Policy Statement	Laboratory personnel are responsible for insuring the specimen submitted for testing is acceptable and adhering to the procedure for performing the test.
Purpose	This procedure provides technical instruction for the performance of the BD Affirm.
Scope	This procedure applies to technical personnel authorized to perform testing. This group includes, but is not limited to Medical Technologists as well as leads and supervisory personnel.
Responsibility	All the above personnel are responsible for following this procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.

Specimen Handling and Retention

Specimen collection is a critical step. Personnel collecting vaginal fluid specimens should be well-trained to minimize the possibility of inadequate specimens. For specimen collection, use only the Affirm VPIII Ambient Temperature Transport System (ATTS). In order to obtain an adequate specimen, the procedure for specimen collection must be followed closely.

Preparation of Collection Tube using ATTS

1. Open the seal on outer plastic pouch of ATTS and remove all components [each plastic pouch contains enough material for the collection and transport of one vaginal specimen].
2. Tear open the foil pouch and remove the ATTS Reagent Dropper.
3. Break ampule in ATTS Reagent Dropper by firmly squeezing vial with finger and thumb.

Caution: Break ampule close to its center one time only. Do not manipulate dropper any further, as the plastic may puncture and injury may occur.

4. Dispense reagent from ATTS Reagent Dropper into Sample Collection Tube.
5. Peel wrapper to expose patient swab. Remove swab. Discard wrapper.
6. Collect patient specimen.

Vaginal Sample Collection

1. Place the patient in position for a pelvic examination. Insert an UNLUBRICATED speculum (WITHOUT JELLY OR WATER) into the vagina to permit visualization of the posterior vaginal fornix.
2. Using the sterile swab, obtain a sample from the posterior vaginal fornix. Twist or roll the swab against the vaginal walls two or three times, ensuring the entire circumference of the swab has touched the vaginal wall. Swab the lateral vaginal wall while removing the swab.
3. Immediately place the swab into the Sample Collection Tube.

4. With the swab touching the BOTTOM of the collection tube, grasp the pre-scored handle of the swab just above the top of the tube and bend until the swab breaks. When the swab is fully inserted into the collection tube, the score mark on the swab is approximately 1 cm above the top of the collection tube. Discard the broken handle into an infectious waste container.
5. Place the cap over the exposed end of the swab and firmly press the cap onto the tube. The cap will “snap” onto the tube when it is properly seated.
6. Label the Sample Collection Tube with a Meditech barcode or patient identification information. Include the time the sample was collected and collector’s name.

Handling

With using the Affirm VPIII Ambient Temperature Transport System (ATTS) the total time between sample collection and proceeding with sample preparation should be no longer than 72 hours when the specimen is stored at ambient (15 to 30°C) or refrigerated conditions (2 to 8°C).

Retention

Once a sample has arrived in the laboratory the following requirements must be met to ensure specimen integrity.

- All samples must be received in the laboratory module of Meditech.
- All samples must be retained in the appropriate rack until tested to reduce chance of specimen loss.
- Samples should not be opened until testing is performed to eliminate chance for contamination or alteration.
- A pending log should be checked to verify that all samples are accounted for in the laboratory.

Reagents and Supplies

Reagents
All reagents are supplied ready for use.

- Probe Analysis Cards (PAC) (24 tests): Individually packaged cards, wrapped in an absorbent paper towel moistened with a solution containing sodium azide (0.1%, w/v) as a preservative. Each card contains the following five beads: Negative Control, *Trichomonas*, *Gardnerella*, *Candida*, and Positive Control.
- Reagent Cassettes (RC) (24 tests): Reagents are sealed in multi-well, foil-covered cassettes. Each cassette has seven wells. From front to back the wells contain:
  - Well No. 1: Patient Sample Reservoir, supplied empty
  - Well No. 2: Hybridization Solution, 350 µL: Color development probe, Formamide, Buffered chaotropic solution
  - Well No. 3: Wash Solution, 750 µL: Detergent, Buffer solution, Preservative (Proclin™)
  - Well No. 4: Conjugate, 500 µL: Enzyme conjugate, Preservative (Proclin)
  - Well No. 5: Wash Solution, 750 µL: Detergent, Buffer solution, Preservative (Proclin)
  - Well No. 6: Wash Solution, 750 µL: Detergent, Buffer solution, Preservative (Proclin)
  - Well No. 7: Substrate Buffer, 500 µL: Buffered Peroxide Solution
- Substrate Solution (S) (Red Cap, 3.4 mL for 24 tests; Bottle): Individually packaged solution in foil pouch; Indicator substrate, Stabilizing agent, Alcohol
- Lysis Solution (L) (Blue Cap, 10.8 mL for 24 tests): Detergent, Buffer solution, Preservative (Proclin)
- Buffer Solution (B) (Green Cap, 15 mL for 24 tests): Buffered chaotropic solution, Formamide
- Filter Tips (FT) (24 tests)
- Sample Collection Caps (SCC) (24 tests)
- Sample Collection Tubes (SCT) (24 tests)

