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Comparison of commercial RT-PCR diagnostic kits for COVID-19

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MAIN FINDINGS

- We assessed basic performance of 7 commercially available COVID-19 RT-PCR kits from Altona Diagnostics, BGI, CerTest Biotec, KH Medical, PrimerDesign, R-Biopharm AG, and Seegene.
- We conclude that all kits included in this study may be used for routine diagnostics of patient samples.
- For diagnostics involving samples with expected low viral loads it might be preferable to use the RT-PCR kits from BGI, KH Medical, R-Biopharm AG, or Seegene.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus emerged in the human population in the final months of 2019 from a, so far unidentified, animal reservoir and has since spread across the globe (1). The SARS-CoV-2 pandemic poses an enormous burden on society, economic and healthcare systems worldwide, and various measures are being taken to control its spread. Many of these measures critically depend on the timely and accurate diagnosis of virus-infected individuals. Real-time reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive and specific assay and therefore preferred (2, 3). Whereas many COVID-19 RT-PCR kits are currently commercially available, an independent assessment of these products is not yet publicly available and direly needed to guide implementation of accurate tests in a diagnostic market that is flooded with new tests. As of 11 April 2020, the FIND organization listed 201 molecular assays on their website as being on the market (www.finddx.org/covid-19/pipeline).

Coronaviruses are positive-stranded RNA viruses that express their replication and transcription complex, including their RNA-dependent RNA polymerase (RdRp), from a single, large open reading frame referred to as ORF1ab (4). The coronavirus structural proteins, including the envelope (E), nucleocapsid (N), and spike (S) proteins, are expressed via the production of subgenomic messenger RNAs, which during certain stages of the replication cycle far outnumber (anti)genomic RNAs. The ORF1ab/RdRp, E, N, and S genes are the targets most frequently used for SARS-CoV-2 detection by RT-PCR. For example, the "Corman" PCR, which was co-developed in our lab and is now routinely used for our in-house diagnostic work, targets a combination of the E-gene and the RdRp-gene (2). In this set-up, the E-gene primer/probe set is specific for bat betacoronaviruses, and therefore detects both SARS-CoV-1 and -2, while the RdRp-gene primer/probe set is specific for SARS-CoV-2.

Here, we provide a comparison of a selection of seven readily available COVID-19 RT-PCR kits from different manufacturers (Table 1). One of these kits (BGI) was recently also included in a comparative study of various SARS-CoV-2 primer/probe sets (5). Most of the selected kits are CE-IVD certified and can be produced in large quantities. Using a dilution series of SARS-CoV-2 RNA we determine the 95% limit of detection (LOD95%) for each of these assays. In addition, a concise panel of clinical samples (n=22) was run to provide a first indication of clinical sensitivity and specificity. Although some kits appeared to perform better than others at identifying clinical samples at very low concentrations of SARS-CoV-2 RNA, all tests were able to identify positive samples with Ct≤34.5 in our in-house E-gene PCR. Therefore, we conclude that all of the RT-PCR kits assessed in this study may be used for routine diagnostics of COVID-19 by experienced molecular diagnostic laboratories.

Table 1. Overview of kits for RT-PCR-based detection of SARS-COV-2 included in the study.

Manufacturer	Country	Catalog number	Storage condition	Regulatory status	Target gene(s)
Altona Diagnostics	Germany	821003	-20°C	RUO[2]	E[1], S
BGI	China	MFG030010	-20°C	CE-IVD	RdRp
CerTest Biotec	Spain	VS-NCO213L	RT	CE-IVD	ORF1ab, N
KH Medical	Korea	RV008	-20°C	CE-IVD	RdRp, S
PrimerDesign	England	Z-Path-COVID-19-CE	RT	CE-IVD	RdRp
R-Biopharm AG	Germany	PG6815RUO	-20°C	RUO[3]	E
Seegene	Korea	RP10244Y	-20°C	CE-IVD	RdRp, N, E[1]

