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Subject **BD Affirm VPIII Operations & Maintenance Procedure** Microbial Identification Test	**Attachments** ☒ Yes ☐ No
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Manual BD Affirm MircoProbe Processor User's Manual	**Last Review Date** February 2018
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Review Responsibility Specialty Department Lead Medical Technologist	**Contact** Lead Medical Technologist

Purpose/ Principle

The Affirm VPIII Microbial Identification Test is a DNA probe test intended for use in the detection and identification of Candida species, Gardnerella vaginalis and Trichomonas vaginalis nucleic acid in the vaginal fluid specimens from patients *with symptoms* of vaginitis/vaginosis.

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. The process ruptures the walls of the organism, releasing the nucleic acid analyte. A second solution, the Buffer Solution (B), is added. The 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 & 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.

Definitions
N/A

Specimen Information

- Specimen types accepted
  - Vaginal swab collected using the Affirm VPIII Ambient Temperature Transport System (ATTS)
- Specimen Collection (To be done by the provider)
- Specimen storage:
  - Up to 72 hours stored at ambient (15-30°) or refrigerated (2-8°)
- Unacceptable specimens:
  - Any other swab types
    - (such as Aptima, etc.)
  - Dry swabs
  - Swabs collected using the sample collection kit contained in the Affirm VPIII test kit.
    - Even though these swabs say “BD Affirm” on the vial, they are smaller vials than the VPIII (ATTS) vial. The stability is only 1 hour because there is no preservative. Credit these samples with “**WVIAL**”. (See diagram to compare differences in size.) The tube on the left shows the size of this smaller tube and should not be accepted.

Reagents/Equipment/Supplies

1. Test Kit:

    a. Reagent Cassette (RC) - Sealed multi well cassette containing Patient sample reservoir, Hybridization solution, Wash solution, Conjugate, Wash solution and Substrate buffer.

    b. Probe Analysis Card (PAC) – Individually packaged cards, wrapped in an absorbent paper towel moistened with a solution containing sodium azide as a preservative. Each card contains the following 5 beads: Negative Control, Trichomonas, Gardnerella, Candida, and Positive Control.

    c. Substrate Solution (S) – Indicator substrate, Stabilizing agent, Alcohol.

    d. Lysis Solution (L) – Detergent, Buffer solution, Preservative (Proclin)

    e. Buffer Solution (B) – Buffered chaotropic solution, Formamide

    f. Filter tips, swabs, collection caps and tubes.

    g. Storage Temperature of Cassettes & Reagents
        i. Not In Use = 2-8° C (stable until expiration date on box)
        ii. In Use (Testing Area) = up to 30° C (for no more than 3 months)
        iii. All components (Cassettes, Reagents, samples, etc.) must be brought to Room Temp (22-28° C) prior to testing.

2. Controls (ordered separately)
    a. Positive Control (swab positive for C. albicans, T. vaginalis and G. vaginalis)
    b. Negative Control (swab negative for C. albicans, T. vaginalis and G. vaginalis)

3. BD MicroProbe Processors with dedicated software.

4. BD MicroProbe Lysis Blocks

5. Vortex

Special Safety & Handling Precautions

1. Handle all samples as if capable of transmitting infection.

2. Dispose of all specimens and material used to perform the test as though they contain an infectious agent.

3. Do not interchange reagent cassettes or buffer, lysis & substrate solutions across multiple lot numbers.

4. Do not use any reagent cassette if its pouch has been perforated. Do not open the cassette's sealed foil pouch until just prior to use.

5. If a space heater is used as a heat source to keep the testing environment in the proper range, it must be turned off and unplugged when testing is not taking place. If any electrical issues arise, notify the Lead and/or the Lab manager.

Quality Control

1. **Internal Quality Controls**
    a. Two internal controls are included 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 the PAC, Reagent Cassette and Processor. In a proper functioning test, the Positive Control bead will be blue and the Negative Control bead will remain colorless after processing.

2. **External Quality Controls**
    a. Gibson BioScience Tri-Valent Controls are available separately for use with the BD Affirm VPIII Assay to verify the performance of the test.
        a. The Positive Control will produce a positive test result for C. albicans, T. vaginalis and G. vaginalis
        b. The Negative Control will produce a negative test result.

    b. External Controls should be performed in the following circumstances:
        a. Monthly (typically the first testing day of the month).
        b. When opening a new test kit lot number.
        c. Whenever a new shipment has been received, regardless of lot number.
        d. In the above circumstances, External Controls should be run on BOTH BD Affirm Micro Processor instruments and documented on each appropriate Logsheet.

    c. Preparation of External Controls
        a. Obtain 1 Positive and 1 Negative control swab from the refrigerator, record lot numbers and expiration in online log.
        b. Place each swab into separate labeled Sample Collection tubes & snap at the scored mark.
        c. Place Sample Collection Caps on the tubes.
        d. Proceed as directed in the Assay Procedure.

3. QC failures.
    a. If there is a failure of the QC, whether it's the External QC or the Internal QC beads on the PAC, patient results cannot be reported. Refer to the "Repeat Job Aid" at the end of this procedure for instructions.

