Title: Report on Method Validation of a Cepheid Xpert® Xpress PCR Assay to Detect SARS-CoV-2

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Pfizer Vaccine Research and Development
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Pearl River, NY

Title: Report on Method Validation of a Cepheid Xpert® Xpress PCR Assay to Detect SARS-CoV-2

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Title: Report on Method Validation of a Cepheid Xpert® Xpress PCR Assay to Detect SARS-CoV-2

SYNOPSIS

This report summarizes the method validation for the Cepheid Xpert® Xpress SARS-CoV-2 PCR test performed using the GeneXpert® and GeneXpert® Infinity instruments at the Pearl River, NY Pfizer Vaccine Research & Development laboratories. The purpose of the Xpert® Xpress SARS-CoV-2 test is to objectively score nasal swabs from persons suspected of virus infection as either positive or negative for SARS-CoV-2. This document summarizes the detection limits, clinical sensitivity, and clinical specificity of the PCR test when used in Pfizer Vaccine Research & Development laboratories. The validation was performed with simulated virus samples, nasal swabs scored SARS-CoV-2 positive or negative by a predicate PCR test, pre-COVID-19 nasal swab samples, and live SARS-CoV-2. This report documents that the Xpert® Xpress SARS-CoV-2 PCR test met pre-specified acceptance criteria outlined in protocol VR-MVP-10076 and is suitable for its intended use as a diagnostic assay for detection of SARS-CoV-2 in clinical samples in support of clinical trials to evaluate the efficacy of Pfizer's SARS-CoV-2 vaccine candidate, or in other SARS-CoV-2 related epidemiological studies.

Key Validation Outcomes

Clinical Performance Parmenter	Observed Result	Expected Results
Detection Limit of Live Virus	100% detection at 0.01 focus forming units (FFU)/mL	0.01 FFU/mL
Detection Limit of AccuPlex™ SARS-CoV-2	100% detection at 250 copies/mL	250 copies/mL
Agreement in Positive Samples	100%	100%
Agreement in Pre-COVID-19 negative samples	100%	100%

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Study Number: N/A

Functional Area: Vaccine Research and Development

Test Facility: Pfizer Vaccine Research & Development
401 North Middletown Road,
Pearl River, NY 10965

Study/Testing Initiation Date: 20 July 2020

Study/Testing Completion Date: 25 July 2020

1. OBJECTIVES

This report summarizes the successful implementation of protocol VR-MVP-10076 [1] to validate the FDA-EUA approved Cepheid Xpert® Xpress SARS-CoV-2 RT-PCR assay as performed on the GeneXpert family of instruments for its intended use in Pfizer's clinical trials designed to evaluate the efficacy of Pfizer's SARS-CoV-2 vaccine candidate and other epidemiological studies to identify subjects infected with COVID-19. The assay method is described in VR-TM-10295 [2] (Method for Identification of SARS-CoV-2 in Nasal Swab Using the Cepheid Xpert® Xpress RT-PCR Assay Performed on the GeneXpert Molecular Diagnostic System). Assay sensitivity, specificity, and limit of detection have been evaluated and overall assay performance is described and summarized in this report.

2. INTRODUCTION

Pfizer, in collaboration with BioNTech, has rapidly developed a promising vaccine candidate to protect against COVID-19. To establish efficacy of the vaccine candidate through a clinical trial, Pfizer selected a commercially available diagnostic assay for detection of SARS-CoV-2 RNA in clinical trial volunteers. The diagnostic test, Xpert® Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA) is currently being marketed under an FDA Emergency Use Authorization (EUA) issued March 20, 2020 [3]. The performance characteristics of the Xpert® Xpress SARS-CoV-2 test are described in the package insert for this device, which has been updated as Cepheid generates additional data [4]. Several recent publications also describe the assay's performance [5, 6, 7, 8, 9]. The results of a multi-center study of sensitivity and accuracy of the Xpert® Xpress SARS-CoV-2 test substantiated Cepheid's diagnostic performance claim of detection of 0.01 plaque forming units per mL of live virus and detection of SARS-CoV-2 while exclusively not detecting other human coronaviruses [5]. In addition, the concordance of positive and negative sample results in the Xpert® Xpress test to other EUA SARS-CoV-2 tests has been published, with percent positive agreement and percent negative agreement consistently above 95% [6, 7, 8, 9].