[1]As does the in-house "Corman" E-gene PCR, these E-gene assays are specific for both SARS-CoV-1 and -2.
[2]According to manufacturer's website the kit is RUO, the FindDx website states CE-IVD certification for this kit.
[3]According to the manufacturer, CE-IVD certification will be applied for in the near future.
Abbreviations: CE-IVD, European conformity label-in vitro diagnostics; E, envelope protein of SARS-CoV-2; RdRp, RNA-dependent RNA polymerase of SARS-CoV-2; N, nucleocapsid protein of SARS-CoV-2; ORF1ab, open reading frame 1 a and b of SARS-CoV-2, includes the RdRp; RNA-dependent RNA polymerase of SARS-CoV-2, part of ORF1ab; RT, room temperature; RT-PCR, reverse-transcription polymerase chain reaction; RUO, research use only; S, spike protein of SARS-CoV-2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

METHODS

Selection of kits

Commercially available COVID-19 RT-PCR kits were identified via the FindDx website (www.finddx.org/covid-19/pipeline, March 2020) and requests for information and sample kits were sent via e-mail to approximately 20 manufacturers and/or distributors, focusing on those kits that had already obtained CE-IVD certification. Promising commercial kits were selected based on: 1) listing on the FindDx website; 2) responsiveness to requests; 3) accessible information (in English); 4) compatibility with different PCR platforms; 5) considerable production capacity. Notably, all of the PCR kits that we had selected for our analysis have in the meantime also been selected for the first round of independent evaluation by FIND (www.finddx.org/covid-19/sarscov2-eval-molecular/, April 2020). All of the kits included in our analysis were provided free of charge and none of the manufacturers were involved in the assessment and interpretation of the results. The selection encompasses both kits that require transport and storage at -20°C and kits that can be transported and stored at room temperature. Target genes for each RT-PCR kit were available in the assay documentation or upon request (for an overview, see Table 1). All PCRs were run on a LightCycler 480 II (LC480II, Roche) and performed according to the manufacturer's instructions for use. Of note however, for some kits (BGI, KH Medical, and Seegene) settings for the LC480II were not provided and were therefore adapted from those provided for another machine.

PCR efficiency and limit of detection

To establish PCR efficiency we first ran a duplicate 10-fold dilution series of viral RNA for each assay. Viral RNA was isolated from SARS-CoV-2 viral particles (Dutch clinical isolate) obtained from cell culture using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche) and diluted in TE buffer. We determined the slope by linear regression in GraphPad Prism and defined the required levels for PCR efficiency (E) and R^2 as >95% and >0.95, respectively. Next, we ran four replicates of a 2-fold dilution series (diluted in yeast carrier RNA in water) to determine the LOD95% by Probit analysis using SPSS Statistics (IBM, version 24). The limited range of the dilution series did not allow for determination of a confidence interval for the LOD95% for all assays, which should therefore be regarded as an approximation and not considered definitive. The starting concentration of the viral RNA (copies/ml) was determined by digital PCR targeting the SARS-CoV-2 RdRp-gene and was specific for the positive sense genomic RNA (2).

Clinical sensitivity and specificity

Finally, a panel of clinical samples with in-house confirmed SARS-CoV-2 ($17.25 \leq Ct \leq 39.6$ for the E-gene during routine diagnostics; n=16) or other respiratory viruses (influenza virus type A (n=2), rhinovirus (n=2), RSV-A and -B) was prepared (for Ct values obtained in initial diagnostics, see supplementary Table S1). RNA was isolated anew from stored clinical samples (naso- and/or oropharyngeal swabs in GLY-medium) using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche) and was assessed with a single replicate to obtain a first indication of clinical specificity and sensitivity. No re-test was performed when the result was

inconclusive according to the manufacturer's instructions for interpretation of the result (n=2). In addition to clinical samples, a panel of viral RNA from related cell cultured human coronaviruses (including SARS1, MERS, NL63, OC43, and 229E) was used to assess cross-reactivity within the coronavirus family (for Ct values of these samples see supplementary Table S1).

