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Multi-center evaluation of cepheid xpert® xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic

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ABSTRACT

Background: With the outbreak of SARS-CoV-2, rapid diagnostics are paramount to contain the current pandemic. The routinely used realtime RT-PCR is sensitive, specific and able to process large batches of samples. However, turnaround time is long and in cases where fast obtained results are critical, molecular point of care tests (POCT) can be an alternative. Here we report on a multicenter evaluation of the Cepheid Xpert Xpress SARS-CoV-2 point-of-care test.

Study design: 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. A sensitivity and specificity panel was tested consisting of a dilution series of SARS-CoV-2 and ten samples containing SARS-CoV-2 and a range of other seasonal respiratory viruses. Additionally, 58 samples of patients positive for SARS-CoV-2 with different viral loads and 30 tested negative samples in all three Dutch laboratories using an in-house RT-PCR, were evaluated using Cepheids Xpert Xpress SARS-CoV-2 cartridges.

Results: Xpert Xpress SARS-CoV-2 point of care test showed equal performance compared to routine in-house testing with a limit of detection (LOD) of 8.26 copies/mL. Other seasonal respiratory viruses were not detected. In clinical samples Xpert Xpress SARS-CoV-2 reaches an agreement of 100 % compared to all in-house RT-PCRs

Conclusion: Cepheids GeneXpert Xpert Xpress SARS-CoV-2 is a valuable addition for laboratories in situations where rapid and accurate diagnostics are of the essence.

1. Background

In December 2019 the emergence of a novel coronavirus was reported in Wuhan, China [1]. Since its emergence, the disease COVID-19 caused by coronavirus SARS-CoV-2 has spread rapidly with at the end of April worldwide more than 3 million cases and deaths reaching 200.000 (2). Clinical symptoms range from mild upper respiratory tract symptoms to severe bilateral pneumonia, with large numbers of patients being admitted to hospital, putting tremendous pressure on health care systems [3–5]. The diagnosis of COVID-19 is based on a combination of clinical symptoms with or without radiological imaging, confirmed by SARS-CoV-2 PCR [6]. Currently, either an RT-PCR targeting the envelope (E-gene) in combination with polymerase (RdRp-gene) is being used as described by Corman et al. [7] or targeting the nucleoprotein (N-gene) as described by the US Centers for Disease Control and Prevention [8]. However several bottlenecks have been reported since its implementation. From a technical point of view, the process is time consuming with sample RNA extraction and PCR runtime of approximately 6 h and a turnaround time of 12 − 24 hours. Since providing fast results is of critical importance in a time of shortage of medical personnel, protective materials and beds on isolation wards and to insure timely and adequate treatment for patients, developing high quality rapid point of care diagnostics is essential. Antigenic tests have the potential in providing quick diagnosis,

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Table 1
Specificity panel composition.

Panel coding	Virus	Target specific Ct or dPCR SARS-CoV-2 RdRp gene copies/mL
EQA−01	Influenza virus A(H3N2)[a]	21.64
EQA−02	SARS-CoV-2	8.26*10^1 copies/mL
EQA−03	CoV-OC43	27.30
EQA−04[b]	SARS-CoV-2	8.26 copies/mL
EQA−05	Rhinovirus A16	25.49
EQA−06	CoV-229E	32.60
EQA−07	No virus	Neg
EQA−08	CoV-NL63	28.84
EQA−09	Influenza virus B-Victoria[a]	28.32
EQA−10	SARS-CoV-2	8.26*10^3 copies/mL

[a] Ct values were for influenza virus A(H3N2) from matrix gene RT-PCR and for influenza virus B/Victoria from hemagglutinin gene RT-PCR.
[b] 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[a]	10−10	8.26*10^-2
Sen. Serie-03	10−4	8.26*10^4
Sen. Serie-04[b]	10−8	8.26
Sen. Serie-05	10−7	8.26*10^1
Sen. Serie-06[a]	10−9	8.26*10^-1
Sen. Serie-07	10−5	8.26*10^3

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

however sensitivity thus far has been lacking which makes them unreliable. Molecular point of care tests reduce the time to result from a couple of hours to less than an hour but with the same performance as real-time RT-PCR in the laboratory [9,10]. The aim of this study is to evaluate Cepheid Xpert Xpress SARS-CoV-2 random access test for use during the current SARS-CoV-2 pandemic in the Netherlands.

2. Materials and methods

2.1. Preparation panels

Preparations for the specificity and sensitivity quality assessment panels for SARS-CoV-2 were done at the RIVM. The virus (hCoV-19/Netherlands/Noord_Brabant_0117R/2020) was first isolated from a throat swab specimen from an RT-PCR positive patient. 100 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 (cytopathic effect) was > 90 %. A next passage was done after a freeze/thaw cycle on VERO E6 (ATCC CRL-1586)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 E6 cells and found to contain 5.62*10^7 TCID50/mL (fifty-percent tissue culture infective dose) infectious virus. The virus was inactivated by incubation for 2 h 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.

