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| GeneXpert Xpress Multiplex Vaginal Panel (MVP) Assay  |
| **Purpose** | This procedure provides instructions for performing the Xpert Xpress Multiplex Vaginal Panel (MVP) assay on the Cepheid GeneXpert system. |
| **Policy Statements** | This procedure applies to all technical staff performing testing on the GeneXpert. |
| **Principle and Clinical Significance** | The Xpert Xpress MVP Assay is an automated *in vitro* diagnostic test for qualitative detection of DNA targets for anaerobic bacteria associated with bacterial vaginosis (BV), *Candida* species associated with vulvovaginal candidiasis, and *Trichomonas vaginalis,* the agent of trichomoniasis. The Xpert Xpress MVP test is performed on the GeneXpert Instrument Systems using clinician-collected and self-collected vaginal swabs from patients who are symptomatic for vaginitis/vaginosis. The Xpert Xpress MVP test utilizes PCR for the amplification of specific DNA targets and utilizes fluorogenic target-specific hybridization probes to detect and differentiate DNA from:* Organisms associated with bacterial vaginosis (detected organisms not reported individually).
* *Atopobium* spp. (*Atopobium vaginae,* *Atopobium* novel species CCUG 55226)
* Bacterial Vaginosis-Associated Bacterium 2 (BVAB2)
* *Megasphaera*-1
* *Candida* spp. (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*, species not differentiated)
* *Candida glabrata/Candida krusei* (species not differentiated)
* *Trichomonas vaginalis*

The Xpert Xpress MVP is intended to aid in the diagnosis of vaginal infections in women with a clinical presentation consistent with bacterial vaginosis, vulvovaginal candidiasis, or trichomoniasis. The most common causes of vaginosis and vaginitis are: 1) proliferation of one or more anaerobic bacterial species in the vaginal tract leading to vaginal discharge without inflammation (22-50% of symptomatic women), known as bacterial vaginosis; 2) vulvovaginal candidiasis (17-39%); and 3) trichomoniasis (4-35%). Symptoms in undiagnosed women may be caused by a broad array of non-infections conditions, including atrophic vaginitis, various vulvar dermatologic conditions, and vulvodynia. Abnormal vaginal discharge has a broad differential diagnosis, and successful treatment typically requires an accurate diagnosis. The GeneXpert automates and integrates sample preparation, nucleic acid extraction, amplification, and detection of the target sequences in clinical specimens by using real-time PCR. The Xpert Xpress MVP test includes reagents for the detection of DNA from BV organisms, *Candida*species, and *Trichomonas vaginalis* from vaginal swab samples. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for an adequate amplification process and to monitor for the presence of inhibitors in the PCR reaction. The SPC also ensures that the PCR reaction conditions are appropriate for the amplification and that the PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.  |
| **Test Code** | **MVPP** |
| **Sample** | 1. **Acceptable specimens:**
* Vaginal swabs collected in the Xpert Swab Specimen Collection Kit (SWAB/G-50)
1. **SDES codes/Specimen type:**
* VAG -vagina
1. **Specimen Collection and Transport:**
* Refer to *Lab Test Directory* on StarNet
1. **Specimen assessment:**
* Refer to the policy MCVI 2.1 *Specimen Rejection Criteria.*
1. **Specimen Storage**
* Room temp or refrigerated (2-28°C): 42 days
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| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual*:1. *Biohazard Containment*
2. *Safety in the Microbiology Laboratory*
* *Biohazardous Spills*
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| **Materials** |

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| Reagents | Supplies | Equipment |
| * Household bleach
* 70% ethanol
 | * Xpert Swab Specimen Collection Kit (SWAB/G-50)
* Xpert Xpress MVP cartridges
* Transfer pipettes
* Simple racks
* Cartridge transfer tray
 | * Biosafety Hood
* Cepheid GeneXpert Instrument and computer
* Printer
* Vortex
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| **Storage** | Store kits at 2-28°C. Kits are stable until the expiration date printed on the outer box.  |
| **Calibration** | Annual “Xpert Check Kit” calibration performed by Cepheid. |
| **Quality Control** | **Daily Quality Control:**Once an Xpert cartridge has been loaded and before the sample processing steps begin, the software checks the optics, the readiness of the module’s mechanical components, and the ambient temperature of the module to assure proper performance of PCR, and the physical integrity of the cartridge. **Quality Control**Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).* **Sample Processing Control (SPC):** Ensures the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the assigned acceptance criteria.
* **Probe Check Control (PCC):** Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the assigned acceptance criteria.

