C
GB00022852	-	-	39,87	-	-	C
GB00013988	-	-	-	-	35,81	D
GB00050578	-	-	-	-	30,44	D
GB00004155	-	-	-	-	33,9	D
GB00041835	-	-	-	-	33,55	D
GB00051516	-	-	-	-	34,61	D
GB00022843	-	-	-	-	33,59	D
GB00023429	-	-	-	-	36,78	D
GB00029708	-	-	33,4	46,55	33,55	E
GB00004097	36,97	-	36,65	-	37,34	E
GB00029704	-	-	38,64	-	34,71	E
GB00004295	-	-	36,36	-	36,61	E
GB00014076	-	-	33,82	-	37,88	E
GB00024641	-	-	35,5	46,23	36,69	E
GB00098662	36,95	37,32	36,63	-	-	F
GB00050577	30,86	-	-	-	-	G


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Table 3: Results for the accuracy from positive controls (hyphens indicate no increase of fluorescence determined by the instrument)

Name	Cp N Gen Run 461 (old Probe)	Cp E Gen Run 461 (old Probe)	Cp N Gen Run 481 (new Probe)	Cp E Gen Run 481 (new Probe)	Cp Erstanalyse	
NTC_EX	-	-	-	-	-	B
NTC_EX	-	-	-	-	-	B
NTC_EX	-	-	-	-	-	B
NTC_EX	-	-	-	-	-	B
NTC	-	-	-	-	-	B
NTC	-	-	-	-	-	B
NTC	-	-	-	-	-	B
NTC	-	-	-	-	-	B
PCR-POS	26,8	26,9	24,3	22,69	27,18	A
PCR-POS	26,39	26,5	24,36	22,61	26,55	A
PCR-POS	26,53	26,63	25,78	23,92	25,66	A
PCR-POS	26,61	26,7	24,6	22,87	28,21	A
POS_EX	29,55	29,68	31,49	29,55	30,2	A
POS_EX	29,57	29,68	31,31	29,6	30,28	A
POS_EX	29,37	29,49	31,29	29,38	28,8	A
POS_EX	29,81	29,93	29,77	27,45	28,39	A
POS_EX	29,79	29,91	31,61	29,39	28,56	A
POS_EX	29,94	30,2	31,97	29,65	28,43	A
POS_EX	30,2	30,35	31,96	30,3	30,43	A
POS_EX	29,97	30,17	31,8	29,69	30,35	A

#### 7.1.2.2 IPC Assay

The results for the accuracy testing can be extracted from table 4 to 7. In 12 of the patient samples with high virus load / low Cp values in the corona assay no signals were generated with probe in old design. With probe in new design in all cases signals were detected, however in some cases with higher Cp values. This results corresponded to the idea that the IPC assay is less robust than the corona specific assay. Cp values from probe with old design are higher as those from probe with new design.




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Table 4: Results for the accuracy testing from patient samples for IPC: Boxes labelled 'red' indicate that no signal was detected with old probe design, but with new probe design. These were samples with low Cp values in Corona specific assays.


Name	Cp new Design	Cp old Design	Delta
GB00003966	26,32	29,98	3,66
GB00004068	26,47	29	2,53
GB00004079	26,33	29,53	3,2
GB00004097	26,73	29,58	2,85
GB00004099	26,61	30,2	3,59
GB00004101	26,15	31,26	5,11
GB00004102	26,68	29,24	2,56
GB00004104	26,13	28,73	2,6
GB00004105	26,33	30,04	3,71
GB00004107	26,45	30,49	4,04
GB00004155	26,4	30,14	3,74
GB00004182	26,73	29,86	3,13
GB00004183	26,22	31,44	5,22
GB00004188	25,9	29,76	3,86
GB00004189	26,34	30,73	4,39
GB00004190	26,14	29,82	3,68
GB00004193	26,92	30,62	3,7
GB00004195	25,96	30,54	4,58
GB00004196	26,93	31,84	4,91
GB00004198	26,41	28,74	2,33
GB00004232	26,32	28,22	1,9
GB00004240	26,56	30,45	3,89
GB00004241	26,39	30,19	3,8
GB00004243	26,28	29,5	3,22
GB00004244	26,24	29,59	3,35
GB00004246	26,27	29,14	2,87
GB00004248	26,65	31,46	4,81
GB00004251	26,51	29,97	3,46
GB00004252	25,93	29,73	3,8
GB00004255	25,9	30,77	4,87
GB00004261	26,3	30,84	4,54
GB00004280	26,15	28,59	2,44
GB00004282	25,91	28,16	2,25
GB00004283	25,83	30,22	4,39
GB00004285	26,58	31,16	4,58
GB00004287	25,98	32,14	6,16