RESULTS

PCR efficiency was above the required level for all kits included in the study. We first assessed PCR efficiency for each target gene assay by running a duplicate 10-fold dilution series of SARS-CoV-2 viral RNA (Figure 1). All assays showed an efficiency ≥96% and R squares were >0.97, which are both well above the pre-defined required level. Since the applied filter settings were not correct for reading the Seegene N-gene assay, we excluded these data from all of our analyses.

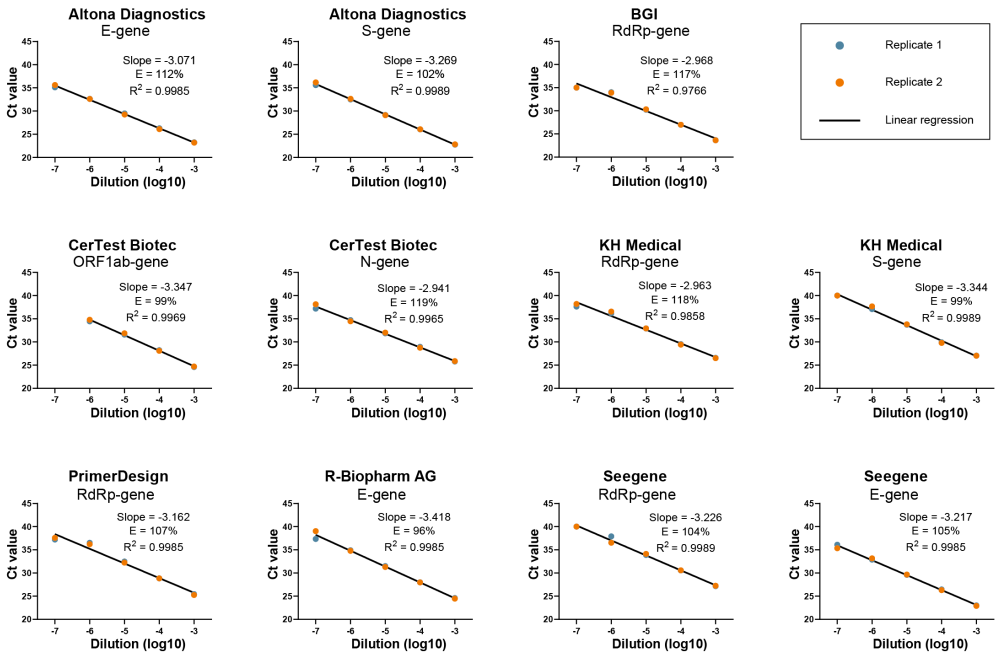

Figure 1. PCR efficiency for seven commercially available RT-PCR kits for the detection of SARS-CoV-2 RNA. PCR efficiency (E) for each target gene was assessed using a duplicate 10-fold dilution series of SARS-CoV-2 viral RNA. Linear regression was performed in Graphpad Prism to obtain the slope and R^2. The percentage efficiency was calculated from the slope using the formula $E = 100*(-1+10^{-1/slope})$. E, envelope protein of SARS-CoV-2; RdRp, RNA-dependent RNA polymerase of SARS-CoV-2; N, nucleocapsid protein of SARS-CoV-2; ORF1ab, open reading frame 1a and b of SARS-CoV-2, includes the RdRp; RNA-dependent RNA polymerase of SARS-CoV-2, part of ORF1ab; S, spike protein of SARS-CoV-2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

The LOD95% varied within a 6-fold range between the kits included in the study. The 10-fold dilution series provided a first indication of the LOD95% for each assay and were used to determine the starting point of a 2-fold dilution series performed with four replicates to come to a more precise estimate (for Ct values, see supplementary Table S2). Probit analysis was performed to estimate the LOD95%, which is shown in Table 2. Notably, due to the limited extent of the dilution series, this analysis did not always provide upper and lower bounds of the estimate and should not be considered definitive. We found that the estimated LOD95% for the various targets of the RT-PCR kits varied within a 6-fold range, with the RT-PCR kit from Altona Diagnostics having the lowest LOD95% at 3.8 copies/ml for both the E- and S-gene assays and the PrimerDesign kit having the highest LOD95% at 23 copies/ml (Table 2). Overall, our in-house "Corman" RT-PCR had the lowest estimated LOD95% at 0.91 copies/ml for the E-gene assay (2).