- Individually wrapped, pre-scored, sterile swabs (24 tests)

Reagent Guidelines

The Affirm VPIII test kit is stable until the expiration date indicated on the kit box when stored at 2 to 8°C. Alternatively, store kits at room temperature (up to 30°C) no more than 3 months. For convenience, store all reagents at room temperature once opened. Only one kit can be left at room temperature at a time. All reagents and PACs must be at 22 to 28°C prior to use. If refrigerated, allow to sit at room temperature a minimum of 30 minutes prior to use. Indicate the date the reagents were removed from the refrigerator and the technologist name on the box.

Note: The Buffer Solution (B) precipitates under refrigeration. Allow the solution to come to room temperature for at least 30 minutes and then agitate the bottle for 10 to 15 seconds until any precipitate is dissolved.

Testing should be performed our of one kit box at a time. Do not mix kit box components, even if it is the same lot number.

Supplies

- Affirm VPIII Ambient Temperature Transport System, (100 systems)
  - Individually wrapped, pre-scored, sterile polyester-tip swab
  - Sample Collection Tube
  - Sample Collection Cap
  - Ambient Temperature Transport Reagent Dropper (in foil pouch).
- BD MicroProbe Processor
- BD MicroProbe Lysis Block
- Isensix Temperature sensors
- Lysis Solution and Buffer Solution
- Timer

Warnings and Precautions

For *in vitro* Diagnostic Use.

Read all instructions carefully before use.

For specimen collection, use only the Affirm VPIII Ambient Temperature Transport System. Use only vaginal fluid specimens from patients with symptoms of vaginitis/vaginosis.

The Isensix temperature monitoring system will ensure that the temperature of the Lysis Block is 80 - 90°C, the testing environment temperature is 22 - 28°C and that the testing environment humidity is 10-85%.

Substrate Solution (S): Substance contains alcohol and is combustible. Keep away from heat, sparks and flame. Keep container tightly closed to prevent evaporation.

Paper Towel surrounding PAC: Towel is moistened with sodium azide (0.1%, w/v). Sodium azide is very toxic by inhalation, in contact with skin and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water.

Reagents contain ingredients that could be irritating or caustic if allowed to come in contact with skin, eyes or mucous membranes. Wear gloves, safety glasses and lab coat, and use standard laboratory precautions when handling. If swallowed, call a physician. In case of skin or eye contact, flush with copious amounts of water.

Pathogenic microorganisms including hepatitis viruses and Human Immunodeficiency Virus may be present in clinical specimens. “Standard Precautions”[17-20] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

Proper handling and disposal methods should be established. Wipe up spillage of patient specimens immediately and disinfect with an appropriate disinfectant. Treat the cleaning materials as biohazardous waste.

The sterile swab should not be used if the packaging is open or damaged. Avoid touching the beads. Avoid contaminating reagent bottles. Do not use a reagent after its expiration date.

Quality Control

The Affirm VPIII Microbial Identification Test includes two internal controls on each PAC: a Positive Control bead and a Negative Control bead. These control beads are tested simultaneously with each patient specimen, ensuring the proper performance of PAC, Reagent Cassette (RC) and Processor. The Positive Control also ensures the absence of specimen interference. The Negative Control also ensures the absence of non-specific binding from the specimen.

In a properly functioning test, the Positive Control bead will be blue and the Negative Control bead remains colorless (i.e., absence of blue color) after processing. If the Positive Control does not turn blue, and/or the Negative Control does not stay colorless, the test results are invalid and patient results should not be reported.

