Department of Health & Social Care

Technical Validation Report for Cepheid Xpert® Xpress SARS CoV 2 assay

Published: September 2021 (Version 1.1)

Contents

Assay description

1. What is the principle and method of the assay (description of the assay according to the manufacturer's Instructions for Use (IFU)?

As stipulated in the IFU XPRSARS-COV2-10 October 2020

The Xpert Xpress SARS-CoV-2 test is a molecular in vitro diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in nasopharyngeal (NP) swab, nasal swab, or nasal wash/aspirate specimens.

The Xpert Xpress SARS-CoV-2 test is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized.

The Xpert Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in NP swab, nasal swab, or nasal wash/aspirate specimen. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional.
The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The NP swab, nasal swab, or nasal wash/aspirate specimen is collected and placed into a transport tube containing 3 mL of viral transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

The results are interpreted automatically by the GeneXpert System and are clearly shown in the View Results window. The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets according to the algorithms shown in Table 1.

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result text	N2	E	SPC
SARS-CoV-2 Positive	+	+/-	+/-
SARS-CoV-2 Presumptive Positive	-	+	+/-
SARS-CoV-2 Negative	-	-	+/-
Invalid	-	-	-

It has been noted that presumptive positive results could result from testing some SARS-CoV-2 N gene variants; guidance on repeat testing is given by the FDA https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests#xpert

2. What is the use for which the device is intended according to the data supplied by the manufacturer on the label, in the IFU, in promotional or sales materials or statements, or as specified by the manufacturer in the performance evaluation?

As stipulated in the IFU 302-3787, Rev. B (Oct 2020)

The Xpert Xpress SARS-CoV-2 test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimen collected from individuals who are suspected of COVID-19 infection.

Results are for the identification of SARS-CoV-2 RNA. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.

Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Xpert Xpress SARS-CoV-2 test is intended to be performed by trained users in both laboratory and near patient testing settings.

3. Is the test a stand-alone device/test or to be used in conjunction with other equipment and is biosafety containment required?

The test is designed to be used with the Cepheid GeneXpert® instrument systems. All human samples should be considered as infectious and handled according to Good Laboratory procedures. If using the swabs and transport media recommended in the IFU (see Section 4 for details), it should be noted that the virus is not inactivated prior to testing. There is a pipetting step that is required to pipette sample from the primary tube to the assay cartridge for processing. If the transport media used to transport and store the sample does not inactivate the sample, this should be performed in a biosafety cabinet.

Type of sample to be used in validation

4. Stipulate the sample type (e.g. whole non-extracted virus, extracted RNA, synthetic RNA, plasmid DNA containing assay target regions) and any sample matrices (e.g. saliva, plasma, nasopharyngeal/oronasal swab [dry or in VTM] etc) in which the material is to be spiked.

   Whole non-extracted virus from nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimen collected from individuals who are suspected of COVID-19 infection in transport medium or saline. The swab types and transport media recommended in the IFU are as follows:

   - Nylon flocked swab (Copan P/N 502CS01, 503CS01) or equivalent
   - Viral transport medium, 3 mL (Copan P/N 330C) or equivalent
   - 0.85% (w/v) saline, 3 mL
   - Sample Collection Kit for Viruses (Cepheid P/N SWAB/B-100, SWAB/M-100, SWAB/F- 100) or equivalent

   Nasopharyngeal swab, nasal swab and nasal wash/aspirate specimens can be stored in viral transport medium or saline, at room temperature (15-30 ˚C) for up to 8 hours and refrigerated (2-8 ˚C) up to 7 days until testing is performed on the GeneXpert Instrument Systems.

   Results of samples tested at 21 sites across England and Scotland were collected for this report

   Of the 21 NHS sites who conducted a verification of the assay, one site used Primestore VTM, as this medium is inactivating, allowing the Cepheid platform to be used in clinical areas. The site noted a slight increase in CT values compared to using non-inactivating media, but due to the sensitivity of the assay this was not thought to be a cause for concern and was unlikely to change the outcome of any test results.