4. **IQCP**
    BD Affirm VPIII Microbial Identification Test follows an Individualized Quality Control Plan (IQCP). The IQCP is a separate set of Documents and is in a separate tab in the manual at the work area. For additional information on IQCP, consult with the Lead of the department.

Calibration and Maintenance

1. Calibration
    a. The BD MicroProbe Processor is factory calibrated. No further calibration or alignment is required under conditions of normal use.
    b. The BD MicroProbe Processor is not designed to be repaired by the user. Any service or repair work must be done by BD authorized service personnel only. Contact your BD Technical Services representative or authorized distributor if you believe your Processor requires service or repair.

2. Maintenance
    a. Weekly: Wipe processor unit, cassette caddy and counters with ELIMINase.
    b. Rinse with DI water followed by 70% isopropyl alcohol and allow to air dry.
    c. If using squirt bottles with products, squirt the liquid onto a gauze or towel. Avoid splattering the work area.

3. Decontamination
    a. If a patient spill occurs, immediately wipe up spill and dispose of in biohazard container.
    b. Wipe down all contaminated surfaces with ELIMINase, followed by 10% bleach and allow to air dry.
    c. Follow with DI water and 70% isopropyl alcohol.
    d. If using squirt bottles with products, squirt the liquid onto a gauze or towel. Avoid splattering the work area.

**Caution – Make sure bleach does not come in contact with the program card or into the program card port. If you suspect liquid has spilled into the card port, contact BD Technical Services for assistance.

Assay Procedure

1. Sample Preparation
    a. Before processing, ensure that all reagents are at 22-28°C. Verify that the environment is between 22-28°C and Humidity is between10-85%. Record temperature and humidity in the online log. A space heater may be needed to ensure testing is performed at the right temperature. Temperatures below 22° may pose a risk of false-positive Gardnerella results.

    b. Ensure specimens, cassettes, reagents and controls (if needed) are at room temperature prior to performing testing. (about 30 minutes)

    c. Verify that the BD MicroProbe Lysis Block is at 85° ± 5°C

    d. Turn on BD MicroProcessor. The Processor arm will move to “home” during this step.

    e. Print worksheet: **CLWET**
        1. Other sites within the family of care also perform the BD Affirm assay. If a specimen arrives from one of these locations, it’s possible the specimen will still be on the worksheet from that location. All worksheets are named similarly with “WET” at the end (SPWET, etc.)
        2. It is acceptable for Central Lab staff to report patient results on another location’s worksheet. Any appending texts, including the location of where the testing was performed, should append correctly in the LIS and/or EPIC. Please verify in EPIC that “Performed at HealthPartners Central Laboratory, etc.” was appended to results.

2. Assay Steps

   a. Verify that there is liquid in the sample collection tube (SCT). (There won't be liquid in the Control tubes until the Lysis is added.) Uncap the tube, making sure swab is still seated in the cap. Add 12 vertical drops of Lysis Solution to the tube. Avoid touching the tip of the bottle to the collection tube.

   b. Replace the cap, with the swab, back into the collection tube and vortex 3-5 seconds.

   c. Set timer and incubate the collection tube in the Lysis Block for 10 minutes. (at least 10 minutes but no longer than 20 minutes).

   d. Remove collection tube from Lysis Block

   e. Gently roll the bottle to mix the Buffer Solution (do not shake). Make sure no crystals are present in the solution. Crystals may appear as a clump at the bottom of the bottle. Uncap the tube, making sure swab is still seated in the cap. Add 12 vertical drops to the collection tube. Avoid touching the tip of the bottle to the collection tube.

   f. Replace the cap, with the swab, back into the collection tube and vortex 3-5 seconds.

   g. Remove as much fluid as possible from the swab by lifting the swab above the fluid level and pressing it firmly against the side of the tube for at least 10 seconds. Dispose of the swab in biohazard container. Place a filter tip (FT) firmly onto the collection tube.
      1. If swab shaft is too short and doesn't stay attached to the collection cap, a tweezers may be used to remove the swab. To ensure no cross-contamination between patients, clean the tweezers with bleach or bleach wipes, followed by water, then towel dry if needed.

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

3. Automated Processing

   a. Remove the Cassette Caddy from the Processor.

   b. Label a Reagent Cassette for each patient on the front of the Cassette. Carefully pull the foil covering off of the Cassette, lifting opposite the bent flap. Remove any residual foil and/or "stringy glue" from the Cassette. Place each Cassette into 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.

   c. Carefully remove a Probe Analysis Card (PAC) from the pouch & label one for each patient to be tested. Lay on a paper towel. Avoid touching the beads. Foil pouch should be tossed in biohazard bin.

   d. Press the RUN key on the Processor. You will be prompted to "Add Substrate." Add 4 drops of Substrate Solution to well #7 of the Reagent Cassette.

   e. Press the RUN key. You will be prompted to "Add Sample." Add the corresponding sample tube to the Reagent Cassette by carefully inverting the Sample Collection Tube and squeezing the entire contents of each tube through the filter tip into well #1. Foam at the filter tip is a good indication that the entire sample was used.

