National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Cepheid, Xpert® Xpress SARS-CoV-2

Multicentre evaluation using specificity and sensitivity panels and stored SARS-CoV-2 positive clinical specimens

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Summary

Background Routinely used real-time RT-PCR for SARS-CoV-2 detection is highly specific and sensitive and can be performed in high throughput setting but requires up to 6 hours to obtain results and a specialised laboratory to perform. When a more rapid result is needed, molecular point of care tests (mPOCT) can be an alternative. Here we report the evaluation of one of the first SARS-CoV-2 mPOCT on the market, the Cepheid GeneXpert Xpert® Xpress SARS-CoV-2.
Methods The Xpert® Xpress SARS-CoV-2 assay was evaluated against the routine in-house real-time RT-PCR assays in three medical microbiology laboratories in The Netherlands. Analytical evaluation of specificity and sensitivity was done using quality assessment panels containing specimens with different viral loads of SARS-CoV-2 and other seasonal respiratory viruses. For clinical evaluation, respiratory tract clinical specimens with different SARS-CoV-2 loads (n=58) or SARS-CoV-2 negative (n=30) from patients with symptoms of acute respiratory infection were selected from the routine diagnostic repositories in the labs.
Results The Xpert® Xpress SARS-CoV-2 assay performed at least equally well compared to the routinely used in-house tests. The LOD was about 8.26 digital copies of positive strand genomic SARS-CoV-2 RNA/ml. Selected seasonal human coronaviruses and other respiratory viruses were not detected. In clinical evaluation full concordant results were obtained with the Xpert® Xpress SARS-CoV-2 assay with routine RT-PCR high and low viral load and negative specimens.
Conclusion The Cepheid GeneXpert Xpert® Xpress SARS-CoV-2 assay has at least equal performance with routine diagnostic real-time RT-PCR assays in three medical microbiology laboratories in the Netherlands. This mPOCT is therefore a valuable addition in the field of COVID-19 diagnostic assays for use in high and low resource settings where a rapid and highly accurate result is critical.

Keywords: SARS-CoV-2, molecular POCT, evaluation, GeneXpert

Updates
27-04-2020: matrix clinical specimens added

1 Background

Rapidly after the discovery of SARS-CoV-2 as the cause of severe respiratory disease in China, now called COVID-19, the first whole genome sequence was publicly shared and real time RT-PCR assays were developed and validated. On March 12, 2020, COVID-19 was declared a pandemic by the World Health Organization.[1] In the Netherlands the COVID-19 expert centre formed by the National Institute for Public Health and the Environment (RIVM) and the Erasmus Medical Centre (Erasmus MC), collaborated in the validation of the real-time RT-PCR assays developed at Charité - Universitätsmedizin Berlin.[2] The E-gene and RdRP-gene RT-PCR assays were used at the expert centre and subsequently rolled out in the existing network of 13 outbreak assistance laboratories across the Netherlands.[3] As a second step, additional laboratories were supplied to create excess testing capacity to cope with increased demands for molecular diagnostics in the progression of the pandemic. Although high throughput real-time RT-PCR is highly useful for testing many patients it still takes up to six hours to obtain a result. Point of care tests by which a result is obtained in far less time, have the potential taking appropriate action more rapidly in situations where timely triage is critical. Antigenic rapid tests take only about 15-30 minutes to obtain a result, however these tests lack sensitivity for individual use and WHO does not currently recommend the use of COVID-19 antigen-detecting rapid diagnostic tests for patient care.[4] Molecular point of care tests can be performed outside the laboratory and reduce the time to result by a couple of hours to less than an hour but with the same performance as real-time RT-PCR in the laboratory.[5.6] In this report we present the results of the multicentre evaluation of one of the first molecular point of care tests on the market in the United States, the Cepheid GeneXpert Xpert® Xpress SARS-CoV-2 assay.[7] The assay is expected to obtain the CE-IVD status shortly.

2 Methods

2.1 Preparation panels

For preparing specificity and sensitivity quality assessment panels for SARS-CoV-2, at RIVM the virus (hCoV-19/Netherlands/Noord_Brabant_0117R/2020) was first isolated from a throat swab specimen from an RT-PCR positive patient. One hundred µl was inoculated on VERO-6 cells monolayer in MEM with Hanks' salts with 10% FCS and 100 units penicillin and streptomycin/ml by 37°C for 7 days after which CPE was >90%. A next passage was done after a freeze/thaw cycle on VERO-6 cells monolayer at 35°C for 4 days until >90% CPE to create a stock volume for further use. After a freeze/thaw cycle at -80°C, the supernatant including cell remnants was homogenized, aliquoted and frozen at -80°C. The stock virus was titrated on VERO-6 cells and found to contain 5.62*10^7 TCID50/ml infectious virus. The virus was inactivated by incubation for 2 hours at 60°C. Inactivation was assessed by virus isolation for 7 days. No virus growth was detected by CPE and RT-PCR. The number of RdRP gene copies in the inactivated virus stock was determined by digital RT-PCR using the reverse RdRP primer for cDNA synthesis and subsequent PCR using the RT-PCR with RdRP primers and SARS-CoV-2 specific probe. The stock contains 8.26*10^8 copies RdRP positive strand RNA/ml.