5. Stipulate if the material is required to be extracted i.e. volume received, volume extracted, volume eluted, elution buffer to be used in the assay.

The GeneXpert® instrument systems provides sample extraction as part of the automated workflow.

6. If possible, stipulate if any interfering substances such as preservatives are likely to be present. Shelf life and number of freeze thaw events should also be stated, if known. Where dry swabs are to be used, samples will need to be collected prospectively; two

swabs per participant, one for a new test and one to be tested using reference method; or one swab for a new test collected within 24 hours of a positive reference method swab.

N/A

Equipment and reagents

7. List all the equipment required that are not supplied by the manufacturer with calibration/service requirements and dates where applicable.

All equipment and instrumentation are supplied.

8. List all the reagents required that are not provided by the manufacturer with shelf-life expiry dates and storage conditions. Include positive and negative control materials.

Specimen collection kits – see Section 4 for details.

Performance characteristics

9. Analytical Sensitivity and Linearity of SARS COV-2 targets

10. Dilution series: This should be calculated using a validated standard dilution series. If this is not possible (as standard material is not available), use 5 clinical positive replicates, with a 5 log10 dilution, plus 5 negatives, plus the use of inhibition controls. If feasible, repeat over several days, different users/machines (feasibility may be limited due to availability of positive material). Where dry swabs are to be used, known amounts of standard material should be added to the swab, and then tested as per IFU. Reference to be made on the reporting of results and for example CT values.

    - Data from IFU XPRSARS-COV2-10 October 2020

    Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Xpress SARS-CoV-2. The LoD of Xpert Xpress SARS-CoV-2 was established using one lot of reagent and limiting dilutions of live SARS-CoV-2 virus (USA_WA1/2020) prepared in viral transport medium and NP swab clinical matrix. The concentration level with observed hit rates greater than or equal to 95% in the LoD determination study were 0.0050 and 0.0200 PFU/mL for the N2 target and E target, respectively (Table 2). Verification of the estimated LoD claim was performed on one reagent lot in replicates of 20 prepared in pooled NP swab clinical matrix. The LoD is the lowest concentration (reported as PFU/mL) of live SARS-CoV-2 virus samples that can be reproducibly distinguished from negative samples ≥ 95% of the time with 95% confidence. The claimed LoD is 0.0200 PFU/mL (Table 2).

Table 2: LoD Determination Using USA-WA1/2020 Strain

Strain	Concentration (PFU/mL)	Total Valid Results	Hit Rate N2 target (%)	Hit Rate E target (%)	Mean CT* N2 target	Mean CT* E target
USA-WA1/2020	0.02	20	100	95	38.3	36.4
	0.005	22	95.5	68.2	40.5	39.1
	0.0025	22	90.9	36.4	41.5	39.6
	0.001	22	50	18.2	42	42
	0.0005	22	45.5	18.2	41.7	41.5
	0.0003	22	18.2	4.5	42.1	44.9
	0.0001	22	9.1	0	42.9	n/a
	0	0	0	0	n/a	n/a

*Calculations only include positive results.

- Data from Site 9

Lower limit of detection (LLOD) was determined using the Qnostics SARS-CoV-2 standard (table 3) and NIBSCs SARS-CoV-2 (19/304) (table 4.). Due to lack of cartridges to complete this work, limited dilution series were run.

Table 3: Dilution Series and LLOD using Qnostics SARS-CoV-2 (Site 9 data)

	Xpert Xpress result		
copies/ml	E gene CT value	N gene CT value	Interpretation
10000	28	30.2	Positive
1000	31.7	34.2	Positive
100	34.5	38.9	Positive
10	0	40.8	Positive
1	Neg	Neg	Negative

Table 4: Dilution Series and LLOD using NIBSCs SARS-CoV-2 (19/304) (Site 9 data)

		Xpert Xpress result	
copies/ml	E gene CT value	N gene CT value	Interpretation
1000000	28.1	28.5	Positive
100000	32.4	32.8	Positive
10000	34.7	35.5	Positive
1000	40	40	Positive
100	neg	41.2	Positive
10	neg	Neg	Negative

11. Linearity and efficiency: Ideally for linearity, the use of a standardised reference panel should be used to ensure effective benchmarking and assure that dilutions are accurate. If an alternative route for establishing linearity is undertaken, the method will need to be documented. For LAMP assays the linearity will need to be standardised via dilutions in appropriate matrixes using untreated virus. Plot the data from Section 10 and calculate linearity and efficiency. Compare the data with that supplied by the manufacturer, if applicable.