External Controls

External controls are used in accordance with local, state, and federal accrediting organizations as applicable. External Controls are performed: weekly, with each new lot, with each new lot shipment, and with any troubleshooting of the testing as required. Gibson Laboratories controls are utilized as external controls. The Tri-Valent Positive swabs are simulated clinical specimens that test for *Trichomonas, Gardnerella* and *Candida*. The Tri-Valent Negative swabs are simulated clinical specimens that contain *Esherichia coli* and test negative for the three species, showing selectivity in the event of fecal cross-contamination. External Controls must be documented on form *CORE 6660 F BD Affirm QC Log.*

Lot Comparisons/Parallel Testing

Before a new reagent lot and/or shipment is put into use, the performance is checked with external controls to ensure that equivalent results are obtained with the new lot. The controls should be tested in the same manner as patient samples. (See procedure section.) Results should be retained in Binder #11 – BD Affirm Vaginal Pathogens.

Procedure

Sample Preparation

1. Verify that the BD MicroProbe Lysis Block is at 80-90°C and that reagents are at room temperature, 22-28°C, and well mixed.
2. Uncap the sample collection tube (SCT) making sure the swab shaft is firmly seated in the cap. **For external controls:** Add the trivalent control swab into an empty sample collection tube. Grasp the pre-scored handle of the swab and bend it until the swab breaks.
3. Add 12 drops of Lysis Solution (L) to the tube. Hold the dropper bottle vertically when adding drops.
4. Place the swab with cap back into the tube and recap to prevent evaporation.
5. Mix the swab in the tube by vortexing or vigorously swirling and moving the swab up and down against the side of the tube for at least 10 seconds.
6. Insert the tube into a well of the Lysis Block to heat.
7. Incubate the tube in the Lysis Block for 10 minutes (at least 10 minutes, but not longer than 20 min). Use a timer for this step. Over processing will lead to denatured DNA and a possible false negative result.
8. Remove the tube from the Lysis Block. CAUTION!! SCT will be hot.
9. Add 12 drops of well-mixed Buffer Solution (B) to the tube containing the swab. Avoid touching the tip of the bottle to the tube.

10. Replace the cap tightly on the tube and mix by vortexing or flicking the tube briskly for 10 seconds.
11. To proceed with automated processing of the *prepared sample*, remove as much fluid as possible from the swab by lifting the swab above the fluid level and pressing the swab firmly against the side of the tube for at least 10 seconds. Dispose of swabs in a biohazard container. Press a Filter Tip (FT) firmly onto each sample collection tube (SCT).

Note: *Prepared specimens* may be stored at room temperature for up to 24 hours.

Starting the Test

Note: Before proceeding, ensure that all reagents are at 22-28°C. With each test run, verify that the testing environment is between 22 and 28°C.

1. If the Affirm VPIII Microbial Identification Test Program Card is not already in the BD MicroProbe Processor, insert the Program Card, printed side up, arrow pointing towards the instrument, into the slot located on the front right side of the instrument.
2. Make sure that the Processor is turned on. If not, turn on the Processor. The Processor arm will move to “home” during this initial step. As you move through the procedure, follow the prompts on the Processor Display. If additional help is needed, press the [HELP] key or call technical support.
3. Remove the Cassette Caddy from the Processor. It is easier to add the samples with the Caddy off the Processor.
4. Select one Reagent Cassette (RC) for each sample to be tested, label with sample identification on the front end of the Reagent Cassette (RC) using a permanent-marking pen. Carefully pull the foil covering off of the Cassette, lifting from the end WITHOUT the upward bent flap. Place the Reagent Cassettes (RC) on the Cassette Caddy, loading from the center to the sides and balance the number of cassettes on each side of the arm as evenly as possible.
5. Add 4 drops of Substrate Solution (S) to well #7 of the Reagent Cassette (RC). Close the bottle cap to avoid evaporation.