Table 2. Estimated limit of detection for SARS-COV-2 in copies/ml for individual assays.

	LOD95% in copy/ml determined in this study[1]			
Company	**E**	**N**	**ORF1ab/RdRp**	**S**
Altona Diagnostics	3.8 (NA)	-	-	3.8 (NA)
BGI	-	-	4.3 (NA)	-
CerTest Biotec	-	4.8 (NA)	18 (13-56)	-
KH Medical	-	-	4.8 (NA)	4.3 (NA)
PrimerDesign	-	-	23 (16-123)	-
R-Biopharm AG	4.3 (NA)	-	-	-
SeeGene	4.8 (NA)	NA[2]	18 (13-56)	-
In-house PCR	0.91 (0.61-2.4)	-	3.1 (2.1-7.3)	-

[1]The copy number was determined by digital PCR for the positive sense RdRp gene. Due to the limited range of the 2-fold dilution series, a confidence interval could not be determined for all assays.
[2]The filter settings for the Seegene N-gene PCR were not correct and these results are therefore excluded.
Abbreviations: E, envelope protein of SARS-CoV-2; LOD95%, 95% limit of detection; N, nucleocapsid protein of SARS-CoV-2; NA, not available; ORF, open reading frame; RdRp, RNA-dependent RNA polymerase of SARS-CoV-2; RT-PCR, reverse-transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S, spike protein of SARS-CoV-2.

The clinical sensitivity appears to vary between the kits included in the study. Next, we analyzed a panel of clinical samples previously submitted for routine SARS-CoV-2 diagnostics (n=16) for which the presence of various amounts of SARS-CoV-2 RNA had been confirmed using our in-house PCR. In addition, we included a panel of clinical samples (n=6) with other confirmed respiratory viral infections, including influenza virus type A, RSV A and B, and rhinovirus. Notably, the new RNA isolation performed on stored clinical samples resulted in increased Ct values (by approximately 1 Ct) compared to the initial diagnostic results for our in-house E-gene PCR. For this reason, even using our in-house PCR we could not confirm the presence of SARS-CoV-2 RNA in 3 out of 16 samples (see Figure 2A and supplementary Table S1). The positive identification rate for the various RT-PCR kits varied from 10 to 13 out of 16 samples (Figure 2A), with R-Biopharm AG performing best (13/16), followed by BGI, KH Medical, and Seegene (12/16), and Altona Diagnostics, CerTest Biotec, and PrimerDesign (10/16). Of note, both CerTest Biotec and Seegene had one "inconclusive" sample according to the manufacturer's instructions for interpretation, which might have tested positive upon re-testing but has now been counted as "negative". All target gene assays were able to positively identify the 10 clinical samples with the highest concentrations of SARS-CoV-2 (Ct≤34.50 in in-house E-gene PCR). For these samples, the different assays showed a similar pattern of Ct values, on average ranging from almost 1 Ct lower (Altona Diagnostics S-gene) to almost 5 Ct higher (KH Medical S-gene) than those obtained with the in-house E-gene PCR (Figure 2B).

None of the assays showed cross-reactivity with circulating respiratory (corona)viruses. Importantly, none of the assays resulted in a positive signal for any of the clinical samples with confirmed non-coronavirus respiratory viral infections (Supplementary Table S1). We also ran a panel consisting of cell culture-derived viral RNA for related human coronaviruses (SARS1, MERS, NL63, OC43, and 229E) to check for cross-reactivity within the coronavirus family. Of these, only the SARS-CoV-1 E-gene was identified, as per design, by assays from Altona Diagnostics, Seegene, and our in-house PCR (Supplementary Table S1).

