PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

BD Affirm™ VPIII Test Procedure

Department of Microbiology

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1.0 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 vaginal fluid specimens. This 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. Two distinct single-stranded probes are used for each organism, a capture probe and a color development probe. These probes 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.

During sample preparation, the sample is treated with a Lysis Solution and heated. This process ruptures the walls of the organism, releasing the nucleic acid analyte. A second solution, the Buffer Solution, 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 along with the PAC, and automated processing begins. The BD MicroProbe™ Processor moves the PAC from one well of the Reagent Cassette to another. Hybridization occurs on the PAC beads in the first and second wells of the Reagent Cassette. Hybridization of the analyte to the capture probe on the bead occurs in well 1, and hybridization of the color development probes occurs in well 2. All unbound samples 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.

2.0 Clinical Significance

The Affirm™ VPIII Microbial Identification Test may be used to aid in the diagnosis and treatment of patients with symptoms of vaginitis/vaginosis.

3.0 Scope

This procedure is classified under CLIA as Moderately Complex. It should be carried out by technical personnel familiarized and trained on all levels of the operation of the BD Affirm™ testing platform. Testing includes but is not limited to: instrument start up, shutdown, routine maintenance, performance checks, basic troubleshooting, QC checks, administrative tasks and record keeping of information vital to verification of instrument and technical proficiency in accordance with the department SOP. Records are to be kept within the employee's record in the department of continued competence and proficiency on the equipment. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document. Follow proper handling, storage, and disposal of specimens and items that come into contact with specimens. Place contaminated materials in a biohazardous waste container.

The reagent(s) and/or chemical(s) that are used in this procedure may be hazardous to your health if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of this procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves
- Laboratory Coat

Disinfectant following procedure:

- Bleach dilution sprayers or wipes can be used for on demand disinfectant.

Reference for spill/decontamination

- MSDS
- Chemical hygiene plan

5.0 Specimen Requirements

Specimen collection is a critical step. For specimen collection, use only the Affirm™ VPIII Ambient Temperature Transport System. Separate swabs should be used for other tests, e.g. culture or microscopic slide samples.

5.1 Collection of Vaginal Samples

1. Open the seal on outer plastic pouch of Affirm™ VPIII Ambient Temperature Transport System and remove all components.
2. Tear open 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 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.
6. Insert an unlubricated speculum (without jelly or water) into the vagina to permit visualization of the posterior fornix. Using the sterile swab, obtain a sample from the posterior vaginal fornix. Twist or roll the swab against the vaginal wall 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.
7. Immediately place the swab into the sample collection tube containing the ATTS reagent.
8. 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. Discard the broken handle into an infectious waste container.
9. 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.
10. Label the sample collection tube with the patient identification information. Include the time the sample was collected.

5.2 Specimen Storage and Transport

When 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 h when the specimen is stored at ambient conditions (15 to 30 °C). The system has also been qualified for transport use at refrigerated conditions 2 to 8 °C.

6.0 Materials

6.1 Equipment and/or Testing System

- BD MicroProbe Processor
- BD MicroProbe Lysis Block
- Thermometer

6.2 Reagents

- BD Affirm™ VPIII Microbial Identification Test kit (Catalog Number 446250) 24 tests

  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. All reagents and PACs must be at 22 to 28 °C prior to use. The Buffer Solution (B) precipitates under refrigeration. Allow the solution to come to room temperature for a minimum of 30 min and then agitate the bottle for 10 to 15 s until precipitate is dissolved. For convenience, store all reagents at room temperature once opened.
  - **Probe Analysis Cards (PAC)**: 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**: Reagents are sealed in multi-well, foil-covered cassettes. Each cassette has seven wells. From front to back the wells contain:
    - Well #1: Patient Sample Reservoir, supplied empty
    - Well #2: Hybridization Solution, 350 µL: Color development probe, Formamide, Buffered chaotropic solution
    - Well #3: Wash Solution, 750 µL: Detergent, Buffer solution, Preservative (Proclin™)
    - Well #4: Conjugate, 500 µL: Enzyme conjugate, Preservative (Proclin)
    - Well #5: Wash Solution, 750 µL: Detergent, Buffer solution, Preservative (Proclin)
    - Well #6: Wash Solution, 750 µL: Detergent, Buffer solution, Preservative (Proclin)
    - Well #7: Substrate Buffer, 500 µL: Buffered Peroxide Solution
  - **Substrate Solution** (Red Cap): Individually packaged solution in foil pouch; Indicator substrate, Stabilizing agent, Alcohol
  - **Lysis Solution** (Blue Cap): Detergent, Buffer solution, Preservative (Proclin)
  - **Buffer Solution** (Green Cap): Buffered chaotropic solution, Formamide
  - Filter Tips