12. Lowest Limits of Detection (LLOD): Where a validated standard dilution series was used LLOD should be calculated, using data from Section 11, in copies/ml (to align with the relevant MHRA TPP). Where clinical positive material is used, copies/ml cannot be calculated; median CT value or dPCR should be given for the lowest dilution detected from the samples used in Section 10.

Table 5: Reported lowest limit of detection (LLOD) from three data sources described in Section 10.

Source	LLOD (Copies/mL)
IFU XPRSARS-COV2-10 October 2020 (table 2)	0.0200 PFU/mL (copies/ml not available)
Site 9 Verification report (using QNostics) (Table 3)	10 copies/mL
Site 9 Verification report (NIBSCs SARS-CoV-2 (19/304)) (Table 4)	100 copies/mL

These results demonstrate that the Xpert Xpress SARS CoV-2 assays detects the virus at a concentration of between 10-100 copies/mL, and does not detect any virus in the negative sample.

Precision and robustness

13. Intra-assay precision: Use the data for 5 replicate values from a single day from 4.1.1 to calculate Standard Deviation & Coefficient of Variation measurement, with the values for the latter to be <10%. To include the use of inhibition controls

- Data from Site 10

The same sample was run on different analysers, same lot.

Table 6: Summary of QC materials ran over a period of 3 consecutive days.

Hospital site within Trust Site	**Date of Run**	**Cepheid**		
		SARS CoV-2		
		Result	E CT	N2 CT
1	20/04/2020	D	19.3	21.7
1	21/04/2020	D	19.3	21.7
2	20/04/2020	D	19.1	21.4
3	20/04/2020	D	19.2	21.6
4	21/04/2020	D	18.9	21.3
5	21/04/2020	D	19.3	21.6

These results indicate that the assay precision as far as it has been tested in this small study is as expected.

14. Inter-assay precision: Use the data for 5 replicate values from multiple days from 4.1.1 for Standard Deviation & Coefficient of Variation with the values for the latter to be <15%.

See Section 13 and Table 6 above.

15. Repeatability: Spike 30 negative samples from different individuals with known amount of agent/positive material (suggested 3x the LLOD), all should be positive.

- Data from IFU XPRSARS-COV2-10 October 2020

The reproducibility of the Xpert Xpress SARS-CoV-2 test was established at three sites using a 5-member panel including one negative sample, two low positive (~1.5x LoD) and two moderate positive (~3x LoD) samples. The negative sample consisted of simulated matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated matrix using either AccuPlex™ SARS-CoV-2 reference material (targeting the N2 and E genes) or inactivated SARS-CoV Urbani strain (targeting the E gene). Testing was conducted over six (6) days, using three (3) lots of Xpert Xpress SARS-CoV-2 cartridges at three (3) participating sites each with two (2) operators to yield a total of 144 observations per panel member (3 Sites x 2 Operators x 3 Lots x 2 Days/Lot x 2 Runs x 2 Reps = 144 observations/panel member). Summary results are shown in table 7.

Table 7: Summary of Reproducibility Results - % Agreement by Study Site/Operator.

Sample	Total percentage agreement by sample
negative	100% (144/144)
SARS-CoV-2 low pos	98.6% (142/144)
SARS-CoV-2 mod pos	100% (144/144)
SARS-CoV-2 low pos	100% (144/144)
SARS-CoV-2 mod pos	100% (144/144)

Analytical specificity (Interferences and cross-reactions)

16. Cross-reactivity to non-target samples/organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases, both closely related (i.e., other coronaviruses), syndromic diseases (i.e., other respiratory viruses and bacteria) and common diseases (i.e. HIV, HBV, HCV, VZV, EBV, CMV) should be tested.