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GB00004288	26,99	29,83	2,84
GB00004290	26,51	30,73	4,22
GB00004291	25,92	31,5	5,58
GB00004295	26,44	31,61	5,17
GB00004850	26,69	30,31	3,62
GB00004851	26,54	29,92	3,38
GB00004852	26,48	29,46	2,98
GB00004853	26,24	33,65	7,41
GB00004855	26,36	31,63	5,27
GB00004863	26,64	30,5	3,86
GB00004868	26,39	34,98	8,59
GB00004871	26,55	29,78	3,23
GB00004873	26,75	30,38	3,63
GB00004874	26,52	31,95	5,43
GB00004882	26,17	30,53	4,36
GB00004883	26,13	33,08	6,95
GB00004884	26,4	29,55	3,15
GB00004885	26,36	30,46	4,1
GB00005250	25,94	-	-
GB00005258	26,74	29,98	3,24
GB00005431	26,63	32,31	5,68
GB00005757	26,41	29,86	3,45
GB00005760	26,32	30,49	4,17
GB00005988	26,28	29,94	3,66
GB00007009	26,34	29,58	3,24
GB00007010	25,3	29,88	4,58
GB00007011	26,45	29,42	2,97
GB00007633	26,43	30,56	4,13
GB00007636	26,25	30,88	4,63
GB00007657	25,99	30,11	4,12
GB00007661	26,55	30,29	3,74
GB00007673	26,15	31,07	4,92
GB00007732	26,99	30,28	3,29
GB00008365	26,44	31,7	5,26
GB00013986	26,51	29,57	3,06
GB00013987	26,54	30,28	3,74
GB00013988	26,46	31,58	5,12
GB00013991	26,9	29,12	2,22
GB00013992	26,43	30,21	3,78
GB00013993	26,64	30,19	3,55
GB00013994	26,47	30,88	4,41
GB00014025	26,39	29,92	3,53

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GB00014042	26,27	30,59	4,32
GB00014043	30,42	-	-
GB00014044	26,45	31,57	5,12
GB00014047	25,86	28,99	3,13
GB00014048	26,95	29,59	2,64
GB00014049	26,3	30,23	3,93
GB00014050	26,29	30,67	4,38
GB00014051	37,29	-	-
GB00014052	26,34	31,14	4,8
GB00014053	26,99	31,85	4,86
GB00014054	26,46	31,63	5,17
GB00014055	26,84	29,51	2,67
GB00014056	26,61	32,7	6,09
GB00014057	26,35	30,96	4,61
GB00014058	26,56	30,42	3,86
GB00014062	26,38	29,61	3,23
GB00014063	26,35	29,14	2,79
GB00014064	26,71	31,33	4,62
GB00014065	26,84	30,98	4,14
GB00014066	26,3	30,16	3,86
GB00014067	26,98	29,91	2,93
GB00014068	26,18	28,87	2,69
GB00014069	26,55	31,31	4,76
GB00014070	25,9	29,47	3,57
GB00014073	26,19	30,78	4,59
GB00014074	26,66	32,99	6,33
GB00014076	26,1	30,84	4,74
GB00014077	25,4	29,61	4,21
GB00014078	25,99	28,97	2,98
GB00014079	26,56	30,9	4,34
GB00014080	26,39	29,77	3,38
GB00014082	26,24	29,47	3,23
GB00014083	26,17	29,92	3,75
GB00014084	26,23	30,52	4,29
GB00014085	26,29	30,51	4,22
GB00015785	26,09	32,59	6,5
GB00015786	25,83	29,39	3,56
GB00015788	35,92	46,97	11,05
GB00015797	26,23	29,15	2,92
GB00015811	26,5	29,65	3,15
GB00015812	26,32	-	-
GB00015814	26,36	29,61	3,25

