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EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Cepheid Xpert Xpress SARS-CoV-2 DoD test
For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

INTENDED USE

The Xpert Xpress SARS-CoV-2 DoD test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, anterior nasal, or mid-turbinate swab or nasal wash/ aspirate) collected from individuals suspected of COVID-19 by their healthcare provider.

Testing of nasopharyngeal, oropharyngeal, anterior nasal, or mid-turbinate swab and nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2 DoD run on the GeneXpert Dx and GeneXpert Infinity systems is limited to U.S. Department of Defense (DoD) designated laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests, or similarly qualified U.S. DoD designated laboratories.

Testing of nasopharyngeal, anterior nasal, or mid-turbinate swab specimens using the Xpert Xpress SARS-CoV-2 DoD test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is limited to U.S. DoD designated laboratories certified under CLIA that meet requirements to perform high, moderate, or waived complexity tests or similarly qualified US DoD designated laboratories. Testing of these specimens is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation or similarly qualified DoD patient care settings.

The Xpert Xpress SARS-CoV-2 DoD test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to eight upper respiratory swab specimens (i.e., nasopharyngeal, oropharyngeal, anterior nasal, or mid-turbinate swabs) collected individually in transport media from individuals suspected of COVID-19 by their healthcare provider. Testing of pooled specimens is limited to U.S. DoD designated laboratories that meet the requirements to perform high complexity tests.

Specimens should only be pooled in areas with low SARS-CoV-2 prevalence, and when testing demand exceeds laboratory capacity or reagent availability. For pooled specimen testing, authorized laboratories will adhere to a protocol for ongoing monitoring of the pooling strategy or limit testing to individuals who are subjected to a detailed infection prevention and control plan.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2; 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. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

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. Negative results from pooled samples should be reported as presumptive. Specimens with low viral genetic material may not be detected in

pooled samples due to decreased sensitivity. If clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, the patient should be considered for individual testing.

Testing with the Xpert Xpress SARS-CoV-2 DoD test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems. The Xpert Xpress SARS-CoV-2 DoD test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Xpert Xpress SARS-CoV-2 DoD test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 DoD test is performed on the GeneXpert Dx, GeneXpert Infinity, and GeneXpert Xpress 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. For a full description of the systems, see the GeneXpert Dx Operator Manual, GeneXpert Infinity System Operator Manual, or GeneXpert Xpress System User's Guide.

The Xpert Xpress SARS-CoV-2 DoD test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument systems. 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 nasopharyngeal, anterior nasal, or mid-turbinate swab specimen and/or nasal wash/aspirate specimen collected and placed into a viral transport tube containing 3 mL 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 Xpert Xpress SARS-CoV-2 cartridge is loaded onto the GeneXpert instrument, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

The Xpert Xpress SARS-CoV-2 DoD test has been validated for up to 8-sample pooling in DoD designated high complexity laboratories using the GeneXpert Dx and GeneXpert Infinity systems. Pooling may be performed with upper respiratory swab specimens (i.e. nasopharyngeal, nasal, mid-turbinate, or oropharyngeal swabs) collected individually in transport media from individuals suspected of COVID-19 by their healthcare provider.

INSTRUMENTS USED WITH TEST (Individual and Pooled Specimen Testing)

- GeneXpert Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual
- For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher
- For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

INSTRUMENTS USED WITH TEST (Individual Specimen Testing)

- GeneXpert Xpress System (Tablet configuration): GeneXpert Xpress II and IV instruments with proprietary GeneXpert Xpress Software Version 5.0 and 5.1, tablet computer device with touchscreen, barcode scanner, external CD drive, wireless printer, Getting Started Guide, and GeneXpert Xpress System User's Guide.
- GeneXpert Xpress System (Hub configuration): GeneXpert Xpress IV instrument, GeneXpert Hub with proprietary GeneXpert Xpress Software Version 6.1 or higher, GeneXpert Hub with integrated computer, touchscreen monitor and barcode scanner, external CD drive, Getting Started Guide, and GeneXpert Xpress System User's Guide.

CONTROLS TO BE USED WITH TEST

Sample Processing Control (SPC): An exogenous control that consists of armored RNA containing an artificial RNA sequence that does not exist in nature. The reagent is in the form of lyophilized beads on-board with the test cartridge. It is processed together with the clinical sample to confirm adequate sample processing and control for the presence of PCR reaction inhibitors. The SPC is required to be detected for a valid negative testing of target analytes.

Probe Check Control (PCC): After sample processing but before the start of the RT-PCR reaction, the GeneXpert systems measure the background fluorescent signal from the probes to monitor reagent bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

External Controls: External controls are not provided with the kit. External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

INTERPRETATION OF RESULTS

The results are interpreted automatically by the GeneXpert systems from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window. The systems automatically verify that internal controls (SPC and PCC) are within the acceptance criteria and provide SARS-CoV-2 test results based on the two gene targets being detected. The possible testing results and the corresponding result call algorithms are shown in **Table 1**.

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

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+/-	+/-
SARS-CoV-2 PRESUMPTIVE POSITIVE	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

An explanation of the SARS-CoV-2 reported results and interpretation is provided in **Table 2**.