A specificity panel was created using dilution series of the inactivated SARS-CoV-2 stock and live virus isolates, from Dutch patients obtained in routine diagnostic setting, of influenza virus A(H3N2) and B/Victoria, CoV-OC43 (betacornavirus), CoV-NL63 and CoV-229E (both alphacoronavirus) and Rhinovirus A16. One specimen was included containing no virus. Dilutions were created in MEM with Hanks' salts and HEp2 cells were added at a concentration of 10,000 cells per ml panel specimen. Details of the panel are listed in Table 1.

A sensitivity panel was created by 10-fold diluting the inactivated SARS-CoV-2 stock in MEM with Hanks' salts from 10-4 – 10-10. Per dilution HEp2 cells were added at a concentration of 10,000 cells per ml. Details of the panel are listed in Table 2.

Table 1. Specificity panel composition. Cq values for seasonal viruses were determined using real-time RT-PCR with Fast-Virus Mastermix after MagNApure 96 RNA extraction with the total nucleic acid kit small volume.

Panel coding	Virus	Target specific Cq or dPCR SARS-CoV-2 RdRP gene copies/ml
EQA_CoV20-01	Influenza virus A(H3N2)	21.64
EQA_CoV20-02	SARS-CoV-2 (d2)	8.26*10^1 copies/ml
EQA_CoV20-03	CoV-OC43	27.30
EQA_CoV20-04[3]	SARS-CoV-2 (d3)	8.26 copies/ml
EQA_CoV20-05	Rhinovirus A16	25.49
EQA_CoV20-06	CoV-229E	32.60
EQA_CoV20-07	No virus	Neg
EQA_CoV20-08	CoV-NL63	28.84
EQA_CoV20-09	Influenza virus B-Victoria	28.32
EQA_CoV20-10	SARS-CoV-2 (d1)	8.26*10^3 copies/ml

[2] Cq values were for influenza virus A(H3N2) from matrix gene RT-PCR and for influenza virus B/Victoria from hemagglutinin gene RT-PCR.
[3] Preliminary rated as educational specimen; meaning there is reasonable doubt that this viral load is detected by all RT-PCR based assays.

Table 2. Sensitivity panel composition.

Panel coding	Dilution factor of stock virus	Number of dPCR copies of SARS-CoV-2 RdRP gene per ml
Sen. Serie-01	10-6	8.26*10^2
Sen. Serie-02[1]	10-10	8.26*10^-2
Sen. Serie-03	10-4	8.26*10^4
Sen. Serie-04[2]	10-8	8.26
Sen. Serie-05	10-7	8.26*10^1
Sen. Serie-06[1]	10-9	8.26*10^-1
Sen. Serie-07	10-5	8.26*10^3

[1] This viral load is highly likely not detected by RT-PCR.
[2] Preliminary rated as educational specimen; meaning there is reasonable doubt that this viral load is detected by all RT-PCR based assays.

2.2 Selection clinical specimens

Clinical specimen were selected from the repository of routinely processed nose (nasopharyngeal or mid-turbinate) and throat (oropharyngeal) swabs collected in virus transport medium (lab 1 GLY, Media Products, Groningen, The Netherlands or labs 2 and 3 UTM, Copan, Brescia, Italy) from patients with acute respiratory infection at each of the three laboratories. Three sets were selected based on the observation that the E-gene RT-PCR is most sensitive: 1) 10 specimens positive for E-gene and RdRP-gene RT-PCR with range of E-gene Cq values; 2) 8-10 specimens positive for E-gene RT-PCR only (low viral load) with range of E-gene Cq values; 3) 10 specimens negative for E-gene and RdRP-gene RT-PCR.