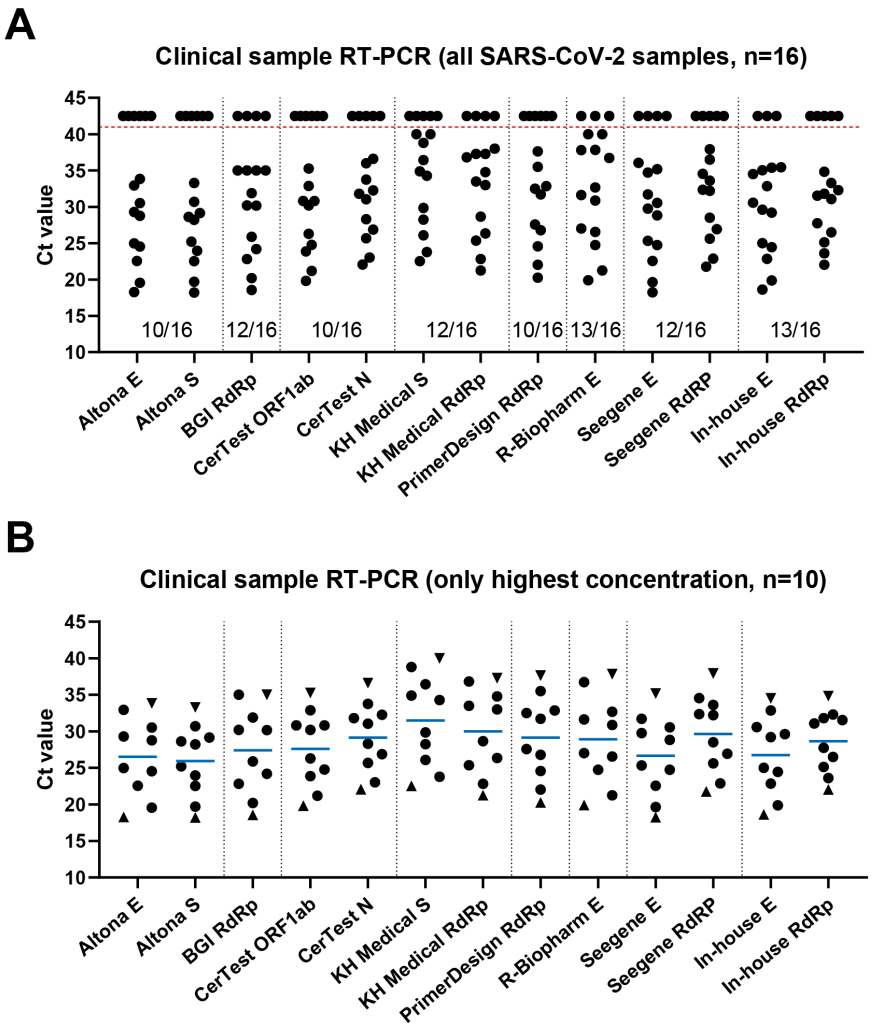

Figure 2. Different RT-PCR kits showed variations in detection rate and Ct values. RNA isolated from stored SARS-CoV-2-positive clinical samples using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche) was subjected to the various RT-PCR assays according to the manufacturer's instructions for use, on a LightCycler 480 II (Roche). **A)** Graph depicts Ct values obtained for all clinical samples (n=16) in all RT-PCR assays. Data points above the red dotted line are negative, for plotting purposes indicated with Ct 42.5. The detection rate of the complete RT-PCR kit is indicated below the data points, e.g. 10/16 means 10 out of 16 samples tested positive according to the instructions for data interpretation provided by the manufacturer. For both the CerTest and Seegene kits, one sample was "inconclusive" according to the manufacturer's guide for interpretation and was therefore counted as "negative", although a signal was observed for at least one target. **B)** Graph depicts only data for those clinical samples (n=10) with the highest concentration of SARS-CoV-2 RNA and which were positively identified by all RT-PCR assays. The blue line shows the mean Ct value for each assay, triangles show the Ct values of the samples with the highest (sample 1) and lowest (sample 10) concentration according to the in-house E-gene PCR. E, envelope protein of SARS-CoV-2; RdRp, RNA-dependent RNA polymerase of SARS-CoV-2; N, nucleocapsid protein of SARS-CoV-2; ORF1ab, open reading frame 1a and b of SARS-CoV-2, includes the RdRp; RNA-dependent RNA polymerase of SARS-CoV-2, part of ORF1ab; S, spike protein of SARS-CoV-2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