6. Match up each Sample Collection Tube (SCT)/Filter Tip (FT) with the corresponding labeled Reagent Cassette (RC). Invert the Sample Collection Tube (SCT) and firmly squeeze the entire contents of each tube through the Filter Tip (FT) into reservoir of well #1 of the appropriate Reagent Cassette (RC). Dispose of patient sample tube in a biohazard container. Foam at the filter tip is a good indication that the entire sample has been delivered.
7. Open pouch containing the PAC, remove PAC slightly from pouch, and label with sample identification on the PAC in the space provided.
8. Place a labeled PAC into Well 1 of each corresponding labeled Reagent Cassette (RC). Avoid touching beads.
9. Carefully replace the Cassette Caddy on the Processor, taking care not to splash reagents. Assure that the Caddy is securely seated on all four locator pins.
10. Press the [RUN] key. You will see several prompts reminding you of critical steps in the process. By hitting the [RUN] you are acknowledging that you completed the steps. The arm of the Processor will start forward. The Processor will automatically pick up and move the PACs through the test procedure. The instrument will begin the processing time sequence and will indicate “Please wait. Processing 32:50” with minutes remaining on the timer indicated. At the end of the processing time, the instrument will beep and present the PAC for removal.
11. Remove the PAC, and gently blot dry with a paper towel. Interpret the results for each specimen as soon as possible after completion of the test. The PAC should be viewed against a white background, under normal intensity lighting.
12. Press [RUN] to home the processor after removing the PAC.

Reporting

Interpretation of Results

Results are determined by the presence or absence of color on the test bead. The presence of any visible blue color on the target organism bead, when viewed against a

white background, is a positive result. The absence of any visible blue color on the target organism bead is a negative result.

A positive result for *Candida*, *Gardnerella* and/or *Trichomonas* means nucleic acid for *Candida* species (*C. albicans*, *C. glabrata*, *C. kefyr*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*), *G. vaginalis* and/or *T. vaginalis*, respectively, is present in the sample and indicates the patient has candidiasis, bacterial vaginosis, and/or trichomoniasis when consistent with clinical signs and symptoms. Simultaneous infections by more than one organism are common.

Negative results for *Candida*, *Gardnerella* or *Trichomonas* tests suggest the patient does not have candidiasis, bacterial vaginosis and/or trichomoniasis, respectively, when consistent with clinical signs and symptoms.

Invalid Controls

In a properly functioning test, the Positive Control bead will be blue and the Negative Control bead remains colorless (i.e., absence of blue color) after processing. If the Positive Control does not turn blue, and/or the Negative Control does not stay colorless, the test results are invalid and patient results should not be reported.

Before reporting any results, always verify that the assay is valid.

Entering the result in Meditech

1. Review the PAC card and ensure that the controls are valid.
2. Log into Meditech and go into Enter Results on the Specimen Desktop.
3. Enter accession number.
4. Enter Negative or Positive for the controls and each species.
5. File Result.

Principle of the Procedure

The Affirm VPIII Microbial Identification Test is based on the principles of nucleic acid hybridization. In nucleic acid hybridization tests, complementary nucleic acid strands align to form specific, double-stranded complexes called hybrids.

The test uses two distinct single-stranded nucleic acid probes for each organism, a capture probe and a color development probe, that are complementary to unique genetic sequences of the target organisms. The capture probes are immobilized on a bead embedded in a Probe Analysis Card (PAC), which contains a separate bead for each target organism. The color development probes are contained in a multi-well Reagent Cassette (RC).

During sample preparation, the sample is treated with the Lysis Solution (L) and heated. This process ruptures the walls of the organism, releasing the nucleic acid analyte. A second solution, the Buffer Solution (B), is added. This solution stabilizes the nucleic acid and establishes the stringency conditions necessary for specific hybridization. At this point, the sample is added to the first well of the Reagent Cassette (RC) along with the PAC, and automated processing begins. The BD MicroProbe™ Processor moves the PAC from one well of the Reagent Cassette (RC) to another. Hybridization occurs on the PAC beads in the first and second wells of the Reagent Cassette (RC). Hybridization of the analyte to the capture probe on the bead occurs in well 1, and the hybridization of the color development probes occurs in well 2. All unbound sample components and probes are washed away in well 3. Enzyme conjugate binds to the captured analyte in well 4. Unbound conjugate is washed away in wells 5 and 6. In well 7, the indicator substrate is converted to a blue-colored product if bound enzyme conjugate is present on the bead. The final step is reading the results of color development on each of the target organism beads and controls.

Limitations

The assay is intended to be used with the Affirm VPIII Ambient Temperature Transport System, the Affirm VPIII Sample Collection Set, or the swabs provided in the Affirm VPIII Microbial Identification Kit. Other methods of collection have not been evaluated.

Optimal test results require appropriate specimen collection. Test results may be affected by improper specimen collection, handling and/or storage conditions. A negative test result does not exclude the possibility of vaginitis/vaginosis.