- Data from IFU XPRSARS-COV2-10 October 2020

In-silico Analysis

An in silico analysis for possible cross-reactions with all the organisms listed in Table 8 was conducted by mapping primers and probes in the Xpert Xpress SARS-CoV-2 test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 8 is expected based on the in silico analysis.

Table 8: Xpert Xpress SARS-CoV-2 Analytical Specificity Microorganisms

Microorganisms from the Same Genetic Family	**High Priority Organisms**
Human coronavirus 229E	229E Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis

Microorganisms from the Same Genetic Family	High Priority Organisms
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-pneumophila
	Bacillus anthracis (Anthrax)
	Moraxella catarrhalis
	Neisseria elongata and meningitidis
	Pseudomonas aeruginosa
	Staphylococcus epidermidis

Microorganisms from the Same Genetic Family	High Priority Organisms
	Staphylococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

- Data was unavailable from any of the sites to confirm these results

Diagnostic sensitivity and specificity (Clinical validation with confirmed positives and negatives)

17. Samples selected for this validation will be appropriate to the assay. Low medium and high viral load samples will be equally distributed to avoid increasing or lowering DSe and DSp.

18. Diagnostic sensitivity: Confirmed clinical samples from patients (positive RT-qPCR result) should be used. Preferably, depending on the availability of samples, ~150 samples should be included to align with MHRA TPP. Clinical sensitivity (95% CI) and positive predictive value (PPV) should be calculated in comparison with a CE marked reference method that itself has sensitivity and specificity and a limit of detection within the specifications of the MHRA TPP. The CT values or equivalent for both the assessed and comparator assays must be included in the validation report.

- Data from multiple verification reports from NHS labs in the UK (21 sites)

404 RT-PCR positive samples and 318 RT-PCR negative samples were evaluated in this analysis using a number of different comparator assays. Table 9 shows the sensitivity and specificity of the assay using the combined results of the analyses.

Table 9: Diagnostic sensitivity and specificity of the Xpert Xpress SARS-CoV-2 assay

		Comparator Assay Result		
		Pos	Neg	
Xpert Xpress Result	**Pos**	402	3	405
	Neg	2	315	317
		404	318	722

sensitivity	99.5
specificity	99.1

The assay gives a sensitivity of 99.5% (95% CI 98.2-99.9, 402/404 RT-PCR positive samples).

Table 10 below shows the spread of CT values across the dynamic range of the assay, where the CT data for both the assay and the comparator assay were available. CT values were only available for 12 of the 21 sites, due to the comparators used.

The sensitivity of the assay meets the desirable criteria in the MHRA TPP for Laboratory Based SARS CoV-2 Viral Detection Tests (https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-laboratory-based-sars-cov-2-viral-detection-tests) for RNA extracted assays, with sensitivity of greater than 99% (with 95% two-sided confidence interval entirely above 97%).

The sensitivity of the assay meets the desirable criteria in the MHRA TPP for Point of Care SARS CoV-2 assays (https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-point-of-care-sars-cov-2-detection-tests) with sensitivity greater than 97% (within confidence intervals of 93-100%).

The sensitivity also meets the TVG criteria for Category 1 performance (sensitivity >97%).

19. Diagnostic specificity: Confirmed clinical samples from patients (negative RT-qPCR result) should be used. Preferably, depending on the availability of samples, ~250 samples should be included to align with MHRA TPP. Clinical specificity (95% CI) and negative predictive value (PPV) should be calculated in comparison with a CE marked reference method that itself has sensitivity and specificity in line with the MHRA TPP. The CT values or equivalent for both the assessed and comparator assays must be included in the validation report.

    See Table 9 above. The specificity of the assay is 99.1% (95% CI 97.3-99.7, 315/318 RT-PCR negative samples). There were four discrepancies (all from one site) where the Cepheid was detected (but only with N-gene target detected in isolation, with CT values >42) and the Comparator was NOT Detected. All four of these discrepant samples were detected at the limit of detection of the assay where variable results owing to stochastic detection at low copy number is anticipated and were removed from the analysis.