**External Quality Control:*** Perform QC using external positive and negative controls every 30 days. Record results in the GeneXpert assay binder on the Log.
* See IQCP document.
* See Quality Control Procedure.

**New Lot/Shipment Quality control:*** Perform QC using external positive and negative controls with each new lot or shipment before putting into service. Record results in the GeneXpert assay binder on the Log.
* See Quality Control Procedure

**Wipe testing control:*** Perform wipe testing every 30 days to monitor for contamination.
* See Quality Control Procedure.

**NOTE:** External quality control may be performed on an as needed basis if certain circumstances arise. Examples include:* Drift in results (e.g., increasing/decreasing positivity rates)
* Potential contamination (negative control)
* After drastic system maintenance
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| **Procedure** | **Cartridge preparation:**1. Clean hood with 10% bleach dilution (made daily) followed by 70% ethanol.
2. Change gloves.
3. Obtain an Xpert Xpress MVP Assay cartridge, transfer pipette, and sample transport tube to be tested.
4. Label the side of the cartridge with a bar-coded foot-label.
5. Open the cartridge lid.
6. Vortex the sample for 5-10 seconds.
7. Open the transfer pipette wrapper on the side of the squeeze bulb.
8. Carefully unscrew the sample lid, completely squeeze the top bulb of the pipette until it is fully flat and place into sample transport tube. Release the top bulb of the pipette to draw up specimen in the transfer pipette (560µL). Excess samples may go into the overflow reservoir bulb.

See **Figure 1**.  Figure 1. Transfer pipette1. Insert the pipette to the bottom of the well in the cartridge and squeeze the top bulb completely to empty the pipette’s content into the cartridge. Continue to squeeze the top bulb until the pipette is removed from the cartridge. Dispose of the pipette. See **Figure 2**.

1. Close the cartridge lid, and set onto the transfer tray.
2. Change gloves and proceed to prepare additional samples or start the test.

NOTES: -Hood surfaces must be cleaned between samples with 10% bleach dilution followed with 70% ethanol if there were any splashes, spills, or uncertainty of cleanliness. -\*\*Start the test within 30 minutes of adding the sample to the cartridge**Starting the test:**1. Ensure clean gloves are on before stepping to the computer work space.
2. If instrument and computer are turned off: start up the instrument by flipping the power switch located in the back of the instrument. Turn on the computer next.
3. Log onto the appropriate Windows account:
	1. User: Cepheid-Admin
	2. Password: cphd
4. The GeneXpert software will launch automatically. If it doesn’t double-click the GeneXpert Dx software shortcut icon on the desktop.
5. Log onto the software.
	1. User: First 6 letters of your first and last name (combined)
	2. Password: First 6 letters of your first and last name (combined)
6. In the GeneXpert System window, click **Create Test.**
7. Navigate to the **Sample ID** box. Scan or type in the sample ID.
8. Scan the barcode on the cartridge.

NOTE: if the barcode on the cartridge does not scan, then repeat the test with a new cartridge.1. If prompted, select Xpert MVP from the **Select Assay MENU.**
2. Select the appropriate test type for samples or controls.
3. Enter additional information in the “notes” field (day of QC, collect date, etc.) if needed.
4. Click **Start Test**.
5. Enter your username and password, if requested.
6. Open the instrument module door with the blinking green light.

NOTE: when setting up for testing you may opt to use any available module.1. With the barcode facing towards you, set the cartridge into the module and close the door.
2. Wait for the test to start and the light to stop blinking. The test will run for 60 minutes.
3. Turn printer on.
4. Remove the cartridge when testing is finished (the light will be off and the system will release the door lock).
5. Dispose of used cartridges into bio-bags and place into biohazard sharps bins.
6. Clean any equipment used (pipettes, racks, transfer tray, etc.), hood, and counters (including keyboard, scanner, and mouse) at the end of the day.

NOTE: Sample processing, testing, and cleaning should follow a unidirectional work-flow to avoid contamination.  |
| **Interpretation/ Results**  | 1. Reports will print automatically after testing has been completed
2. If report does not print, check to see if printer is on
3. To reprint reports: Click on **View Results** on the top menu bar of the GeneXpert Dx software, select Report from the bottom menu bar, select report you want to print, click Preview PDF, and click printer icon to print.
4. Place large patient label on results.