2.2. Specificity panel

The specificity panel was created using dilutions of the inactivated SARS-CoV-2 stock and live virus isolates of influenza virus A(H3N2) and B/Victoria, CoV−OC43 (betacoronavirus), CoV-NL63, CoV-229E (both alphacoronaviruses) and Rhinovirus A16. One of the samples contained no virus. Dilutions were created in MEM with Hanks' salts, HEp2 cells were added at a concentration of 10,000 cells per ml panel specimen. Details of the panel are listed in Table 1. Ct 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.

2.3. Sensitivity panel

A sensitivity panel was created by 10-fold diluting the inactivated SARS-CoV-2 stock in MEM with Hanks' salts from 10−4 to 10−10. For each dilution HEp2 cells were added at a concentration of 10,000 cells per ml. Details of the panel are listed in Table 2. Concentrations of copies per sample were determined using dPCR performed on positive-strain genomic RNA. The RdRp-PCR also detects negative-strain genomic RNA and the E-gene PCR additionally detects sub-genome messengers.

2.4. Selection clinical specimens

Upper respiratory tract samples of patients tested for SARS-CoV-2 between January 2020 and March 2020 were included in the evaluation. Samples were primarily taken by nasopharyngeal or mid-turbinate, and oropharyngeal swabs and collected in UTM or GLY medium. Samples had been stored at −80 °C. Test were performed at three laboratories; RadboudUMC in Nijmegen, PAMM in Veldhoven and the RIVM in Bilthoven (laboratories were numbered randomly). Three sets of samples were selected based on the observation that the E-gene RT-PCR is most sensitive [7]. The first set consisted of 30 samples positive for both E-gene and RdRp-gene with a range of Ct-values. The second set consisted of 28 samples positive for E-gene only and the third set consisted of 30 samples tested negative for E-gene and RdRp-gene (Table 3).

Table 3
RT-PCR Ct range of clinical samples.

Clinical samples SARS-CoV-2	N	E-gene Average Ct (range)	RdRp-gene Average Ct (range)	N1-gene Average Ct (range)[a]
SARS-CoV-2 E-gene +/RdRp gene +	30	26,7(17,1–34,0)	28,8 (16,2–34,2)	29 (24,0−34,0)
SARS-CoV-2 E-gene +/RdRp gene -	28	33,5(25,8–37,6)	NA	NA
SARS-CoV-2 E-gene - /RdRp-gene -	30	NA	NA	NA

[a] One out of 3 laboratories used N1-gene in addition to E-gene in the in-house RT-PCR.

Table 4
Platforms used for routine testing of panels and clinical samples of SARS-CoV-2.

	Isolation platform	Extraction kit	PCR platform	RT-PCR Mastermix
Laboratory 1	MagNApure 96 (Roche)	MagNApure 96 DNA and Viral NA Small Volume	Roche LC480 II	Life Technologies Taqman FastVirus 1-step mastermix
Laboratory 2[a]	Roche COBAS4800	CT/NG extraction protocol	Roche LC480 II	Roche LightCycler Multiplex RNA Virus Master
Laboratory 3[b]	BioMérieux NucliSens easyMAG	EasyMAG extraction reagents	Thermo Fisher QuantStudio 6	Life Technologies Taqman FastVirus 1-step mastermix

[a] Laboratory 2 started with E-gene and RdRp-gene and mid-March moved on to E-gene testing only.
[b] Laboratory 3 started with E-gene and RdRp-gene and at the beginning of April moved on to E-gene and CDC N1-gene primer and probes.

2.5. Testing

Both the sensitivity and specificity panels were distributed blinded. They 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 routine diagnostic procedure using the locally implemented RT-PCR for E-gene and/or RdRp-gene and/or N-gene (Table 4). Panels and clinical specimens were tested on Cepheid GeneXpert systems using the Xpert Xpress SARS-CoV-2 test according to the manufacturer's instruction. In short, the Xpert Xpress SARS-CoV-2 assay targets two genes, the E-gene (Sarbeco specific) and N2-gene (SARS-CoV-2 specific), input in the cartridges requires 300 µl of sample in virus transport medium. Xpert Xpress SARS-CoV-2 kit insert considers E-gene only positive specimens 'SARS-CoV-2 presumptive positive' and requests retesting, N2 only positives are deemed positive. The Xpert Xpress SARS-CoV-2 For Use Under an "U.S. Food and Drug Administration Emergency Use Authorization (EUA) Only" was bought from Cepheid Europe for pre-CE-IVD release evaluation.

