Expected results specificity and sensitivity panels SARS-CoV-2

Note The primers and probe of the RdRP-gene SARS-CoV-2 RT-PCR have been modified form the original 5.1.2e 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 (many thanks to 5.1.2e, JBZ, for sharing her ideas on this). These primers can no longer be combined with the 5.1.2e et al. Sarbeco RdRP probe for a Sarbeco-specific RT-PCR.

Table 1. Contents of specificity panel #6 and Ct values in real-time RT-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 PCRs were performed in fourfold; after the Ct value the number of positive results obtained is given between brackets.

Panel coding	Virus[1]	Target specific Ct[2]	E-gene Ct	RdRP-gene SARS-CoV-2 probe Ct
EQA_CoV20-01	CoV-NL63	28.10 (4)	Neg	Neg
EQA_CoV20-02	CoV-229E	17.22 (4)	Neg	Neg
EQA_CoV20-03[3]	SARS-CoV-2 (d3)	NA	36.95 (2)	35.59 (2)
EQA_CoV20-04	SARS-CoV-2 (d2)	NA	34.80 (4)	34.88 (4)
EQA_CoV20-05	CoV-OC43	27.77 (4)	Neg	Neg
EQA_CoV20-06	SARS-CoV-2 (d2)	NA	34.68 (4)	34.74 (4)
EQA_CoV20-07	Influenza virus A(H3N2)	22.76 (4)	Neg	Neg
EQA_CoV20-08	No virus	Neg	Neg	Neg
EQA_CoV20-09	SARS-CoV-1	NA	28.57 (4)	Neg
EQA_CoV20-10	SARS-CoV-2 (d1)	NA	28.52 (4)	28.37 (4)

[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. d1, d2 and d3 are comparable in viral load with Sen.Series3-01, -04 and -03 respectively of sensitivity panel #3. SARS-CoV-2 is heat inactivated; SARS-CoV-1 is RNA stabilized with yeast tRNA.

[2] For influenza virus A(H3N2) matrix gene; NA = not applicable.

[3] Provisional indication: Educational sample. If the sample is tested multiple times the E-gene and RdRP-gene RT-PCR assays can show a negative result for some of the replicates.

Table 2. Composition of sensitivity panel #3, comprised of a dilution series of inactivated SARS-CoV-2 stock, and results with Ct values in real-time RT-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 prior to panel distribution. The SARS-CoV-2 PCRs are performed in fourfold; after the Ct value the number of positive results obtained is given between brackets.

Panel coding	Dilution stock virus	Number of dPCR copies RdRP target per ml panel specimen[1]	Ct, average (number of positive tests)	
			E-gene (Sarbeco)	RdRP-gene (SARS-CoV-2)
Sen.Series3-07	10-4	4.56*10^5	25.82 (4)	25.69 (4)
Sen.Series3-01	10-5	4.56*10^4	29.28 (4)	29.32 (4)
Sen.Series3-05	10-6	4.56*10^3	33.21 (4)	34.31 (4)
Sen.Series3-04	10-7	4.56*10^2	34.21 (4)	36.10 (4)
Sen.Series3-03[2]	10-8	4.56*10^1	Neg	37.31 (3)
Sen.Series3-02	10-9	4.56*10^0	Neg	Neg
Sen.Series3-06	10-10	4.56*10^-1	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] Provisional indication: Educational sample. After we have received more results from laboratory testing of this panel a definitive status can be given to this sample.