**Result Interpretation**1. The results reported are interpreted automatically by the GeneXpert Instrument System.
	1. NOTE: SPC does not need to pass for a positive result to be valid.
	2. NOTE: SPC does need to pass for a negative result to be valid.
2. Refer to **Table 1** for result interpretation.

**Table 1: MVP Instrument Results and Interpretations** **Reasons to retest the original sample:**1. An INVALID result (SPC failure). This may indicate:
	1. The sample was not properly processed.
	2. PCR was inhibited.
2. An ERROR result. This may indicate:
	1. The reaction tube was filled improperly.
	2. A reagent probe integrity problem was detected.
	3. The maximum pressure limit was exceeded.
	4. A valve positioning error was detected.
3. NO RESULT:
	1. This result indicated that insufficient data were collected (e.g. test stopped while in progress or power failure occurred).

NOTE: Record any failures on the “GeneXpert Service and Error Log” log. **Retesting procedure:** 1. Obtain the original sample and a new cartridge.
2. Retest the sample according to the instructions in this SOP.
3. Report results according to **Table 2** below.

**Table 2: Retesting results and interpretation**

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| **Initial result** | **Repeat Result**  | **Report**  |
| **INVALID** | INVALID | Unresolved  |
| VALID | Valid results |
| **ERROR** | ERROR or INVALID | Unresolved |
| VALID | Valid results |
| **NO RESULT** | NO RESULT, ERROR or INVALID | N/A – repeat testing |

1. See the instructions below for reporting unresolved results.

NOTE: Record any failure, errors, and repeat testing on the “GeneXpert Service and Error Log” log.  |
| **Result Reporting** | 1. Ensure that the printer is turned on.
	1. Reports will print automatically.
	2. Put large patient label on report.
2. Valid results will automatically transmit to the LIS and be auto-verified.

**NOTE**: you must check your results upon completion of testing to ensure validity of results 1. At the end of the shift call a completed worksheet for MVPP, check results, and staple to GeneXpert Report. Place in the GeneXpert MVP result binder.
2. Store samples in fridge:
	1. Mark positive samples on top of caps.
3. Discard old samples after 2 weeks.
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| **Critical Results** | No critical result values.  |
| **Reporting Invalid (unresolved) Results** | 1. Notify the care provider of the unresolved result.
2. Log into Sunquest to release results.
3. Select Result Entry from Menu options
4. In the Configuration field select CGX from the dropdown box.
5. Click on the  button located in the lower right corner to populate the transmitted results.
6. Review messages located on the top and results. Compare results to the GeneXpert report.
7. The result will be reported as **unresolved** (UNRE) and the following code SIA will automatically append: “This sample is inhibitory to amplification and the results are inconclusive. Consider repeat collection if clinically indicated.”
8. Add the code CAL to one of the results, press tab, enter semi-colon and record who the result was relayed to and the date/time.
9. Check the release box.
10. Click  button located on the lower left corner. Click  when the “Verify Release Destination” window opens.
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| **Reporting Invalid (Unresolved) and Quantity not Sufficient Results** | 1. When only 1 pink Cepheid Collection device is received for MVPP and CGPCR testing, an Invalid/Error occurs and there is not enough sample to repeat testing:
* Report UNRE (Unresolved) and the following code SIA will automatically append.
* Add QNS (Quantity not sufficient)
* Free text :FOR REPEAT TESTING
* Notify the care provider of the unresolved and QNS result.
* Follow instructions above or enter manually.
* If entering manually, you will need to result each test box.

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| **Correcting Results** | 1. Open Result Entry, select the Manual resulting mode (top left corner) from the configuration drop down, select the appropriate test. Click  in the lower right corner.
2. Enter the Specimen ID, enter Tab and click Yes to modify the result.
3. Change the incorrect result. The corrected result comment will automatically append. Add the CAL comment, press tab, enter a semi-colon and record who was called and the time/date.