The method validation protocol, VR-MVP-10076 (Method Validation Protocol for the Cepheid SARS-CoV-2 PCR Assay [1]), describes the methods used to evaluate the sensitivity, specificity and limit of detection of the FDA-EUA approved Cepheid Xpert® Xpress SARS-CoV-2 test performed on the GeneXpert family of instruments. Given that the assay is FDA-EUA approved, the protocol was based on information provided in Cepheid's EUA (March 20, 2020 [3, 4]) and other publications [7, 9] to demonstrate that the assay meets the sensitivity, specificity, and LOD described in EUA 200047/A001 when performed in the testing facilities at Pfizer Vaccine Research and Development, Pearl River, NY.

This report summarizes the results and conclusions of the executed validation protocol.

3. GLOSSARY

Table 1. Terms and Definitions

Term	Definition
Ct value	$Cycle_{threshold}$ is defined as the number of cycles required for the fluorescent signal (as measured in the reaction curve) to exceed the background fluorescence in the assay. The Ct value is the point (number of cycles) where the reaction curve crosses the threshold.
LOD	Limit of detection – the minimum FFU/mL that can be reliably classified as being positive with a confidence level of 95%
FFU	Focus Forming Unit. A single infectious SARS-CoV-2 viral particle, as measured by the replication of its progeny on a localized area of a cell monolayer, detected by fluorescence in a virus titration.
PCC	Probe Check Control. 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.
SPC	Sample Processing Control. A target included in the Cepheid SARS-CoV-2 assay. The SPC verifies that sample processing is adequate and detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional.

4. MATERIALS AND METHODS

Documents VR-TM-10295 [2] and VR-SOP-LC-11286 [10] describe a laboratory method for detection of SARS-CoV-2 in nasal swabs using the FDA EUA diagnostic Xpert® Xpress SARS-CoV-2 Assay (Cepheid, Sunnyvale, CA). The Xpert® Xpress SARS-CoV-2 test is marketed as a rapid, automated in vitro diagnostic test for the qualitative detection of SARS-CoV-2 gene sequences (N2 and E) in nasal swab samples. The test is intended to be used in Pfizer's clinical trials and other epidemiology studies to identify subjects infected with COVID-19. Detection of RNA sequences specific for SARS-CoV-2 is carried out by real-time multiplex RT-PCR following a single-step sample processing protocol and was performed according to the manufacturer's instructions.

The reagents described in Table 2 were used in the validation. The equipment used in the validation included GeneXpert IV units (b)(4) which were controlled by a (b) (4) and operated as a (b) (4), manually loaded instrument. In addition, GeneXpert IV unit

(b) (4) was operated manually in a (b) (4) laboratory, and GeneXpert Infinity (b) (4) was used with automated cartridge loading.

Table 2. Reagents and Sources

Reagent	Use	Source
AccuPlex™ SARS-CoV-2 Reference Material Kit, 0505-0126	External Run Control and spike for LOD Experiments	SeraCare, Milford, MA
(b) (4)	(b) (4)	(b) (4)
Nasal swabs from SARS-CoV-2 positive donors	Clinical Sensitivity Experiments	
Nasal swabs from SARS-CoV-2 negative donors	Clinical Specificity Experiments	
(b) (4)		
Nasal swabs from healthy donors collected pre-pandemic (b) (4)	Clinical Specificity Experiments	Pfizer Vaccine Research & Development, Pearl River NY
Xpert® Xpress SARS-CoV-2 Assay	Assay Kits	Cepheid, Sunnyvale, CA

4.1. Live SARS-CoV-2 Reporter Virus

(b) (4) The live virus was used to confirm the limit of detection of the Xpert® Xpress test (Supportive Table 10.2).

