Materials and Method

Preparation SARS-CoV-2 dilution

SARS-CoV-2 stock hCoV-19/Netherlands/NoordBrabant_10003/2020; 5.62*10^7 TCID50/ml; 1.73*10^10 digitale kopieën RdRp-gen positief strengs RNA/ml; 1.28*10^10 digitale kopieën E-gen positief strengs RNA/ml is diluted in a 10-fold series (10^{-1} to 10^{-8}) in viral transport medium GLY (Mediaproducts B.V., Groningen, The Netherlands) with an end volume of 9 ml. The 10-fold series are vortex for 1 minute at room temperature.

Evaluation protocol RIVM

From each dilution 350 µl is added to the 1 ml pre-filled tube with LIAISON® SARS-CoV-2 Sample Inactivation Buffer (n=3). After incubating for at least 2 hours the procedure is follow as described in the prescription supplied by the manufacture.

From each dilution 350 µl is added to the inactivation buffer of the Roche SARS-CoV-2 rapid antigen test (Hoffmann-La Roche, Basel, Switzerland)(n=3). After adding the SARS-CoV-2 the procedure is follow as described in the prescription supplied by the manufacture.

Three nasopharynx swabs (FLOQswab 503CS01, COPAN Italia S.p.A., Brescia, Italy) are dipped into each SARS-CoV-2 dilution. After dipping into the 15 ml tube (Greiner Bio-One Laboratories, Kremsmünster, Austria) make sure that there is no drop hanging of the nasopharynx swab. The nasopharynx swabs are Place and soak the swab into the inactivation buffer of the Roche SARS-CoV-2 rapid antigen test. Roll/rotate the swab at least 5 times. After adding the nasopharynx swabs the procedure is follow as described in the prescription supplied by the manufacture.

To quantify the Ct-value of the dilution series, from each dilution 200 µl was mixed with 275 µl lysis buffer which includes 25 µl Equine Arteritis Virus (EAV) and 450 µl was extracted on a MagNA Pure 96 Instrument (Hoffmann-La Roche, Basel, Switzerland) using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Hoffmann-La Roche, Basel, Switzerland) and eluted in a volume of 50 µl. The E-gene/EAV multiplex PCR was used to test inhibition of EAV amplification. E-gene primers and probes were as described by Corman et al (1); RdRp-gene primers and probes have been modified form the original Corman primers and probe conferring the primers SARS-CoV-2 specific and the analytical sensitivity comparable to that of the E-gen RT-PCR. Modified primers and probe: RdRp_SARS-F2 GTGAAATGGTCATGTGTGGCGG; RdRp_SARS-R2 CAAATGTTAAAAACACTATTAGCATAAGCA; RdRp_SARS-P2.2 CCAGGTGGAACCTCATCAGGAGATGC; EAV as described by Scheltinga et al (2). Reaction condition of Corman et al are described in Table 1 and 2 . All tests are run on the Light Cycler 480 I (LC480) (Hoffmann-La Roche, Basel, Switzerland) and performed according to the manufacturer's instruction.

Evaluation protocol ETZ

From each dilution 1 ml is added to the 1 ml pre-filled tube with LIAISON® SARS-CoV-2 Sample Inactivation Buffer (n=3). After incubating for at least 2 hours the procedure is follow as described in the prescription supplied by the manufacture.

Three nasopharynx swabs (FLOQswab 503CS01, COPAN Italia S.p.A., Brescia, Italy) are dipped into each SARS-CoV-2 dilution. After dipping into the 15 ml tube (Greiner Bio-One Laboratories, Kremsmünster, Austria) make sure that there is no drop hanging of the nasopharynx swab. The nasopharynx swabs are Place and soak the swab into the pre-filled tube with LIAISON® SARS-CoV-2

Sample Inactivation Buffer. Roll/rotate the swab at least 5 times. After incubating for at least 2 hours the procedure is follow as described in the prescription supplied by the manufacture.