2.3 Testing

Both panels were distributed blinded. The panels were shipped frozen on dry ice to ensure the same number of freeze thaw cycles for all three laboratories. At all three laboratories the panel specimens were processed as clinical specimens in the normal diagnostic procedure using the locally implemented E-gene (Sarbeco specific) and RdRP-gene (Sarbeco specific primers with SARS-CoV-2 specific probe) RT-PCR assays as described by 5.1.2e et al. [2] and routine platforms, lab 1 Roche MagNApure with MagNApure 96 DNA and Viral NA Small Volume kit extraction followed by in-house PCR on Roche LC480 II using Life Technologies Taqman FastVirus 1-step mastermix, lab 2 Roche COBAS4800 with CT/GT kit extraction followed by in-house PCR on Roche LC480 II using Roche LightCycler Multiplex RNA Virus Master and later on moved to E-gene assay only, and lab 3 BioMérieux NucliSens easyMAG with easyMAG extraction reagents followed by in-house PCR on Thermo Fisher QuantStudio 6 using Life Technologies Taqman FastVirus 1-step mastermix. Later on lab 3 replaced the RdRP-gene assay by the CDC N1-gene SARS-CoV-2 specific test primers and probes [8]. The SARS-CoV-2 positive and negative specimens were selected from biobanks of previously tested specimens at the three laboratories using these assays. Panels and clinical specimens were tested on Cepheid GeneXpert systems using the GeneXpert Xpert® Xpress SARS-CoV-2 assay. The GeneXpert Xpert® Xpress SARS-CoV-2 assay 'For Use Under an Emergency Use Authorization (EUA) Only' was bought from Cepheid Europe for pre-CE-IVD release evaluation, allowing other laboratories that want to implement the assay to just perform a simple few cartridges consuming entrance quality verification to fulfil ISO 18159 requirements once the assay is released on the European market. The GeneXpert Xpert® Xpress SARS-CoV-2 assay targets two genes, the E-gene (Sarbeco specific) and N2-gene (SARS-CoV-2 specific).

3 Results and discussion

3.1 Specificity

None of the assays used by the three labs, Xpert® Xpress SARS-CoV-2 included, were positive with the included seasonal respiratory viruses. At all three labs, all three SARS-CoV-2 containing specimens were positive in the routine in-house assays (Figure 1). Except for one specimen that was N2-gene positive only at lab 3, all three specimens were positive for both targets in the Xpert® Xpress SARS-CoV-2 assay at all three labs (Figure 1). The N2-gene only positive specimen was retested with Xpert® Xpress SARS-CoV-2 at lab 3 and then found positive for both targets with E-gene at Cq 39.8 and N2-gene at Cq 42.2.

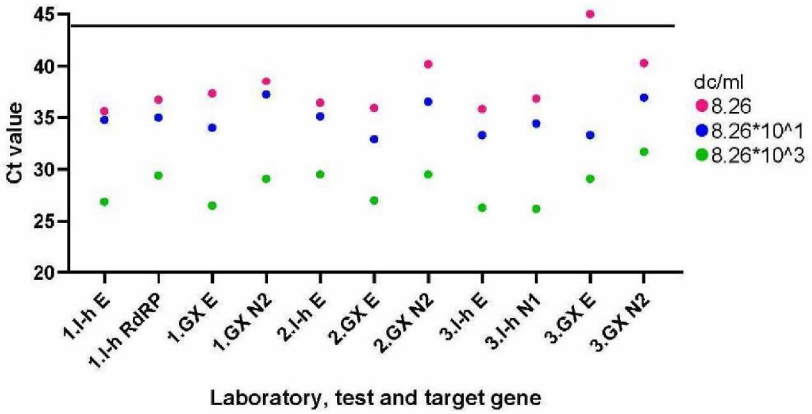

Figure 1. Detection of SARS-CoV-2 RNA in specificity panel by GeneXpert Xpert® Xpress SARS-CoV-2 assay as compared to in-house assay in a three-centre study. Labs indicated with 1, 2 and 3; I-h = In-house RT-PCR with target gene indicated; GX = GeneXpert Xpert® Xpress SARS-CoV-2; negative results indicated with Cq value 45; dc/ml = digital copies genomic RNA per ml. 3.GX E 8.26 dc/ml was positive after repeat testing with Cq 39.8.

3.2 Sensitivity

Despite that for one lab the in-house assay dropped off at the 10-8 dilution in the sensitivity panel, the 10-8 dilution in this panel was detected by the in-house assay of the other two labs and the Xpert® Xpress SARS-CoV-2 assay in all three labs. For lab 3, the 10-8 dilution was detected in the specificity panel (Figure 1), suggesting this dilution which has been prepared separately for both panels from the same virus stock is close to the LOD of the in-house assay of lab 3, and the Xpert® Xpress SARS-CoV-2 as well as outlined below. The 10-9 dilution was negative using the in-house assays, but positive for one target (E-gene or N2-gene) in the Xpert® Xpress SARS-CoV-2 assay in each of the three labs (Figure 2). As the Xpert® Xpress SARS-CoV-2 kit insert

considers E-gene only positive specimens 'SARS-CoV-2 presumptive positive' and requests retesting [7], all three labs retested this specimen in the Xpert® Xpress SARS-CoV-2 assay; at all three labs with negative result for both target genes. This clearly indicates this viral load is well below the LOD of the Xpert® Xpress SARS-CoV-2 assay.