4.2. Clinical Specimens from SARS-CoV-2 Positive Donors

Remnant clinical specimens (Supportive Table 10.4) were purchased from (b) (4) for use in experiments to assess the Xpert® Xpress SARS-CoV-2 percent positive agreement to other molecular-based assays. The specimens were certified by the vendor to have received positive results in a predicate PCR assay, the (b) (4) SARS-CoV-2 PCR test.

4.3. Clinical Specimens from SARS-CoV-2 Negative Donors

Remnant clinical specimens (Supportive Table 10.5) were purchased from (b) (4) for use in experiments to assess the Xpert® Xpress SARS-CoV percent negative agreement to other molecular-based assays. The specimens were certified by the vendor to have received negative results in a predicate PCR assay, the (b) (4) SARS-CoV-2 PCR test.

4.4. Pre-Pandemic Nasal Swabs from Healthy Donors

Pfizer Vaccine Research & Development, Pearl River collected nasal swabs from healthy donors (Supportive Table 10.6) in the (b) (4) (b) (4) (b) (4). The swabs were stored at -80°C before testing. The specimens were used in experiments to assess the Xpert® Xpress SARS-CoV test rate of false positive results.

4.5. Methods

Test method VR-TM-10295 [2] describes the procedures to perform the Cepheid Xpert® Xpress SARS-CoV-2 Assay on nasal swabs collected from patients suspected of having COVID-19. Barcoded samples are received frozen from Sample Management and processed for testing as follows (see VR-TM-10295 [2] for details):

1. Scan the barcode of the specimen vial.

2. Thaw the patient sample containing nasal swab and media (~1 mL) at room temperature.

3. Vortex the patient sample tube and roll the swab against the side of the tube to express as much media as possible from the swab. Remove the swab from the tube and discard.

4. Using a transfer pipette from the Cepheid assay kit, squeeze the top bulb of the pipette completely and place the pipette tip in the specimen transport tube. Release the top bulb of the pipette to fill the pipette with sample from the patient specimen tube.

5. Squeeze the top bulb of the pipette completely again to empty the contents of the pipette into the large cartridge opening and close the cartridge lid.

6. Scan the barcode of the assay cartridge, and load into the instrument/sample module. Initiate the test via the Cepheid GeneXpert Dx version 4.7b or higher or Xpertise software.

4.6. Data Handling

4.6.1. Data Processing and Outputs via Instrument Software

1. Data are presented in qualitative ("Positive", "Negative") and semi-quantitative (Ct value) format for the 2 assay targets (N2 and E). For the validation, the results were interpreted automatically by the GeneXpert Dx software and shown in the View Results window. The Xpert® Xpress SARS-CoV-2 test provided results based on the detection of the two gene targets according to the algorithm shown in Table 3.

Table 3. Xpert® Xpress SARS-CoV-2 Possible Results

(b) (4)

a. Sample processing control. +/- indicates that the positive coronavirus result is accepted as valid for either a positive or negative result from the SPC. A strong positive result for either N2 or E can consume the available PCR reagents in the cartridge and prevent the SPC from amplifying.

(b) (4)

2. Semi-Quantitative data in the form of Ct values from positive and negative samples were captured for each sample run. The Ct values were used to determine average values, standard deviations and 95% confidence intervals for some validation data.

5. RESULTS AND DISCUSSION

Attachments in support of this validation are described in Table 4. Detection limit, clinical sensitivity and specificity data used for the validation analysis are listed in VR-MVR-10080-ATT01 (see Section 8).

Table 4. List of Attachments

Name	Content
VR-MVR-10080-ATT01 (see Section 8)	Data Listing
VR-MVR-10080-ATT02 (see Section 8)	Staff Member Roles and Batch Listing
VR-MVR-10080-Supplemental Info [14]	Cepheid Letter of Authorization
VR-MVR-10080-Supplemental Info 02 [15]	Xpert Xpress SARS-CoV-2 Assay Cut-off

The strategy, design, and acceptance criteria for the validation were described in VR-MVP-10076 [1], Method Validation Protocol for the Cepheid SARS-CoV-2 PCR Assay. The validation was intended to demonstrate that the Xpert® Xpress SARS-CoV-2 test could be used with acceptable sensitivity, specificity, and detection limits when performed in the Pearl River, NY Pfizer Vaccine Research & Development laboratories. To meet that goal, simulated samples were prepared from pooled nasal swab media or sterile (b) (4) (b) (4) spiked with AccuPlex™ SARS-CoV-2 Reference Material. Live virus samples were created by (b) (4) (Section 4.1) in (b) (4), and

remnant clinical samples were purchased. Finally, pre-COVID-19 samples collected from healthy volunteers in (b) (4) were tested (b) (4) All samples were tested according to methods outlined in VR-TM-10295 [2] and VR-SOP-LC-11286 [10].