Table 2. Xpert Xpress SARS-CoV-2 DoD Test Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. • The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because coronavirus target amplification occurred • Probe Check: PASS; all probe check results pass
SARS-CoV-2 PRESUMPTIVE POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. • The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because a target amplification has occurred. • Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. • The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting • SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting • Probe Check: PASS; all probe check results pass
INVALID	SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in IFU. • SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting • Probe Check – PASS; all probe check results pass
ERROR	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in IFU. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
NO RESULT	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in IFU. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

The Xpert Xpress SARS-CoV-2 DoD test includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens if the signal from the target nucleic acid reaches a predetermined threshold before the full 45 PCR cycles have been completed. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen and its results may not be reported.

INTERPRETATION OF RESULTS – SPECIMEN POOLING

SARS-CoV-2 POSITIVE: Specimens with a positive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

SARS-CoV-2 PRESUMPTIVE POSITIVE: Specimens with a presumptive positive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

SARS-CoV-2 NEGATIVE: Report the pooled negative result as presumptive. Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result and results are necessary for patient management, then the patient should be considered for individual testing. The test report should indicate sample pooling was used for any specimens with reported negative results.

Non-determinate Result (INVALID, ERROR, or NO RESULT): Specimens with a non-determinate result must be tested individually prior to reporting a result. However, in instances of a non-determinate test result, repeat testing of the pooled specimens may be appropriate depending on the laboratory workflow and required result reporting time.

PERFORMANCE EVALUATION

The Cepheid Xpert Xpress SARS-CoV-2 DoD test is identical to the Cepheid Xpert Xpress SARS-CoV-2 test, except the Cepheid Xpert Xpress SARS-CoV-2 DoD test has been validated for up to 8-specimen pooling in U.S. Department of Defense (DoD) designated high-complexity laboratories. Analytical and Clinical validation studies for the Cepheid Xpert Xpress SARS-CoV-2 DoD test were previously performed and are described in the Instructions for Use (IFU) of the Cepheid Xpert Xpress SARS-CoV-2 test. The IFU for the Cepheid Xpert Xpress SARS-CoV-2 test is publicly available at: https://www.fda.gov/media/136314/download

Validation of up to 8-Specimen pooling for the Cepheid Xpert Xpress SARS-CoV-2 DoD test is described below. Additionally, recent inclusivity *in silico* analyses and FDA Reference Panel results were provided and are outlined below.

1. **Clinical Study Validation of 5- and 8-Sample Pooling**
   Validation of 5- and 8-sample pooling was performed by testing 20 positive and 160 negative residual nasopharyngeal swabs (NPS) in VTM/UTM collected from individuals suspected of SARS-CoV-2 and tested as part of standard of care. The twenty known positive samples were characterized by an FDA authorized high sensitivity SARS-CoV-2 RT-PCR assay and were chosen to represent a range of clinically relevant concentrations. All 20 positive samples were also tested individually with the Xpert

Xpress SARS-CoV-2 DoD assay. Comparator negative samples were not individually retested with the Xpert Xpress SARS-CoV-2 DoD assay.

For validation of pooling, 20 negative pools of 5 samples and 20 negative pools of 8 samples were generated from 160 samples that tested negative on the FDA authorized high sensitivity SARS-CoV-2 RT-PCR assay when individually tested. All negative sample pools were confirmed negative on the Xpert Xpress SARS-CoV-2 DoD assay.

Individual positive samples were then combined with 160 samples shown to be negative by an FDA authorized high sensitivity SARS-CoV-2 RT-PCR assay (individually tested) to make twenty 5-sample and twenty 8-sample pools. Pools were then tested with the Xpert Xpress SARS-CoV-2 DoD test. Approximately twenty-five percent (25%) of the samples were low positives, defined as samples with an Ct value within 2-4 Ct of the mean N2 target LoD of the Xpert Xpress SARS-CoV-2 DoD test. Pooled test results were compared to individual test results to evaluate the effect pooling has on SARS-CoV-2 detection. Results are described independently for 5- and 8-sample pooling, below.

5-Sample Pool Results

For the 5-sample pool study, 95% (19/20) of expected positive pools were detected (**Table 3**). The single sample that was missed by 5-sample pooling had an N2 Ct value of 40.8 and an E Ct value of 44.6 when tested individually with the Xpert Xpress SARS-CoV-2 DoD test. These results are acceptable.