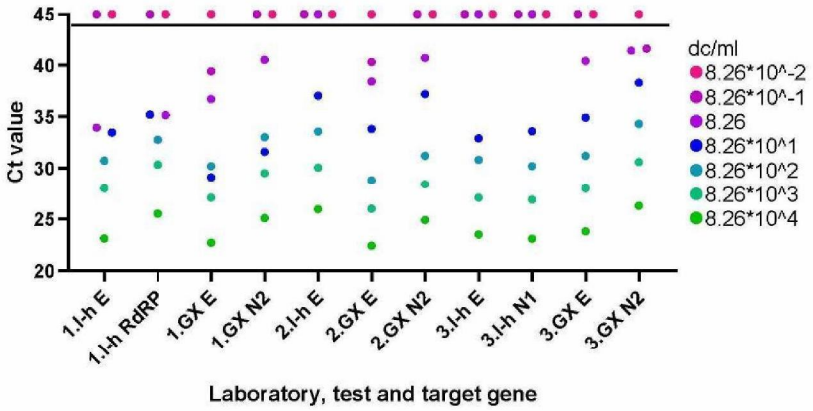

Figure 2. Detection of SARS-CoV-2 RNA in the sensitivity panel by GeneXpert Xpert® Xpress SARS-CoV-2 assay as compared to in-house assay in a three-centre study. Labs indicated with 1, 2 and 3; I-h = In-house RT-PCR with target gene indicated; GX = GeneXpert Xpert® Xpress SARS-CoV-2; negative results indicated with Cq value 45; dc/ml = digital copies genomic RNA per ml. 1.GX N2, 2.GX N2 and 3.GX E 8.26*10^-1 were negative after repeat testing.

3.3 Clinical specimens

At all three labs the 10 clinical specimens previously identified as SARS-CoV-2 negative were also negative in the Xpert® Xpress SARS-CoV-2 assay (not shown). Seven of the SARS-CoV-2 negative specimens selected by lab 1 were positive with considerable viral load for influenza virus A(H3N2), A(H1N1)pdm09 or B/Victoria, RSV-A, RSV-B, rhinovirus or enterovirus D68 and thereby providing additional information about the specificity of the Xpert® Xpress SARS-CoV-2 assay.

The 10 specimens with higher viral load as indicated by positivity in the in-house RT-PCR assay for the E-gene and the lesser sensitive RdRP-gene or the N1-gene RT-PCR assay were all positive for both targets in the Xpert® Xpress SARS-CoV-2 assay (Figure 3).

For the 8-10 low viral load clinical specimens selected at each laboratory, the results were a little bit divers (Figure 4). Specimens selected by lab 1 had lower viral load than those selected by the other two laboratories, likely because of the slight difference in sensitivity of the in-house assays (Figure 2). This might partly explain the following observation. The low viral load specimens selected by laboratories 2 and 3 were all positive for both target genes in the Xpert® Xpress SARS-CoV-2 assay. For the eight selected specimens by lab 1, two specimens were only positive for one of the target genes in the Xpert® Xpress SARS-CoV-2 assay; one in the E-gene assay only (by Cepheid

considered SARS-CoV-2 presumptive positive) and one in the N2-gene assay only (by Cepheid considered SARS-CoV-2 positive) (Figure 4).