The following sections describe the experimental designs, statistical analyses, acceptance criteria, and results for confirming the limit of detection and evaluating sensitivity and specificity of the Xpert® Xpress SARS-CoV-2 test.

5.1. Detection Limits

5.1.1. Detection Limits Study Design

The LOD of the Xpert® Xpress SARS-CoV-2 test was confirmed to the manufacturer's claims using both AccuPlex™ SARS-CoV-2 Reference Material and live (b) (4) (Section 4.1). For all experiments, (b) (4) Xpert® Xpress SARS-CoV-2 test kit was used. For AccuPlex™ SARS-CoV-2 Reference Material, (b) (4) of AccuPlex™ SARS-CoV-2 was prepared in pooled negative nasal swab clinical matrix (Section 4.3). A total of (b) (4) samples were prepared (Supportive Table 10.1). (b) (4) were prepared at 250 copies/mL, which is the LOD of the Xpert® Xpress SARS-CoV-2 test claimed in the Cepheid EUA. An additional (b)(4) samples were prepared at (b) (4), and another (b)(4) samples at (b) (4).

For live virus, (b) (4) samples were prepared (Supportive Table 10.2), (b) (4) at 0.01 FFU per mL, the LOD of the Xpert® Xpress SARS-CoV-2 test claimed in the Cepheid EUA; (b) (4) samples at (b) (4), and (b)(4) samples at (b) (4).

5.1.2. Statistical Analysis

The LOD is defined as the minimum amount of input AccuPlex™ SARS-CoV-2 Reference material or live reporter virus that yields a 95% probability of a positive result. The confirmation of LOD requires an independent percent positive rate that is at or above the estimated LOD level.

5.1.3. Acceptance Criteria

Results are considered descriptive in nature and compared to results provided in Cepheid's EUA document (EUA#200047/A001) [1, 4].

5.1.4. Detection Limits Results

The data collected to confirm the detection limits on simulated samples (Supportive Table 10.1) is summarized in Table 5. On AccuPlex SARS-CoV-2, (b) (4) samples at (b) (4) were detected. (b) (4) were detected, and (b) (4) samples at (b) (4) were detected. This result confirms the published results of detection at 250 copies/mL established by Cepheid [4] and confirmed by an independent clinical testing lab [7].

Table 5. Detection Limit on AccuPlex™ SARS-CoV-2 Reference Material

Description	N	Positive	Positive Rate (%)
(b) (4)	(b) (4)	(b) (4)	80.0
1xLOD (250 copies/ml AccuPlex™)			100.0
(b) (4)			100.0

The data collected on detection limits of live virus in samples (Supportive Table 10.2) is summarized in Table 6. On live virus, (b) (4) samples at 0.01 FFU/mL were detected. (b) (4) at (b) (4) were detected, and (b) (4) samples at (b) (4) were detected. These results confirm the published results of detection at 0.01 FFU/mL established by Cepheid [4].

Table 6. Detection Limit on Live Virus

Description	N	Positive	Positive Rate (%)
(b) (4)	(b) (4)	(b) (4)	75.0
LOD (0.01 FFU/ml Live virus)			100.0
(b) (4)			100.0

5.2. Clinical Performance

5.2.1. Clinical Performance Study Design

Similar to the design of the detection limit experiments, the clinical accuracy of the Xpert® Xpress SARS-CoV-2 PCR assay was confirmed to the manufacturer's claims using both simulated samples prepared with AccuPlex™ SARS-CoV-2 reference material and with remnant clinical samples.