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
TCID50/ml	5.62E+06	5.62E+05	5.62E+04	5.62E+03	5.62E+02	5.62E+01	5.62E+00	5.62E-01
RdRp-gene Copies/ml	1.73E+09	1.73E+08	1.73E+07	1.73E+06	1.73E+05	1.73E+04	1.73E+03	1.73E+02
Ct-value RdRp-**gene** qRT-PCR **RIVM**[1]	12.78	16.82	20.81	24.15	27.97	31.60	35.32	36.74
Ct-value RdRp-**gene** qRT-PCR ETZ[2]	10.23	13.17	16.75	20.20	24.33	28.02	31.21	35.31
1	(3/3)	(3/3)	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)
2	(3/3)	(3/3)	(3/3)	(1/3)[3]	(0/3)	(0/3)	(0/3)	(0/3)
3	(3/3)	(3/3)	(3/3)	(3/3)	(1/3)[4]	(0/3)	(0/3)	(0/3)
	>100000	>100000	10753-12422	1524-1700	173-232	54-67	47-58	39-45
4	(3/3)	(3/3)	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)
	>100000	>100000	20926-22301	2319-2614	297-352	64-82	31-40	34-40
5	(3/3)	(3/3)	(3/3)	(3/3)	(0/3)	(0/3)	(0/3)	(0/3)
	>100000	14436-16112	2247-2437	328-272	86-89	53-62	52-60	56-65

To quantify the Ct-value of the dilution, from each dilution three times 500 µl is heat inactivated for 2 hours at 60°C. from each sample 400 µl is used to extracted on a Abbott ALINITY M system (Abbott Laboratories, Chicago, United States of America). The Alinity m SARS-CoV-2 Assay (Abbott Laboratories, Chicago, United States of America) is used to determine the Ct-value of the samples.

Clinical field evaluation

To compare the Ct-values from different SARS-CoV-2 molecular platforms, each platform needs to test the sensitivity panel in four-fold that is provided by the RIVM. The Abbott Alinity m system and The Alinity m SARS-CoV-2 Assay are used to compare the sensitivity of LIAISON® SARS-CoV-2 Ag assay.

***Table 1**. For the SARS-CoV-2 the primers and probes obtained from Biolegio were premixed at a final concentration of 10 µM primers and 5 µM probes.*

E-gene/EAV qRT PCR	µl	RdRp-gene qRT-PCR	µl
4x Taqman Fast Virus MM	5	**4x Taqman Fast Virus MM**	5
E+EAV PP Mix	3	**RdRp PP Mix**	3
PCR grade water	7	**PCR grade water**	7
Specimen nucleic acid	5	**Specimen nucleic acid**	5
Total volume	20	**Total volume**	20

***Table 2** . Table 2. Amplification temperature protocol for SARS-CoV-2 E-gene and RdRp-gene target with LC480 mark I.*

PCR Program	Segment number	Temp Target (°C)	Hold Time (sec.)	Slope (°C/sec.)	Acquisition mode
Reverse Transcription	**1**	**50**	**900**	**EXTERNAL***	
Denaturation/Inactivation	**1**	**95**	**120**	**EXTERNAL***	
Denaturation	1	95	60	4.4	None

LC 480

Table 4. *Results of the diluted SARS-CoV-2 stock read out by Q-test (Roche) and* LIAISON, *The dilution is done in triplicate in each SARS-CoV-2 Ag assay. The table shows how often the SARS-CoV-2 Rapid-Ag test has become positive.*

[1] *qRT-PCR is done in triplicate, ct-value is an average.*
[2] *qRT-PCR is done in duplex, ct-value is an average.*
[3] *This SARS-CoV-2 Rapid-Ag test has a weak signal.*
[4] *LIAISON® SARS-CoV-2 Ag assay threshold is 200 RFU. two of the three samples were just below 200 RFU.*

References

1. Corman VM, Landt O, Kaiser M, Molenkamp R, 5.1.2e, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, 5.1.2e, Reusken C, Koopmans MP, Drosten C. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3):2000045.
2. S.A. Scheltinga, K.E. Templeton, M.F.C. Beersma, E.C.J. Claas Diagnosis of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR. Journal of Clinical Virology 33 (2005), 2004 Aug; 17: 306-311.