Expected results specificity- and sensitivitypanel SARS-CoV-2

Table 1. Contents of specificity panel and Ct values in a Real-Time PCR using Fast-Virus Mastermix (Thermo Fisher) after extraction of 200 µl on a MagNA Pure 96 with total nucleic acid kit small volume (Roche), elution in 50 µl and 5 µl extraction per reaction. The SARS-CoV-2 PCRs are performed in triplicate; after the Ct value, the amount of positive results obtained is given between brackets.

Panel coding	Virus[1]	Target specific Ct[2]	E-gene Ct	RdRP gene Sarbeco probe Ct	RdRP-gene SARS-CoV-2 probe Ct
EQA_CoV20-01	CoV-OC43	30.79	Neg	Neg	Neg
EQA_CoV20-02	No virus	Neg	Neg	Neg	Neg
EQA_CoV20-03	SARS-CoV-2 (d1)	NA	28.18 (2)	30.98 (2)	29.91 (2)
EQA_CoV20-04[3]	SARS-CoV-2 (d3)	NA	34.87 (1)	Neg	33.59 (2)
EQA_CoV20-05	Influenzavirus A(H3N2)	21.84	Neg	Neg	Neg
EQA_CoV20-06	SARS-CoV-2 (d2)	NA	33.14 (2)	Neg	33.19 (2)
EQA_CoV20-07	CoV-229E	27.72	Neg	Neg	Neg
EQA_CoV20-08	Influenzavirus B-Victoria	28.84	Neg	Neg	Neg
EQA_CoV20-09	CoV-NL63	24.09	Neg	Neg	Neg
EQA_CoV20-10	Rhinovirus A16	23.40	Neg	Neg	Neg

[1] d1, d2 and d3 show that d2 is a 1:100 dilution of d1 and that d3 is a 1:10 dilution of d2. SARS-CoV-2 is heat inactivated. d1, d2 and d3 are comparable with Sen. Serie-02, -07 and -01 in concentrationfrom sensitivity panel #2

[2] For influenzavirus A(H3N2) matrix gene and for influenzavirus B/Victoria hemagglutinine gene; NA = not applicable.

[3] Provisory indication: Educative sample. If the sample is tested multiple times, the E-gene and RdRP-gene probes can show a negative result for some of the tests.

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

Panel coding	Dilution runcontrol	Amount of copies RdRP targets is established using dPCR/ml monster[1]	Ct, average (amount of positive tests)	
			E-gene (Sarbeco)	RdRP-gene (SARS-CoV-2)[2]
Sen. Serie-04	10-4	8.26*10^4	24.68 (3)	25.03 (3)
Sen. Serie-02	10-5	8.26*10^3	28.26 (3)	28.71 (3)
Sen. Serie-06	10-6	8.26*10^2	30.82 (3)	31.43 (3)
Sen. Serie-07	10-7	8.26*10^1	33.11 (3)	34.69 (3)
Sen. Serie-01[3]	10-8	8.26	35.16 (1)	37.38 (3)
Sen. Serie-05	10-9	8.26*10^-1	Neg	Neg
Sen. Serie-03	10-10	8.26*10^-2	Neg	Neg

[1] dPCR is performed on positive sense genomic RNA; the dPCR is also capable of detecting negative sense genomic RNA. Besides detecting negative sense genomic RNA, the E-gene PCR picks up subgenomic messengers as well, likely making the number of target templates for the diagnostic PCR in the samples higher

[2] Modified primers and probe making the PCR and the probe specific to SARS-COV-2 with analytical sensitivity comparable to E-gen RT-PCR: RdRp_SARS-F2 GTGAAATGGTCATGTGTGGCGG; RdRp_SARS-R2 CAAATGTTAAAAACACTATTAGCATAAGCA; RdRp_SARS-P2.2 CCAGGTGGAACCTCATCAGGAGATGC (many thanks to [redacted], JBZ, for sharing her ideas on this). These primers can no longer be combined with the Corman et al. Sarbeco RdRP probe to make a Sarbeco-specific RT-PCR.

[3] Provisory indication: Educative sample. After we have received more results from laboratory testing this panel, a definitive status can be given to this sample.