5.2.2. Statistical Analysis

5.2.2.1. Percent Positive Agreement

For both simulated samples and clinical samples, the percent positive agreement and its 95% lower confidence bound were calculated for each target concentration. The mean Ct values and 95% confidence were calculate for each of the E and N2 nucleic acid targets.

The Positive Percent Agreement (PPA) was defined as the percentage of the positive samples detected by the assay:

PPA = 100*Number of Agreed Positives/Total Number Positive Samples by Predicate Method.

The one-sided lower 95% confidence bound for PPA was determined by the exact (b) (4) method.

5.2.2.2. Percent Negative Agreement

The percent negative agreement and its 95% lower confidence bound were calculated for negative samples.

The Negative Percent Agreement (NPA) was defined as the percentage of the negative samples confirmed by the assay:

NPA = 100*Number of Agreed Negatives/Total Number Negative Samples by Predicate Method.

The one-sided lower 95% confidence bound for NPA was determined by the exact (b) (4) method.

5.2.3. Clinical Performance Acceptance Criteria

Results are considered descriptive in nature and compared to results provided in Cepheid's EUA document (EUA#200047/A001) [1, 4].

5.2.4. Clinical Performance Results

5.2.4.1. Clinical Performance / Simulated Samples – AccuPlex™ SARS-CoV-2 Reference Material

The data collected to assess the assay's clinical accuracy on simulated samples are summarized in Table 7. (b) (4) All of the samples above the LOD were detected which confirms the clinical accuracy of the assay established by the manufacturer [4] and multiple published studies [5, 6, 7, 9].

Table 7. Clinical Performance on Simulated Positive Samples

Description	N	Positive	Positive % Agreement	One-Sided 95% CI Lower Limit (%)
(b) (4)				

5.2.4.2. Clinical Performance – Patient Samples

The data collected to assess the assay's clinical accuracy on positive samples is summarized in Table 8 and Table 9. (b) (4) remnant clinical samples (Supportive Table 10.4), previously determined to be positive using the (b) (4) SARS-CoV-2 PCR test as a predicate PCR method, were tested with one lot of Xpert® Xpress SARS-CoV-2 test kit. Overall, all

(b) (4) samples were detected in the Xpert® Xpress SARS-CoV-2 test (Table 8), and both gene targets returned mean Ct values below Cepheid's published positivity cutoff (Ct < 45).

Table 8. Clinical Performance on Positive Patient Samples

Description	N	Positive	Positive % Agreement	One-Sided 95% CI Lower Limit (%)
Pos Nasal Swab	(b) (4)	(b) (4)	100.0	90.5

Table 9. Cts and Confidence Intervals for Positive Clinical Samples

Target	N	Mean Ct	Standard Deviation	95% CI
E	(b) (4)	24.5	6.62	(22.1, 27.0)
N2	(b) (4)	26.7	6.02	(24.4, 28.9)

The data collected to assess the assay's clinical accuracy on negative samples is summarized in Table 10. (b) (4) remnant clinical samples (Supportive Table 10.5), previously determined to be negative using the (b) (4) SARS-CoV-2 PCR test as a predicate PCR method, were tested using one lot of Xpert® Xpress SARS-CoV-2 test. (b) (4) out of (b) (4) samples were negative in the Xpert® Xpress SARS-CoV-2 test. One sample was unable to be tested due to a cartridge error during processing. One sample had a Ct of 41 for the N2 target only and is considered positive per VR-SOP-LC-11286 [10].

Table 10. Clinical Performance on Negative Patient Samples

Description	N	Negative	Negative % Agreement	One-Sided 95% CI Lower Limit (%)
Neg Nasal Swab	(b) (4)	(b) (4)	96.4	84.1

5.2.4.3. Clinical Performance – Pre-COVID-19 Samples

The single negative patient sample described in Section 5.2.4.2 with an N2 target positivity created concerns about the false positive rate of the Xpert® Xpress SARS-CoV-2 test. To address this question, (b) (4) samples collected from healthy individuals in (b) (4) (Supportive Table 10.6), before SARS-CoV-2 infection was identified in the US, were tested using one lot of Xpert® Xpress SARS-CoV-2 kit.