    The specificity of the assay meets the desired criteria in the MHRA TPP for Laboratory Based SARS CoV-2 Viral Detection Tests (https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-laboratory-based-sars-cov-2-viral-detection-tests) for RNA extracted assays, with specificity of greater than 99% (with 95% two-sided confidence interval entirely above 97%).

    The specificity of the assay meets the desired criteria in the MHRA TPP Point of Care SARS CoV-2 assays (https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/target-product-profile-point-of-care-sars-cov-2-detection-tests) with specificity greater than 99% (within confidence intervals of 97-100%).

The specificity also meets the TVG criteria for Category 1 performance (specificity >99%).

Table 10 CT spread of the assay against the comparator assay across the dynamic range

CT	Site 2			Site 3			Site 4			Site 5		
	Hologic result	Pos match on Cepheid	sens %	Cepheid Gene Xpert 4 plex	Pos match on Cepheid	sens %	Elitech result	Pos match on Cepheid	sens %	Viasure result	Pos match on Cepheid	sens %
<25	8	8	100.0	45	45	100.0	8	8	100.0	0	0	
25-30	8	8	100.0	8	8	100.0	9	9	100.0	13	13	100.0
30-35	6	6	100.0	8	8	100.0	6	6	100.0	5	5	100.0
>35	3	3	100.0	5	5	100.0	1	0	0.0	2	2	100.0
Undefined	16	16		0			0			0		

CT	Site 6			Site 7			Site 8			Site 9		
	Viasure/ cobas 8800	Pos match on Cepheid	sens %	AusDx	Pos match on Cepheid	sens %	samples	Pos match on Cepheid	sens %	AusDx	Pos match on Cepheid	sens %
<25	9	9	100.0	14	14	100.0	15	15	100.0	7	7	100.0
25-30	4	4	100.0	0	0		14	14	100.0	4	4	100.0
30-35	5	5	100.0	0	0		0			7	7	100.0
>35	0			0	0		0			4	4	100.0
Undefined	0			6	6		5	5		42	42	

CT	Site 10			Site 11			Site 12			Site 13		
	Abbott	Pos match on Cepheid	sens %	Local reference test	Pos match on Cepheid	sens %	Abbott M2000	Pos match on Cepheid	sens %	Viasure	Pos match on Cepheid	sens %
<25	5	5	100.0	1	1	100.0	9	9	100.0	1	1	100.0
25-30	0			3	3	100.0	2	1	50.0	0		
30-35	0			0			1	1	100.0	1	1	100.0
>35	0			0			0			0		
Undefined	0			0			0			0		

CT	Total all sites		
	Total comparator Samples	Total positive Matches	Total sens %
<25	122	122	100.0
25-30	65	64	98.5
30-35	39	39	100.0
>35	15	14	93.3
Undefined	69	69	

Summary

The Cepheid Xpert® Xpress SARS-CoV-2 assay has been independently verified at 21 NHS labs in the UK. The collated data from the verification reports are presented in this desktop validation report. The assay meets both the MHRA TPP for Laboratory Based SARS CoV-2 Viral Detection tests for RNA extracted assays and the MHRA TPP for Point of Care Assays with a diagnostic sensitivity of 99.5% (95% CI 98.2-99.9) and a diagnostic specificity of 99.1% (95% CI 97.3-99.7). A comparison with both TPP's is presented as this assay may be used in both situations.

The lowest limit of detection was found to be 10-100 copies/mL for N gene and 100-1000 copies/ml for E gene. This meets the desired criteria of ≤100 copies/mL for the assay in both the MHRA TPP for Laboratory Based SARS CoV-2 tests for RNA extracted assays and the MHRA TPP for Point of Care assays.

© Crown copyright 2018

Published to GOV.UK in pdf format only.

[Directorate/Division/Branch]

www.gov.uk/dhsc

This publication is licensed under the terms of the Open Government Licence v3.0 except where otherwise stated. To view this licence, visit nationalarchives.gov.uk/doc/open-government-licence/version/3

Where we have identified any third party copyright information you will need to obtain permission from the copyright holders concerned.