3. Results

3.1. Specificity panel

None of the samples containing respiratory viruses other than SARS-CoV-2 were tested positive by the routine diagnostic tests and the Xpert Xpress SARS-CoV-2 assay. Three of the samples containing different concentrations of heat inactivated SARS-CoV-2 virus tested positive at all laboratories in both the in-house RT-PCR and the GeneXpert. One sample containing 8.26 cp/mL of SARS-CoV-2 gave an N2-only result in one out of three laboratories. The N2-gene only positive specimen was retested with Xpert Xpress SARS-CoV-2 at lab 3 and was positive for both targets with E-gene at Ct 39.8 and N2-gene at Ct 42.2.(Fig. 1).In the other two laboratories, this sample gave positive results in both the routine diagnostic test and the Xpress assay.

3.2. Sensitivity panel

Xpert Xpress SARS-CoV-2 was able to detect SARS-CoV-2 at a concentration of $8.26*10^{-1}$ cp/mL in all three labs. At this dilution all in-house RT-PCRs tested negative. The Xpert Xpress SARS-CoV-2 resulted in a positive or 'presumptive positive'(E-gene only) at all three laboratories for this dilution [11]. All three labs retested this specimen on a different day (implicating freeze-thaw step) in the Xpert Xpress SARS-CoV-2 assay with negative result for both target genes. Ct values reported by the individual in-house PCRs varied for similar dilutions of viral load, with laboratory one being able to detect samples at 8.26 cp/mL where the other two laboratories failed to detect SARS-CoV-2. (Fig. 2)

3.3. Clinical samples

All samples both E-gene and RdRp gene positive in the in-house PCR were positive in the Xpert Xpress SARS-CoV-2 (Fig. 3). All samples negative in the in-house SARS − COV-2 RT-PCR were negative in the Xpert Xpress SARS-CoV-2. 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. For the panel consisting of E-gene positive and RdRp-gene negative samples, most but not all were positive in the Xpert Xpress SARS-CoV-2. Two samples in laboratory 1 showed an N2-gene only and E-gene only result (Fig. 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&4). When retesting these samples in the in-house RT-PCR they were negative.

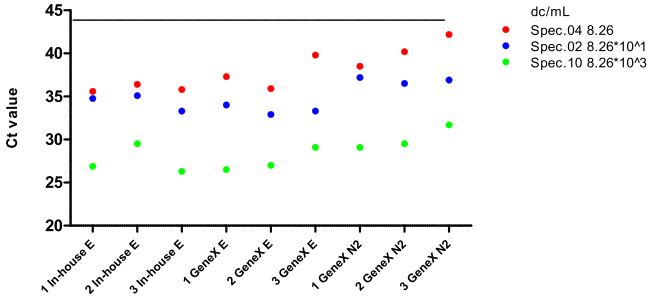

Fig. 1. Results of SARS-CoV-2 containing specimens of the specificity panel. Labs indicated with 1, 2 and 3; In-house = In house RT-PCR with target gene indicated; GeneX = Xpert Xpress SARS-CoV-2; negative results indicated with Ct value 45.

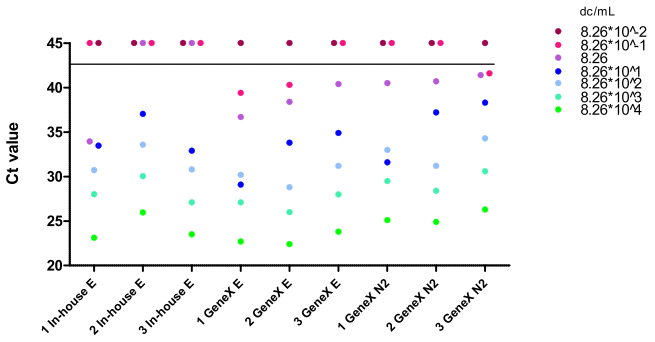

Fig. 2. Results of the sensitivity panel for in-house E-gene (in-house E) and Xpert Xpress SARS-CoV-2 (GeneX E and N2). Individual labs are indicated with 1, 2 and 3; negative results indicated at a Ct value 45.