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GB00015817	25,49	29,42	3,93
GB00015819	25,7	30,32	4,62
GB00015885	26,48	29,8	3,32
GB00020759	26,43	30,26	3,83
GB00021089	26,16	30,18	4,02
GB00021097	26,56	30,16	3,6
GB00021197	26,5	30,31	3,81
GB00021198	25,99	28,4	2,41
GB00021212	26,75	30,61	3,86
GB00022812	26,53	29,35	2,82
GB00022821	26,6	29,69	3,09
GB00022825	26,64	29,02	2,38
GB00022827	26,27	30,02	3,75
GB00022843	26,52	31,76	5,24
GB00022846	26,53	30,46	3,93
GB00022848	26	29,01	3,01
GB00022852	26,28	29,21	2,93
GB00022854	26,3	31,28	4,98
GB00022872	29,06	41,56	12,5
GB00022960	34,15	-	-
GB00023159	26,38	30,93	4,55
GB00023161	26,23	28,96	2,73
GB00023180	26,32	31,18	4,86
GB00023279	26,35	31,18	4,83
GB00023295	26,91	32,41	5,5
GB00023297	-	-	-
GB00023298	25,72	31,57	5,85
GB00023299	25,33	29,9	4,57
GB00023305	26,8	29,96	3,16
GB00023306	26,63	29,8	3,17
GB00023308	26,23	31,84	5,61
GB00023343	26,14	30,89	4,75
GB00023371	26,76	28,2	1,44
GB00023375	26,74	30,77	4,03
GB00023377	26,26	29,89	3,63
GB00023378	26,16	29,37	3,21
GB00023381	26,46	29,38	2,92
GB00023382	26,2	29,57	3,37
GB00023383	26,28	29,63	3,35
GB00023401	26,19	29,8	3,61
GB00023405	26,45	30,85	4,4
GB00023407	26,78	32,58	5,8

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GB00023410	26,27	31,74	5,47
GB00023412	26,32	29,84	3,52
GB00023414	26,53	30,84	4,31
GB00023420	26,41	29,05	2,64
GB00023421	25,94	29,96	4,02
GB00023423	26,45	30,46	4,01
GB00023429	26,38	29,94	3,56
GB00023430	27,43	33,9	6,47
GB00023437	26,18	28,72	2,54
GB00023455	26,6	29,19	2,59
GB00023457	26,35	33,43	7,08
GB00023475	25,88	29,27	3,39
GB00023481	26,32	32,27	5,95
GB00023483	26,44	29,74	3,3
GB00023484	26,45	31,76	5,31
GB00023487	26,41	29,53	3,12
GB00024636	25,96	30,67	4,71
GB00024637	25,98	29,92	3,94
GB00024638	26,79	31,3	4,51
GB00024639	26,28	30,25	3,97
GB00024640	26,29	29,38	3,09
GB00024641	26,46	29,9	3,44
GB00024642	25,98	29,1	3,12
GB00026610	26,12	29,84	3,72
GB00026669	26,92	30,69	3,77
GB00026686	26,93	30,21	3,28
GB00026700	26,29	29,54	3,25
GB00026709	26,41	29,76	3,35
GB00029467	26,93	30,99	4,06
GB00029506	25,59	27,88	2,29
GB00029507	29,04	33,59	4,55
GB00029509	26,36	31,84	5,48
GB00029663	26,48	28,98	2,5
GB00029664	26,9	31,11	4,21
GB00029665	26,39	30,2	3,81
GB00029668	26	30,52	4,52
GB00029672	26,58	29,32	2,74
GB00029673	26,64	28,34	1,7
GB00029676	41,04	-	-
GB00029677	33,45	41,51	8,06
GB00029678	26,52	29,34	2,82
GB00029679	26,61	30,94	4,33

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GB00029680	26,35	28,8	2,45
GB00029682	26,36	30,89	4,53
GB00029688	25,87	28,66	2,79
GB00029689	25,97	28,39	2,42
GB00029690	26,31	29,08	2,77
GB00029691	25,92	30,21	4,29
GB00029693	26,43	29,53	3,1
GB00029703	26,56	29,89	3,33
GB00029704	26,27	28,85	2,58
GB00029708	26,13	30,24	4,11
GB00029711	32,55	36,35	3,8
GB00041809	26,75	29,79	3,04
GB00041813	26,52	30,37	3,85
GB00041816	26,76	29,58	2,82
GB00041817	26,18	31,17	4,99
GB00041822	26,5	31,07	4,57
GB00041829	26,57	28,77	2,2
GB00041830	39,63	-	-
GB00041831	26,33	29,96	3,63
GB00041835	26,38	31,18	4,8
GB00041850	26,15	29,07	2,92
GB00041854	26,95	30	3,05
GB00041860	26,84	30,97	4,13
GB00041867	26,36	30,23	3,87
GB00041868	26,62	30,95	4,33
GB00041870	27,06	-	-
GB00041874	26,66	28,55	1,89
GB00041877	25,88	28,26	2,38
GB00041878	26,53	30,31	3,78
GB00041880	26,46	31,17	4,71
GB00041884	26,19	30,44	4,25
GB00041891	26,39	29,47	3,08
GB00041892	26,61	29,82	3,21
GB00041894	25,87	30,55	4,68
GB00041895	26,51	31,72	5,21
GB00041898	26,7	28,3	1,6
GB00041899	25,46	28,94	3,48
GB00041901	25,73	29	3,27
GB00049976	26,54	31,26	4,72
GB00049977	26,33	30,36	4,03
GB00050126	26,52	30,31	3,79
GB00050131	26,54	29,89	3,35