All (b) (4) samples produced negative results in the Xpert® Xpress SARS-CoV-2 test, giving a false positive rate of 0% (Table 11).

Table 11. Clinical Performance on Pre-COVID-19 Samples

Description	N	Negative	Negative % Agreement	One-Sided 95% CI Lower Limit (%)
Pre-COVID-19 swab	(b) (4)	(b) (4)	100.0	95.8

6. CONCLUSION

This validation report provides documented evidence that the Xpert® Xpress SARS-CoV-2 test, when performed by qualified personnel in accordance with standard operating procedures VR-TM-10295 [2] and VR-SOP-LC-11286 [10] is suitable for its intended purpose of testing of nasal swabs from persons with suspected infection or exposure to SARS-CoV-2. This assay will be used to test samples from Pfizer's clinical efficacy trial of a SARS-CoV-2 vaccine candidate and other epidemiological studies.

7. DEVIATIONS

1. VR-MVP-10076 'Method Validation Protocol for the Cepheid SARS-CoV-2 PCR Assay" section 6.3 'Clinical Specimens from SARS-CoV-2 Positive Donors' states remnant clinical specimens were purchased for use in experiments to define the LOD and percent agreement of the assay. This was an error. Those specimens were used to define the percent agreement only and not to defne the LOD.

2. A deviation to the rules for sample scoring documented in VR-MVP-10076 [1] occurred in this validation. VR-MVP-10076 indicated Pfizer Vaccine Research & Development will consider a sample positive for SARS-CoV-2 only if both the N2 and E gene targets are detected. After reviewing the data for the LOD confirmation shown in VR-MVR-10080-ATT01 (see Section 8) it became evident that following that rule would inappropriately exclude actual positive samples. Although the E target has potential to detect the original SARS coronavirus and bat coronavirus, our results demonstrate that spiked, known SARS-CoV-2 samples can be scored E+/N2- at concentrations close to the limit of detection. In consultation with Cepheid and after reviewing additional published data [9] Pfizer Vaccine Research & Development decided to follow the scoring recommendations in the manufacturer's instructions for use [4]. The impact of this deviation was better data agreement between LOD and percent sensitivity and specificity.

3. During testing of clinical performance, the robotic arm that feeds cartridges into the analysis instrument had a mechanical failure, which resulted in 12 samples sitting more than 30 minutes before processing started. There was no negative clinical matrix available to create additional samples for repeat testing, so new samples were created in sterile (b) (4). To address concerns that the matrix change would confound the results, a new sample set with (b) (4) samples at the LOD, (b) (4) (b) (4) was prepared and tested. Samples (b) (4) were negative nasal swab media, and (b) (4) were sterile (b) (4). The impact of the deviation was an unacceptably low N for analysis. Creation of the new sample set permitted retesting with a higher N for analysis of clinical performance.

4. The validation protocol specified that (b) (4) negative clinical samples would be tested as part of clinical performance/ percent negative agreement. Only (b) (4) samples could be obtained in time for the validation, and one of the samples was received from the vendor containing 130 µl, an insufficient volume for testing. The impact of the deviation is that the percent negative agreement calculation was limited to (b) (4) samples. The impact was mitigated by testing pre-COVID-19 swabs in addition to the (b) (4) negative swabs.

5. VR-MVP-10076 "Method Validation Protocol for the Cepheid SARS-CoV-2 PCR Assay" section 6.4 stated remnant clinical specimens were purchased for use in experiments to define the LOD, sensitivity and percent agreement of the assay. Shipping and purchasing delays due to the pandemic resulted in the remnant clinical specimens not arriving in time for the validation experiments. The experiments to define LOD and sensitivity used (b) (4) as matrix instead of remnant clincal specimens.

6. During validation testing an additional (b) (4) nasal swabs samples were tested that pre-date the existence of COVID-19 by approximately 2.5 years. These samples would be considered better predictors of negative results when assessing assay clinical specificity; therefore, these (b) (4) samples were added to the validation testing and documented in this report as a separate analysis of assay clinical specificity.