f. Press the RUN key. You will be prompted to “Place PAC.” Place the corresponding labelled PAC into well #1 of the Cassette Caddy. Avoid touching the beads.

g. Press the RUN key. You will be prompted to “Place Caddy.” Carefully place the Cassette Caddy on the Processor, taking care not to splash reagents. Assure that the caddy is securely seated on all four locator pins.

h. Press the RUN key twice. The arm of the Processor will start to move forward. The Processor will automatically pick up and move the PAC’s through the test procedure. At the end of the processing time, the instrument will beep and present the PAC for removal. (Approximately 32 minutes for processing)

i. At the end of processing the machine will beep. First, remove the PACs and gently place on a paper towel. Press the RUN key to stop beeping, arm will return to home position. Interpret the results for each specimen as soon as possible after the completion of the test. The PAC should be viewed against a white background, under normal lighting.

4. End of Day

    a. Before turning off the Processor, remove all PACs and Cassettes and press the RUN key. This will allow the arm to return to the home position.

Interpretation of Results

Results are determined by the presence or absence of color on the test bead.

a. Positive - The presence of any blue color on the target organism bead, when viewed against a white background, is a positive result. Positive results may be lighter or darker than the procedural control.

b. Negative – The absence of any visible blue color on the target organism bead is a negative result.

Results / Computer Entry

1. Results will be manually entered using function MEM on worksheet CLWET

    a. Answer control prompts with Y if they are acceptable.
        - INTP (Internal Positive Control)
        - INTN (Internal Negative Control)

    b. Answer each test prompts with POS or NEG
        - TVAG (Trichomonas vaginalis)
        - GARD (Gardnerella vaginalis)
        - CNDID (Candida species)

2. Discard PAC, Reagent Cassette, and any other test materials into a biohazard container.

3. After results are entered, print a completed worksheet. Clip the incomplete & completed worksheets together and leave in the Med Tech stacker.

Reference Ranges (Expected Values)

Negative for Trichomonas, Gardnerella, or Candida species

Limitations of Procedure & Performance Characteristics

1. False results may occur when ATTS specimens are held longer than 72 hours at either ambient (15-30°C) or refrigerated (2-8°C).

2. Prepared specimens held longer than 24 hours at room temperature prior to processing may give inaccurate results.

3. Sensitivity and Specificity of each organism based on multiple studies is as follows:
    a. Trichomonas vaginalis
        i. Sensitivity varies between 90% – 93%
        ii. Specificity ~ 99.9%
    b. Gardnerella vaginalis
        i. Sensitivity varies between 84% - 89%
        ii. Specificity varies between 99% - 100%
    c. Candida species
        i. Sensitivity varies between 81% - 82%
        ii. Specificity ~ 98.3%

4. In clinical studies, no evidence of interference was determined for vaginal lubricants, douches, menses or spermicides. In analytical studies, there was no evidence of interference with water based vaginal lubricants.

Related Documents / Attachments
BD Affirm VPIII Test Procedure Job Aid – Quick Reference Guide

References & Vendor Contact information

1. BD Affirm VPIII Package Insert
2. BD MicroProbe Processor – Instrument User's Manual
3. BD Affirm VPIII Training Packet
4. BD Affirm Repeat Job Aid – (Appendix at end of Procedure)

5. BD Diagnostics
   7 Loveton Circle
   Sparks, MD 21152
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Approved by
Laboratory Medical Director or Designee

BD Affirm Repeat Job Aid / Appendix

The following information can be found in various BD Affirm-related Procedures and/or Documents, and has been approved by BD Tech Support.

When is it "Okay" to repeat a sample?

1. If internal QC bead fails on either a patient or the External QC.
    a. The blue dot for the Pos QC is Negative
    b. The Neg QC dot turns blue – and shouldn't

2. Mechanical issues
    a. Foil not completely removed on Cassette and PAC doesn't "dunk" properly.
    b. Instrument stops "dunking" or doesn't pick up the PAC.

3. Failure to add the Substrate at well # 7
    a. Failure altogether
    b. If Substrate is added to a different well instead of well # 7.

4. If all beads are all positive (or all negative)
    a. All beads (controls and organisms) turn the same color – all clear or all blue.
    b. If after repeating, the same thing happens again (all beads are the same color – most especially if all beads are blue) – then credit the sample with:
        PINTF-RSUG
        Possible Interfering Substances. Recollection Suggested.

When is it "<u>Not</u> Okay" to repeat a sample?

1. If any reagent is added out of order.
    a. Example – Buffer is added ahead of Lysis, etc.
    b. All samples must be credited and recollected. Credit with NTRUN (Lab Error, Test not performed).

2. For any other situation not listed above – call Tech Support. They will determine whether or not it's okay to repeat a sample of if the sample needs recollection.

Instructions for "How" to repeat a sample.

1. Carefully retrieve ALL of the sample from Well 1 (liquid from patient swab) with a clean disposable pipette and place into well 1 of a new reagent cassette.

2. Discard the used cassette and process the new cassette by adding substrate to Well 7 of the new cassette and using a new PAC.