DISCUSSION

Here we provide a comparison of seven commercially available RT-PCR kits for the detection of SARS-CoV-2 in clinical samples. All RT-PCR kits performed satisfactorily regarding PCR efficiency (≥96%) and the estimated LOD95% varied within a 6-fold range between kits (3.8-23 copies/ml). Notably, the copy number concentration of the standard was determined by digital PCR on the positive sense RdRp gene and therefore provides an indication of the number of viral particles per ml. The actual copy number for each RT-PCR target and accompanying limit of detection may vary depending on, for example, the amount of subgenomic messenger RNA-containing cells that are present in the (clinical) sample.

From a selection of clinical samples with various concentrations of viral RNA, all RT-PCR kits were able to positively identify the ten samples with the highest concentrations of SARS-CoV-2 RNA (Ct≤34.5 in our in-house E-gene PCR). To provide an indication on clinical relevance of this finding: from our in-house diagnostic data on patients presenting with COVID-19 symptoms, it appears that from all individuals testing positive for our in-house E-gene PCR (n=416) the proportion of individuals with a Ct value >34.5 is approximately 3.6% (unpublished data). The R-Biopharm AG kit positively identified the highest number of clinical samples, i.e. 13 out of 16, comparable with our in-house PCR. Three kits were able to positively identify 12 out of 16 samples (BGI, KH Medical, Seegene). Notably, we performed our analysis using only a small number of clinical samples and we therefore advise that diagnostic laboratories in the field conduct additional and more extensive in-house clinical validations upon implementation of novel RT-PCR kits. Importantly, none of the assays showed cross-reactivity towards a panel of other respiratory (corona)viruses, except for the expected cross-reactivity with the SARS-CoV-1 E-gene. Since the latter virus is no longer known to be circulating in the human population, we consider this cross-reactivity acceptable.

Considering our findings, we believe that all of the commercially available RT-PCR kits included in this study can be used for routine diagnostics of symptomatic COVID-19 patients. When performing virus diagnostics in populations that may be expected to display low viral loads, such as health-care workers with mild or no symptoms or patients during later stages of the infection (6), it might be advisable to use those kits that performed best regarding the positive identification of clinical samples, i.e. RT-PCR kits from R-Biopharm AG, BGI, KH Medical, and Seegene.

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Table S1. Preliminary clinical sensitivity and specificity analysis including Ct values for seven commercial RT-PCR kits for detection of SARS-CoV-2.