7. VR-MVP-10076 "Method Validation Protocol for the Cepheid SARS-CoV-2 PCR Assay" section 7.1.1 states "SARS-CoV-2 prepared in pooled NP swab clinical matrix". It was an oversight that the protocol did not specifically state that the pooled clinical matrix was negative for SARS-CoV-2, as specified in Section 5.1.1 of this report.

8. SUPPORTING DOCUMENTATION

1. VR-MVR-10080-ATT01, Supplemental Information for VR-MVR-10080- Validation Data Listing.

2. VR-MVR-10080-ATT02, Supplemental Information for VR-MVR-10080 – Roles and Responsibilities for Method Validation.

9. SUPPLEMENTAL INFORMATION

1. VR-MVP-10076, Method Validation Protocol for the Cepheid SARS-CoV-2 PCR Assay.

2. VR-TM-10295, Method for Identification of SARS-CoV-2 in Nasal Swab using the Cepheid Xpert® Xpress RT-PCR Assay Performed on the GeneXpert Molecular Diagnostic System.

3. USDHHS, Response to Cepheid application for Emergency Use Authorization 200047/A001. March 20, 2020.

4. Xpert® Xpress SARS-CoV-2 Instructions For Use. 302-3562, Rev C. April 2020. Downloaded from https://www.cepheid.com/ /en_US/package-inserts/1615 on June 8, 2020.

5. Loeffelholz MJ, Alland D, Butler-Wu SM, et al. Multicenter evaluation of the Cepheid Xpert Xpress SARS-CoV-2 test. J Clin Microbiol 2020;58(8)(Jul):e00926-20.

6. Lieberman JA, Pepper G, Naccache SN et al. Comparison of commercially available and laboratory-developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories. J Clin Microbiol 2020;58(8)(Jul):e00821-20.

7. Zhen W, Smith E, Manji R, et al. Clinical evaluation of three sample-to-answer platforms for detection of SARS-CoV-2. J Clin Microbiol 2020;58(8)(Jul):e00783-20.

8. Moran A, Beavis KG, Matushek SM, et al. Detection of SARS-CoV-2 by use of the Cepheid Xpert Xpress SARS-CoV-2 and Roche cobas SARS-CoV-2 assays. J Clin Microbiol 2020;58(8)(Jul): e00772-20.

9. Lowe CF, Matic N, Ritchie G et al. Detection of low levels of SARS-CoV-2 RNA from nasopharyngeal swabs using three commercial molecular assays. J Clin Virol 2020;128:104387.

10. VR-SOP-LC-11286, Data Review for Cepheid SARS-CoV-2 RT-PCR Assay.

11. VR-SOP-LC-11294, Preparation and Assessment of SARS-CoV-2 Stocks.

12. (b) (4)

13. (b) (4)

14. VR-MVR-10080-Supplemental Info - Cepheid Letter of Authorization.

15. VR-MVR-10080-Supplemental Info 02 - Xpert Xpress SARS-CoV-2 Assay Cut-off.

10. SUPPORTIVE TABLES

10.1. Detection Limit Simulated Samples

Sample Number	Sample ID	Sample Number	Sample ID
(b) (4)			

10.2. Detection Limit Live Virus Samples

Sample Number	Sample ID	Sample Number	Sample ID
(b) (4)			

10.3. Clinical Performance Simulated Samples

Sample Number	Sample ID	Sample Number	Sample ID	Sample Number	Sample ID
(b) (4)					

10.3. Clinical Performance Simulated Samples

Sample Number	Sample ID	Sample Number	Sample ID	Sample Number	Sample ID
(b) (4)					

10.4. Positive Clinical Samples

Sample Number	Sample ID	Sample Number	Sample ID
(b) (4)			

10.5. Negative Clinical Samples

Sample Number	Sample ID	Sample Number	Sample ID
(b) (4)			

a. This sample was not tested because it was received from the vendor with insufficient volume.

10.6. Pre-COVID-19 Samples

Sample Number	Sample ID	Sample Number	Sample ID	Sample Number	Sample ID
(b) (4)					

10.6. Pre-COVID-19 Samples

Sample Number	Sample ID	Sample Number	Sample ID	Sample Number	Sample ID
(b) (4)					

Document Approval Record

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