SOP_QM_MethodVal_A04_1.0_VR_EN	
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GB00050132	25,65	30,05	4,4
GB00050133	26,55	30,64	4,09
GB00050180	25,85	29,44	3,59
GB00050195	26,46	29,46	3
GB00050201	26,99	30,93	3,94
GB00050211	26,27	28,9	2,63
GB00050226	26,31	30,52	4,21
GB00050227	26,14	32,13	5,99
GB00050381	26,3	29,24	2,94
GB00050382	26,48	32,08	5,6
GB00050384	26,43	30,23	3,8
GB00050385	26,82	31,56	4,74
GB00050512	26,65	30,01	3,36
GB00050514	26,47	30,99	4,52
GB00050529	25,99	29,36	3,37
GB00050548	26,61	32,42	5,81
GB00050553	26,19	32,24	6,05
GB00050568	26,62	30,99	4,37
GB00050569	26,44	30,64	4,2
GB00050570	26,3	29,81	3,51
GB00050572	26,32	29,32	3
GB00050577	26,3	-	-
GB00050578	25,96	30,29	4,33
GB00050584	26,35	30,58	4,23
GB00050591	25,98	29,05	3,07
GB00050594	26,4	30,82	4,42
GB00050595	26,43	31	4,57
GB00050596	26,35	26,24	-0,11
GB00050972	25,63	30,29	4,66
GB00051264	26,25	29,67	3,42
GB00051269	26,9	29,71	2,81
GB00051331	26,34	29,34	3
GB00051340	26,42	31,47	5,05
GB00051361	29,39	31,88	2,49
GB00051363	26,49	32,4	5,91
GB00051513	26,47	30,27	3,8
GB00051515	26,43	29,4	2,97
GB00051516	26,69	30,82	4,13
GB00051517	32,31	40,7	8,39
GB00051518	25,76	30,28	4,52
GB00051520	26,42	30,48	4,06
GB00051521	26,52	30,86	4,34

SOP_QM_MethodVal_A04_1.0_VR_EN	
<b>Revalidation Report</b> <b>Detection of SARS-Cov2 RNA by RT-PCR</b>	Validation ID: APG20_021 Version: 1.0

GB00051544	25,78	31,36	5,58
GB00051545	26,67	31,27	4,6
GB00051550	28,01	37,91	9,9
GB00051559	26,28	30,29	4,01
GB00051560	26,11	30,62	4,51
GB00051561	26,17	30,8	4,63
GB00051562	37,43	-	-
GB00051570	26,2	30,61	4,41
GB00051571	26,79	31,97	5,18
GB00051849	25,87	31,35	5,48
GB00051850	44,23	-	-
GB00052775	26,35	29,85	3,5
GB00052799	26,61	34,05	7,44
GB00052802	25,81	31,65	5,84
GB00052805	26,5	29,74	3,24
GB00052807	26,74	32,79	6,05
GB00052808	26,27	30,88	4,61
GB00052809	26,85	26,51	-0,34
GB00052818	26,55	30,67	4,12
GB00098252	26,32	33,58	7,26
GB00098295	26,19	29,96	3,77
GB00098303	26,41	31,33	4,92
GB00098312	26,58	29,32	2,74
GB00098316	26,32	29,77	3,45
GB00098487	25,92	28,85	2,93
GB00098493	26,48	29,88	3,4
GB00098563	26,39	29,86	3,47
GB00098618	26,64	30,86	4,22
GB00098619	26,31	29,51	3,2
GB00098620	26,66	30	3,34
GB00098642	26,51	29,74	3,23
GB00098643	26,65	31,27	4,62
GB00098651	26,34	30,97	4,63
GB00098658	26,47	31,8	5,33
GB00098661	25,87	29,53	3,66
GB00098662	26,29	29,6	3,31
GB00098663	26,14	28,58	2,44
GB00098681	26,33	29,83	3,5