		Altona Diagnostics		BGI	CerTest		KH Medical		PrimerDesign	R-Biopharm AG	Seegene			In-house		Result from initial routine diagnostics
		E	**S**	**RdRp**	**ORF1ab**	**N**	**RdRp**	**S**	**RdRp**	**E**	**E**	**RdRp**	**N[1]**	**E**	**RdRp**	**E**
1	SARS-CoV-2	18.26	18.20	18.56	19.81	22.05	22.54	21.25	20.26	19.91	18.23	21.76	NA	18.61	22.03	17.25
2	SARS-CoV-2	19.54	19.70	20.19	21.20	23.01	23.78	22.82	22.03	21.24	19.64	22.88	NA	19.86	23.62	18.98
3	SARS-CoV-2	22.56	22.52	22.82	23.86	25.69	26.08	25.35	24.56	24.74	22.55	25.62	NA	22.85	25.13	22.17
4	SARS-CoV-2	24.55	23.95	24.20	24.78	26.86	28.23	26.35	26.78	26.54	24.74	26.94	NA	24.45	26.48	24.04
5	SARS-CoV-2	24.99	25.20	25.87	26.30	28.29	29.86	28.65	27.58	27.02	25.34	28.52	NA	25.02	27.74	24.95
6	SARS-CoV-2	28.77	28.20	30.21	30.20	31.05	34.29	32.99	32.50	30.88	28.83	32.21	NA	29.22	31.81	28.84
7	SARS-CoV-2	29.30	28.63	30.18	30.81	32.28	34.90	33.49	31.72	31.64	29.77	32.35	NA	29.61	31.53	29.54
8	SARS-CoV-2	30.52	29.16	31.88	30.80	31.81	36.45	34.78	32.85	32.69	30.56	33.62	NA	30.57	31.07	29.70
9	SARS-CoV-2	32.93	30.70	35.00	32.89	33.75	38.79	36.82	35.50	36.74	31.71	34.54	NA	32.84	32.30	32.26
10	SARS-CoV-2	33.85	33.28	35.00	35.27	36.62	40.00	37.30	37.64	37.84	35.18	37.92	NA	34.50	34.83	33.50
11	SARS-CoV-2	neg	neg	35.00	neg	neg	neg	37.28	neg	40.00	34.72	neg	neg	35.04	33.29	33.10
12	SARS-CoV-2	neg	neg	neg	neg	neg	neg	neg	neg	40.00	36.07	neg	NA	35.46	neg	33.60
13	SARS-CoV-2	neg	neg	35.00	neg	36.00	40.00	38.00	neg	37.81	neg	36.50	NA	35.38	neg	34.00
14	SARS-CoV-2	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	34.20
15	SARS-CoV-2 + InfA	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	35.40 + 34.20
16	SARS-CoV-2	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	39.60
																Pathogen-specific
17	Influenza virus type A	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	26.82
18	Influenza virus type A	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	16.81
19	RSV A	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	29.24
20	RSV B	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	20.02
21	Rhinovirus	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	16.64
22	Rhinovirus	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	25.90
23	Negative control	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	-
24	Negative control	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	-
																Pathogen-specific
25	CoV OC43	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	31.67
26	CoV NL63	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	27.2
27	CoV SARS1	21.61[2]	neg	neg	neg	neg	neg	neg	neg	neg	20.72[2]	neg	NA	pos[2]	neg	21.6
28	CoV MERS	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	34.16
29	CoV 229E	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	NA	neg	neg	33.18

[1]The filter settings for the Seegene N-gene PCR were not correct and these results are therefore excluded.
[2]These assays are expected to cross-react with SARS-CoV-1 since they are designed to be specific for bat beta-coronaviruses.

Abbreviations: Ct, threshold cycle; E, envelope protein of SARS-CoV-2; InfA, Influenza virus type A; N, nucleocapsid protein of SARS-CoV-2; NA, not available; Neg, negative; ORF, open reading frame; Pos, positive; RdRp, RNA-dependent RNA-polymerase of SARS-CoV-2; RT-PCR, reverse-transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome virus 2; S, spike protein of SARS-CoV-2.

Table S2. Ct values obtained for the 2-fold dilution series of SARS-CoV-2 RNA.