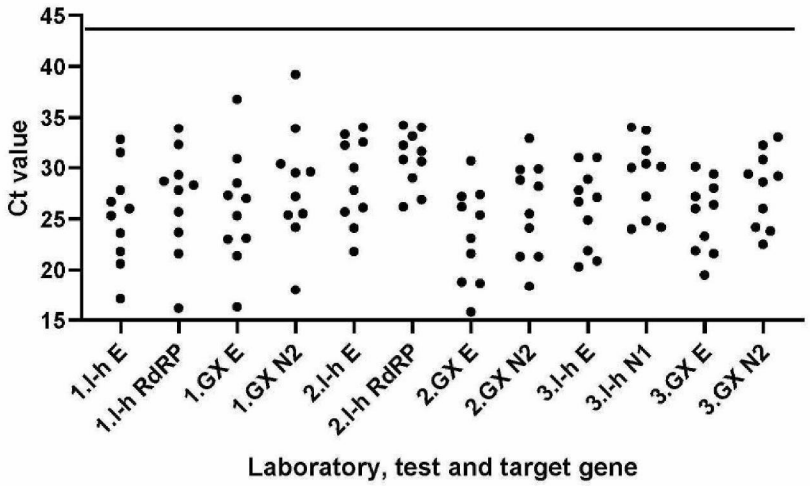

Figure 3. Detection of SARS-CoV-2 RNA in 30 relatively high viral load specimens, 10 selected at each participating lab, by GeneXpert Xpert® Xpress SARS-CoV-2 assay as compared to in-house assay in a three-centre study. Labs indicated with 1, 2 and 3; I-h = In-house RT-PCR with target gene indicated; GX = GeneXpert Xpert® Xpress SARS-CoV-2; negative results indicated with Cq value 45.

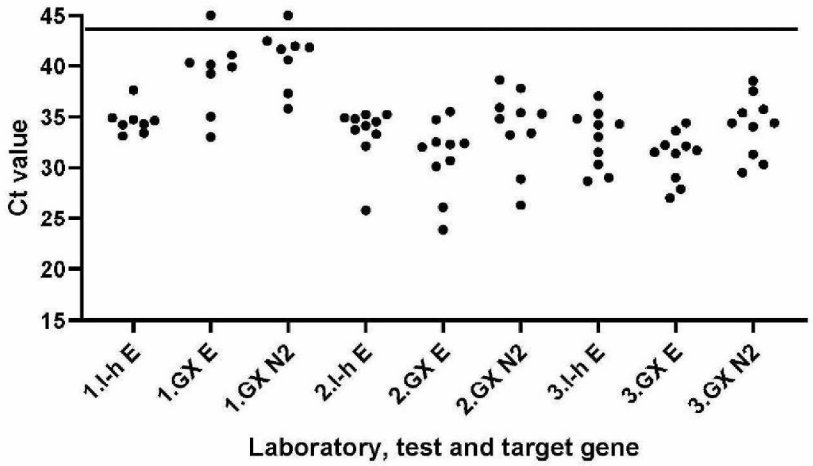

Figure 4. Detection of SARS-CoV-2 RNA in 33 relatively low viral load specimens, eight selected at lab 1 and 10 selected at labs 2 and 3, by GeneXpert Xpert® Xpress SARS-CoV-2 assay as compared to in-house assay in a three-centre study. Labs indicated with 1, 2 and 3; I-h = In-house RT-PCR with target gene indicated; GX = GeneXpert Xpert® Xpress SARS-CoV-2; negative results indicated with Cq value 45.

The cause of E-only positivity for assays using the 5.1.2e et al. primers and probes is the slight lower sensitivity of the RdRP-gene primers and probes than the E-gene primers and probe [2] and the likely abundance of subgenomic E-gene transcripts targeted by E-gene primers and probe compared to genomic RNA targeted by the RdRP primers and probe. WHO indicates that in areas where SARS-CoV-2 is widespread the use of RT-PCR for one discriminating target is sufficient.[9] ECDC adds that confirmation with a second target or retesting or resampling is only necessary if the interpretation of the first result is technically difficult or if the Cq value is above 35 with the first target.[10] In the Netherlands, the E-gene only positive specimens with clear S-shaped amplification curve are given the absence of circulation of other SARS-related betacoronaviruses among humans and the abundance of circulation of SARS-CoV-2 considered positive for SARS-CoV-2 and patients with such results notified as COVID-19 cases.[11] Due to shortages in reagents and plastics many laboratories in The Netherlands have therefore switched to use the E-gene RT-PCR only.[11] Accordingly, we suggest that in The Netherlands, and in general in countries were SARS-CoV-2 is widespread circulating, a specimen with a low viral load that is repeatedly E-gene only positive with clear S-shaped amplification curve in the Xpert® Xpress SARS-CoV-2 assay can be considered positive for SARS-CoV-2. In regions where SARS-CoV-2 is sporadically detected, the set rules in the Xpert® Xpress SARS-CoV-2 kit insert for E-gene only positive specimen should be followed.[7]

4 Conclusion

The Cepheid GeneXpert Xpert® Xpress SARS-CoV-2 assay performed at least equally well compared to routinely used diagnostic real-time RT-PCR assays in three medical microbiology labs in the Netherlands. This assay is therefore a valuable addition in the field of COVID-19 diagnostic tests for use where a rapid highly accurate result is critical.

5 References

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