SOP_QM_MethodVal_A04_1.0_VR_EN	
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Table 5: Results for the accuracy testing from controls for IPC


Name	Cp new Design	Cp old Design	Delta Cp
NTC	25,89	30,69	4,8
NTC	25,85	30,44	4,59
NTC	22,97	30,93	7,96
NTC	27,07	30,81	3,74
NTC_EX	26,42	30,42	4
NTC_EX	26,2	30,9	4,7
NTC_EX	25,83	29,78	3,95
NTC_EX	25,84	28,84	3
PCR-POS	25,92	28,1	2,18
PCR-POS	26	31,18	5,18
PCR-POS	25,92	30,84	4,92
PCR-POS	26,99	30,24	3,25
POS_EX	26,47	31,29	4,82
POS_EX	26,4	30,86	4,46
POS_EX	26,37	30,82	4,45
POS_EX	26,19	30,52	4,33
POS_EX	25,4	30,63	5,23
POS_EX	25,9	29,45	3,55
POS_EX	25,9	30,56	4,66
POS_EX	25,93	29,92	3,99

Table 6: Mean / Min and Max Cp values from patient samples for IPC

Patient samples	New Design	Old Design	Delta
Mean Value	26,73	30,51	3,78
Min Value	25,30	26,24	0,94
Max Value	44,23	46,97	2,74

Table 7: Mean / Min and Max Cp values from controls for IPC

Controls	New Design	Old Design	Delta
Mean Value PCR POS	26,21	30,09	3,88
Mean Value PCR Neg	25,45	30,72	5,27
Mean Value EX POS	26,07	30,51	4,44
Mean Value EX Neg	26,07	29,99	3,91

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## 8 Deviations

N.A.

## 9 Discussion

In summary, the described experiments demonstrate that the new design of the IPC specific probe has no significant influence on the performance of the corona specific assay.

The differences in detection of very low / single copy numbers between the assay with the new and the old probe design and the R BioPharm kit are in the same range as at the comparison of the in house assay with other kits or between other commercially available kits, including IVD kits.

## Conclusion

The modified SARS-CoV2 in-house assay in combination with oligonucleotides and FAM, HEX and ATTO647N-A labelled probes (**APG20\_020**) including the new designed probe for the IPC assay from Eurofins Genomics Europe Synthesis GmbH (**APG20\_021**) is hereby technically approved for routine analysis and **can substitute SARS CoV2 assay APG20\_015**, approved test from 27.04.2020.

## 10 Records


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

Location:

5.1.2h

5.1.2h

Storage: 10 years, department APG

SOP_QM_MethodVal_A04_1.0_VR_EN	
<b>Revalidation Report</b> <b>Detection of SARS-Cov2 RNA by RT-PCR</b>	Validation ID: APG20_021 Version: 1.0

## 11 References and Related Documents

### SOPs:

- SOP\_APG\_DNA-QuantiFluor\_1.0
- SOP\_APG\_LightCycler480II\_1.0
- SOP\_APG\_Methodentwicklung\_1.0
- SOP\_APG\_SARS-CoV-2\_1.0

### Analysis / Method Specification:

N.A.

### References:

- EMA guideline for bioanalytical method validation, 21 July 2011, Effective 1 February 2012. EMEA/CHMP/EWP/192217/2009
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- Rabenau HF, Kessler HH, Kortenbusch M, Steinhorst A, Raggam RB, Berger A. Verification and validation of diagnostic laboratory tests in clinical virology. J Clin Virol 2007;40:93–8.
- Mattocks CJ, Morris MA, Matthijs G, Swinnen, E, Corveleyen, A, Dequeker, E, et al. A standardized framework for the validation and verification of clinical molecular genetic tests. Eur J Hum Genet 2010;18:1276-88.
- Halling KC, Schrijver I, Persons DL, Verification and validation for molecular diagnostic assays. Arch Pathol Lab Med 2012;136:11-3.
- INSTAND e.V.; Extra INSTAND-Ringversuch (340) Virusgenom-Nachweis SARS-CoV-2 – April 2020; Zwischenauswertung vom 16.04.2020 (RvNr. 202003)