Concentration (copies/ml)[1]	Altona Diagnostics								BGI				CerTest Biotec							
	E				S				RdRp				RdRp				N			
230	32.78	32.70	32.79	32.66	32.02	32.21	32.19	32.36	33.85	34.26	33.48	34.03	33.6	33.78	33.93	33.92	34.47	34.53	34.5	34.43
115	33.20	33.92	33.32	33.46	32.70	32.98	32.68	32.49	35	35	35	35	35.25	35.52	34.9	35.44	35.19	35.72	36.15	35.83
57.5	34.03	34.18	33.98	34.20	33.52	33.48	33.16	33.47	35	35	35	35	35.86	36.65	36.57	35.55	35.96	36.01	36.48	36.04
28.8	34.71	34.63	35.11	34.12	33.74	33.88	34.54	33.58	35	35	35	35	37.25	37.46	37.12	36.53	36.34	36.41	37.91	36.56
14.4	35.02	34.91	34.70	34.94	33.97	34.45	33.76	34.05	35	35	35	35	37.23	38.03	36.65	neg	37.68	36.6	37.73	37.91
7.19	34.77	35.51	35.24	34.63	34.98	34.43	34.10	33.74	35	35	35	35	neg	neg	37.29	38.85	38.79	37.81	37.87	37.71
3.59	35.59	35.02	35.47	35.60	34.03	34.36	34.30	34.44	35	35	neg	35	neg	neg	neg	neg	neg	neg	40	37.83
1.80	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

	KH Medical								PrimerDesign				R-Biopharm AG			
	S				RdRp				RdRp				E			
230	36.29	36.13	36.16	35.97	35.63	35.85	36.08	35.76	35.05	34.64	35.02	35.03	34.48	35.03	34.59	34.76
115	37.00	36.79	36.99	36.81	36.63	36.55	36.89	36.45	36.2	35.32	35.48	35.94	35.76	35.82	35.73	35.84
57.5	38.86	37.89	38.37	39.29	37.86	37.97	38.31	37.84	36.49	36.1	37.27	39.3	38.41	36.85	36.87	36.43
28.8	38.37	40.00	40.00	39.22	39.04	38.15	38.27	40.00	40	39.36	36.43	36.83	40.00	37.87	37.24	37.85
14.4	40.00	38.36	40.00	40.00	39.21	38.32	38.86	40.00	neg	neg	39.06	37.22	40.00	40.00	38.84	40.00
7.19	40.00	40.00	38.41	40.00	40.00	40.00	38.48	40.00	neg	neg	neg	39.26	37.82	40.00	39.01	40.00
3.59	40.00	neg	40.00	40.00	40.00	40.00	neg	neg	neg	neg	neg	neg	40.00	40.00	neg	40.00
1.80	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

	Seegene								In-house							
	E				RdRp				E				RdRp			
230	33.09	32.97	33.01	32.63	36.97	36.45	36.64	36.26	32.30	32.83	32.64	32.66	30.01	30.09	30.20	30.17
115	33.54	33.98	33.61	33.99	37.93	38.17	37.24	37.70	33.19	33.47	33.54	33.53	31.53	31.87	31.55	32.01
57.5	34.78	35.09	34.10	34.89	37.32	38.70	40.00	37.15	34.04	34.21	34.46	34.43	32.59	32.86	32.49	32.79
28.8	36.26	35.31	35.64	35.22	38.95	40.00	40.00	39.30	35.04	34.85	34.94	35.65	32.86	33.15	33.28	33.20
14.4	36.59	36.05	36.62	35.90	neg	40.00	40.00	40.00	36.90	35.45	34.62	35.26	33.85	34.14	33.62	33.73
7.19	37.01	36.89	37.74	37.73	neg	40.00	neg	40.00	34.92	35.86	35.53	36.14	33.78	34.25	34.06	34.10
3.59	neg	36.65	36.15	neg	neg	neg	neg	neg	35.33	36.90	35.71	33.80	34.56	33.98	34.48	35.22
1.80	neg	neg	neg	neg	neg	neg	neg	neg	33.97	34.64	33.99	35.17	neg	34.21	34.45	neg
0.898									34.65	34.74	35.56	34.57	neg	34.57	neg	neg
0.449									neg	34.67	neg	neg	34.95	neg	neg	neg
0.225									neg	34.59	34.92	neg	neg	neg	neg	34.59
0.112									neg	neg	neg	neg	neg	neg	neg	neg
0.056									neg	neg	neg	neg	neg	neg	neg	neg
0.028									neg	neg	neg	neg	neg	neg	neg	neg

[1]The copy number was determined by digital PCR for the positive sense RdRp gene.