With the Affirm VPIII Ambient Temperature Transport System specimens held longer than 72 hours at ambient (15 to 30°C) or refrigerated (2 to 8°C) conditions may cause false results. When using the Affirm VPIII Sample Collection Kit or the swabs provided in the Affirm VPIII Microbial Identification Kit, specimens held longer than
One hour at room temperature or four hours at 2 to 8°C prior to preparation may cause false results. Prepared specimens held longer than 24 hours at room temperature prior to processing may give inaccurate results. When performing this test, the temperature of the testing environment must be 22 to 28°C to reduce the chance of false positive results.

A negative result for *Candida*, *Gardnerella* and/or *Trichomonas* indicates nucleic acid from less than 1×10^4 *Candida* cells, 2×10^5 CFU of *G. vaginalis* or 5×10^3 trichomonads, respectively, may be present in the patient sample.

The Affirm VPIII Microbial Identification Test detects the presence of *G. vaginalis* at concentrations of greater than 2×10^5 CFU per patient sample. The diagnostic value of this level of detection is not definitive.

The presence of *G. vaginalis*, although suggestive, is not diagnostic for bacterial vaginosis. As in many clinical situations, diagnosis should not be based on the results of

a single laboratory test. Results should be interpreted in conjunction with other clinical and laboratory data available to the clinician such as pH, amine odor, clue cells and vaginal discharge characteristics.

Women with vaginal discharge should be evaluated for risk factors of cervicitis and pelvic inflammatory disease, and if present, evaluated for other organisms, including *N. gonorrhoeae* and *C. trachomatis.*

Vaginitis/Vaginosis is most frequently caused by *G. vaginalis*, *Candida* species, and *T. vaginalis*. Vaginitis symptoms may also be seen in toxic shock syndrome (caused by *Staphylococcus aureus*) or may be caused by non-specific factors or by specific organisms. Mixed infections may occur. Therefore, a test indicating the presence of *Candida* species, *G. vaginalis*, and/or *T. vaginalis*, does not rule out the presence of other organisms, including *Mobiluncus mulieris, Mycoplasma hominis,* and/or *Prevotella bivia.*

Cryptococcus neoformans at concentrations greater than 1×10^8 yeast/mL react with the Affirm VPIII Microbial Identification Test for *Candida* species. *C. neoformans* is only rarely encountered in the vagina.

M. mulieris at concentrations greater than 4×10^6 bacteria/mL and *Bifidobacterium dentium* at concentrations greater than 8×10^5 bacteria/mL may react non-specifically with the Affirm VPIII Microbial Identification Test for *G. vaginalis*. *B. dentium* is rarely encountered in the vagina.

The Affirm VPIII Microbial Identification Test method is for use with vaginal fluid specimens from patients with symptoms of vaginitis/vaginosis. Performance with other specimens or other patient populations has not been established.

The performance of this test on patient specimens collected during or immediately after antimicrobial therapy is unknown. The presence or absence of *Candida* species, *G. vaginalis* or *T. vaginalis* cannot be used as a test for therapeutic success or failure.

Adulteration of reagents or failure to follow instructions exactly as set forth in the directions for use may adversely affect performance as described in the labeling.

Interfering Substances

In clinical studies, no evidence of interference was determined for vaginal lubricants, douches, menses or spermicides.

Maintenance

The BD Affirm requires no special maintenance procedures other that wiping reagent spills and weekly surface cleaning to remove any dirt and grime. Because of the possibility of blue-colored reagent deposits as a result of reagent reactions, spills and splatters should be wiped up immediately to prevent staining.

Disinfection/Decontamination

1. Set the unit to STANDBY and disconnect the power. Immediately removed any spilled material with by wiping with a paper towel or gauze and dispose in a biohazard container.
2. Wipe all surfaces with a 10% bleach solution and allow to air dry.
3. After the disinfection time is complete, wipe the processor thoroughly with a moist towel to remove the residual disinfectant.
4. Wipe the surfaces with 70% isopropyl alcohol and allow to air dry.

Note: If it is suspected that any liquid material has spilled onto the program card or into the program card port, contact BD Technical Services. Do NOT use the system if such a spill is suspected.

Technical Information

In the United States, telephone Technical Services, toll free (800) 638-8663.

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