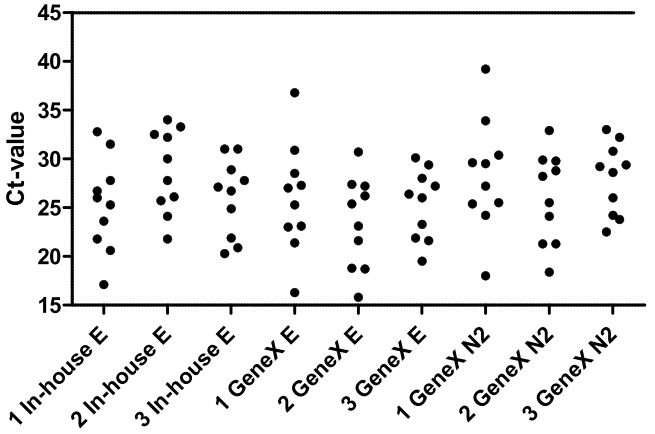

Fig. 3. Results of clinical samples with high SARS-CoV-2 viral load. Panel consists of samples tested in in-house RT-PCR positive for E-gene and RdRp-gene. In-house E-gene (in-house E) and Xpert Xpress SARS-CoV-2 (GeneX E and N2). Individual labs are indicated with 1, 2 and 3; negative results indicated at a Ct value 45.

4. Discussion

In this study we show that the Xpert Xpress SARS-CoV-2 is a random-access system suitable for molecular Point-of-Care testing that is highly specific to and sensitive for the detection of SARS-CoV-2, compared to in-house testing, and furthermore has a run-time of 45 – 50 min with hands on time limited to 2 – 3 min. Current routine molecular diagnostics for SARS-CoV-2 are able to perform high throughput processing, however turnaround time is relatively high which hinders patient management and infection control policies.

In clinical samples Xpert Xpress SARS-CoV-2 reaches an agreement

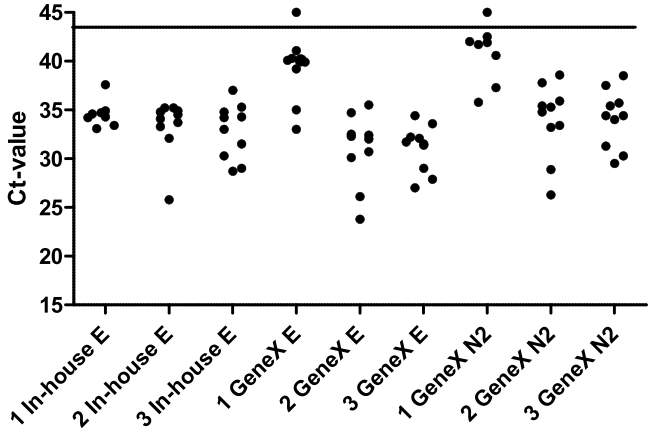

Fig. 4. Results of clinical samples with lower SARS-CoV-2 viral loads, for in-house E-gene (in-house E) and Xpert Xpress SARS-CoV-2 (GeneX E and N2). Individual labs are indicated with 1, 2 and 3; negative results indicated at a Ct value 45. Xpert E-only and N2-only results were for 2 different specimens.

of 100 % compared to all in-house RT-PCRs and the assay outperforms routinely used diagnostic platforms in the sensitivity panel. The two samples with E-gene and N2-gene only results in Xpert Xpress SARS-CoV-2 were retested in in-house RT-PCR, thus freeze-thawing, these turned out to be negative. This indicates that viral loads of these sample are at the limit of detection which for the Xpert Xpress SARS-CoV-2 was found to be around 8.26 cp/mL and that multiple freeze-thaw steps of samples understandably has a significant impact on detection.

In our evaluation, the LOD was lower (8.26 cp/mL) compared to the LOD provided by the manufacturer (250cp/mL). Additionally, a recent evaluation of multiple molecular point of care tests showed an LOD of 100cp/mL [11–13]. This discrepancy is most likely due to the different methods used for determining input concentrations. Digital PCR used to determine the number of copies per mL was performed on positive-strain genomic RNA. The RdRp PCR also detects negative strain genomic RNA and the E-gene PCR additionally detects subgenome messenger RNA which is why the true number of target templates for the diagnostic PCR in the sensitivity panel is probably higher. This could explain the differences in LOD.

Xpert Xpress SARS-CoV-2 is for use on nasopharynx swabs/nasal wash samples in Cepheid viral transport medium. The current evaluation on clinical samples was done using both UTM and GLY medium, as choice of transport medium is limited during the current pandemic, which shows reliable results. Equally so in the sensitivity and specificity panel using MEM with Hanks' salts.

Due to shortages in reagents and plastics many laboratories in The Netherlands have switched to use the E-gene RT-PCR only [14]. Accordingly, we suggest that in countries where SARS-CoV-2 is widespread and in the absence of circulation of other SARS-related beta-coronaviruses among humans, 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 conclusion, Xpert Xpress SARS-CoV-2 is a sensitive and specific random access, cartridge-based assay with a short turnaround time, enabling direct input for patient care and infection control.

Declaration of Competing Interest

None.

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