Table 3. Clinical Performance of the Xpert Xpress SARS-CoV-2 DoD Test with 5-Sample Pools

	Individual Specimen Result		
5-Sample Pool Result	**Positive**	**Negative**	**Total**
Positive	19	0	19
Negative	1*	20	21
Total	20	20	40
PPA[1]	95.0% (95% CI: 76.4%, 99.1%)		
NPA[2]	100.0% (95% CI: 83.9%, 100.0%)		

*The Ct values for the one specimen in the 5-sample pool that was missed on the Xpert Xpress SARS-CoV-2 DoD test were: N2 - 40.8 and an E - 44.6

[1]PPA was calculated using the Xpert Xpress SARS-CoV-2 DoD individual test results

[2]NPA was calculated using test results from an FDA authorized high sensitivity SARS-CoV-2 RT-PCR assay

8-Sample Pool Results

For the 8-sample pool study, 90% (18/20) of expected positive pools were detected (**Table 4**). The two samples that were missed by 8-sample pooling had the following Ct values, as determined by individual testing with the Xpert Xpress SARS-CoV-2 DoD test:

1. N2 gene – 40.8, E gene – 44.6
2. N2 gene – 35.1, E gene – 33.6

Table 4. Clinical Performance of the Xpert Xpress SARS-CoV-2 DoD Test with 8-Sample Pools

	Individual Specimen Result		
8-Sample Pool Result	**Positive**	**Negative**	**Total**
Positive	18	0	18
Negative	2*	20	22
Total	20	20	40
PPA[1]	90.0% (95% CI: 69.9% - 97.2%)		
NPA[2]	100.0% (95% CI: 83.9% - 100.0%)		

*The Ct values for the two specimens in the 8-sample pool that were missed on the Xpert Xpress SARS-CoV-2 DoD test were:

- N2 gene – 40.8, E gene – 44.6
- N2 gene – 35.1, E gene – 33.6

[1]PPA was calculated using the Xpert Xpress SARS-CoV-2 DoD individual test results

[2]NPA was calculated using individual test results obtained with an FDA authorized high sensitivity SARS-CoV-2 RT-PCR assay

These results are acceptable.

2. *In Silico* Inclusivity Study Results

Cepheid provided revised *in silico* analyses for the Xpert Xpress SARS-CoV-2 DoD test because the EUA for the Xpert Xpress SARS-CoV-2 test contained inclusivity information based on a limited number of SARS-CoV-2 sequences available early during the pandemic. For the updated analysis, 110,206 SARS-CoV-2 sequences were downloaded from the GISAID database on October 21st, 2020. Some fraction of the GISAID sequences are tagged as 'high coverage", whereas others are tagged as having low coverage. While most sequences represent the complete genome of the virus, others are partial. After removal of partial sequences, 109,992 full length sequences (as defined by GISAID) for the E gene and 109,995 full length sequences (as defined by GISAID) for the N2 target gene remained and were used for *in silico* analysis.

<u>N2 target</u>

Two hundred eleven (211) matching sequences were excluded from the 110,206 sequences due to ambiguity codes and very low BLAST scores that fell below the minimum threshold for BLAST, which reduced the total to 109,995 sequences. The BLAST file provided in this EUA supplement included all matches from the BLAST analysis including the low scores that represent partial and low-quality matches. The Xpert Xpress SARS-CoV-2 DoD assay had 97.29% (107,076 / 109,995) match to the evaluated sequences, except for 2,919 sequences that had a single mismatch and sixty-three (63) sequences with two or more mismatches. The 63 sequences with additional mismatches were:

- One sequence contained two mismatches in the probe binding region and a third mismatch between oligos;
- Two sequences contained one mismatch in the forward primer region and a second mismatch in the reverse primer region;
- Six sequences contained one mismatch in the forward primer region and a second mismatch between oligos;

- Twenty-seven sequences contained one mismatch in the probe binding region and a second mismatch in the reverse primer region; and
- Twenty-seven sequences contained one mismatch in the forward primer region and a second mismatch in the probe binding region.

None of these mismatches are predicted to have a negative impact on the performance of the assay.

E target

Two hundred fourteen (214) matching sequences were excluded from the 110,206 sequences due to ambiguity codes and very low BLAST scores that fell below the minimum threshold for BLAST, which reduced the total to 109,992 sequences. The BLAST file provided in this EUA supplement included all matches from the BLAST analysis including the low scores that represent partial and low-quality matches. The Xpert Xpress SARS-CoV-2 DoD assay had 99.14% (109,045 / 109,992) match to the sequences, except for 926 sequences that had a single mismatch and 21 sequences with additional mismatches. The 21 sequences with additional mismatches were:

- One sequence contained one mismatch in the forward primer region and a second mismatch between oligos;
- One sequence contained one mismatch in the probe binding region and a second mismatch between oligos;
- One sequence contained two mismatches in the probe binding region,
- One sequence contained a 3-nucleotide deletion and multiple mismatches at the 3'-end of the probe region;
- Two sequences contained two mismatches in the forward primer region;
- Three sequences contained a 6-nucleotide deletion in the probe binding region with multiple mismatches at the 3' end of the amplicon; and
- Twelve sequences contained an 'AA' di-nucleotide, but this lies between the oligonucleotides used in the assay.

None of these mismatches are predicted to have a negative impact on the performance of the assay. These results are acceptable.

3. FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The samples were tested using the GeneXpert Dx System. The results are summarized in **Table 5**.

Table 5. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD (NDU/mL)	Cross-Reactivity
SARS-CoV-2	NP Swab	$5.4x10^3$	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

WARNINGS

- This product has not been FDA cleared or approved; but has been authorized by FDA under an EUA for use by authorized laboratories;

- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and,

- This product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

LIMITATIONS

- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.