 1. Click . Click  when the “Verify Release Destination” window opens.
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| **Limitations** | * The Xpert Xpress MVP test has been validated using the procedures provided in this Instructions for Use only. Modification to these procedures may alter the performance of the test.
* The Xpert Xpress MVP test has been validated with vaginal swabs collected with the Xpert Swab Specimen Collection kit
* Testing of vaginal swab specimens with the Xpert Xpress MVP test is not intended to replace an exam by a clinician. Vaginal infections may results from other causes or concurrent infections may occur.
* As with many diagnostic tests, results from the Xpert Xpress MVP test should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
* Public health recommendations should be consulted regrading testing for additional sexually transmitted diseases for patients with a positive result for bacterial vaginosis (BV) or *T. vaginalis* with the Xpert Xpress MVP test.
* The Xpert Xpress MVP test targets three anaerobic microorganisms that are associated with BV. Other organisms that are not detected by the Xpert Xpress MVP test have also been reported to be associated with BV.
* A *Candid*a group positive results can be due to one or multiple *Candida* species.
* Candida species can be present as commensal organisms in women; the Xpert Xpress MVP positive results for Candida should be considered in conjunction with other clinical and patient information to determine the disease status.
* The BV organism target of the Xpert Xpress MVP test can be commensal in women; Xpert Xpress MVP positive results for bacterial vaginosis should be considered in conjunction with other clinical and patient information to determine the disease status.
* Erroneous test results might occur from improper specimen collection, technical error, sample mix-up, or because the number of organisms in the specimen is not detected by the test. Careful compliance with the Instructions for Use and to the Xpert Swab Collection Kit instructions document are necessary to avoid erroneous results.
* A negative test results does not exclude the possibility of infection because test results may be affected by improper specimen collection, technical error, sample mix-up, concurrent antibiotic therapy, or the number of organisms in the specimen that may be below the sensitivity of the tests.
* False negative results may occur if the organism is present at levels below the analytical limit of detection or below the cut-off concentration.
* Mutations of other changes within the regions of the microbial genomes covered by the primers and/or probes in the Xpert Xpress MVP test may result in failure to detect the target organisms.
* The effects of other potential variable such as vaginal discharge, use of tampons, douching, and specimen collection variables have not been determined.
* The Xpert Xpress MVP test provided qualitative results. No correlation can be drawn between the magnitude of the Ct value and the number of cells in an infected sample.
* The Xpert Xpress MVP test performance has been evaluated in patients 18 years of age and older (including pregnant women).
* The Xpert Xpress MVP test has not been validated for use with vaginal swab specimens collected by patients at home. The self-collected vaginal swab specimen application is limited to healthcare facilities where support/counseling is available to explain procedures and precautions.
* Five strains of *Candida albicans* evaluated in the Inclusivity Study were detected by the Xpert Xpress MVP test. Three of the strains were only detected at concentration higher than 3xLoD.
* Eleven strains of *Atopobium spp*. evaluated in the Inclusivity Study were detected by the Xpert Xpress MVP test. Four of the strains were only detected at concentrations higher than 3xnear cut-off concentration.
* *Candida orthopsilosis*, a recently described species that has been grouped previously with C. parapsilosis, was found to cross-react with the Xpert Xpress MVP test at levels above 1x102 CFU/mL. *Pentatrichomonas hominis* (a commensal of the large intestine) was found to cross-react with the Xpert Xpress MVP test at levels above 5x104 cell/mL. *Trichomonas tenax* (a commensal of the oral cavity) was found to cross-react with the Xpert Xpress MVP test at level above 10 cells/mL.
* Interference with Xpert Xpress MVP test was observed in the presence of mucin (from porcine stomach).
* The analyte target may persist *in vivo*, independent of pathogen viability. Detection of the analyte does not imply that the corresponding pathogen is infectious, or is the causative agent of the clinical symptoms.
* The Xpert Xpress MVP test cannot be used to assess therapeutic success or failure since target nucleic acids may persist following antimicrobial therapy.
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| **Method Performance Specifications** | According to the manufacturer (per the package insert) – Overall specifications are shown in **Table 3**:**Table 3: Overall Performance of Xpert Xpress MVP Test** |
| **References** | Xpert Xpress MVP Instructions for Use, 301-8994, Rev. E, October 2022. Sunnyvale, CA: Cepheid. |
| **Alternate Methods** | 1. *Trichomonas* PCR (TVPCR)
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| **Proficiency Testing** | CAP Molecular Vaginal Panel (MVP): 3 shipments a year with 5 samples. |
| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer.
 | 1. Direct observation
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Susan DeMeyere | 6/6/2023 | Initial Version |
| 2 | Susan DeMeyere | 4/19/2024 | Added resulting instructions for UNRE and QNS with 1 pink collection device. |
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| **Archived by:** |  | **Archived Date:** |  |