6.3 Control Materials and Usage

External controls are prepared using a mixture of *Candida albicans* ATCC 90028, *Gardnerella vaginalis* ATCC 14018, and *Trichomonas vaginalis*. Controls are prepared inhouse and frozen at -70 °C until ready for use.

7.0 Interfering Substances

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.

8.0 Warnings and Precautions

- For specimen collection, use only the Affirm™ VPIII Ambient Temperature Transport System.
- With each test run, monitor the temperature of Lysis Block, 85 ± 5 °C and verify that the testing environment temperature is between 22 and 28 °C.
- 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 coming 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" 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.
- Avoid touching the beads. Avoid contaminating tips of dropper bottles. Do not use a reagent after its expiration date.

9.0 Procedure

9.1 Specimen Processing

1. Upon receipt, verify that the specimen collection date is within 72 h window.
2. Receive the specimens and place them in the Affirm holding rack.
3. Samples can be tested anytime. They may be batched or run individually. However, **specimens must be tested within the 72 h window from collection to assay**.

9.2 Sample Preparation

1. Verify that the BD MicroProbe Lysis Block is at 85 ± 5 °C, and that reagents are at 22-28 °C and well mixed.
2. Uncap the Ambient Temperature Transport System collection tube, making sure the swab shaft is firmly seated in the cap. Add 400 µL of Lysis Solution to the tube.
3. Mix the swab in the tube by vigorously swirling and moving the swab up and down against the side of the tube for at least 10 s.
4. Place the swab with the cap back into the tube and recap to prevent evaporation.
5. Insert the tube into a well of the Lysis Block to heat.
6. Incubate the tube in the Lysis Block for 10 min (at least 10 min, but not longer than 20 min). Use a timer for this step.
7. Remove the tube from the Lysis Block.
8. Add 600 µL of well-mixed Buffer Solution to the tube containing the swab.
9. Replace the cap tightly on the tube and mix by flicking the tube briskly 10 times.
10. 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 s. Dispose of swabs in a biohazard container. Press a Filter Tip firmly onto each sample tube.

9.3 Automated Processing

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. Make sure that the Processor is off when inserting the program card. There are no lights on the card panel if the Processor is off.
2. Turn the Processor on. 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.
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 for each sample to be tested, label with patient/sample identification on the front end of the Reagent Cassette using a permanent marking pen or an accession label. Carefully pull the foil covering off of the cassette, lifting from the end without the upward bent flap. Place the Reagent Cassettes 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. Open a pouch containing PAC for each sample, remove PAC slightly from pouch, and label with patient/sample identification on the PAC in the space provided.

6. Press [RUN] key. You will be prompted to “Add Substrate.” Add 100μL of Substrate Solution to well #7 of the reagent cassette.
7. Press the [RUN] key. You will be prompted to “Add Sample.” Match up each sample tube/filter tip with the corresponding labeled Reagent Cassette. Invert the sample collection tube and firmly squeeze the entire contents of each tube through the filter tip into the reservoir of well #1 of the appropriate Reagent Cassette. Dispose of the patient sample tube in a biohazard container. Foam at the filter tip is a good indication that the entire sample has been delivered.
8. Press the [RUN] key. You will be prompted to “Place PAC.” Place a labeled PAC into Well 1 of each corresponding labeled reagent cassette. Avoid touching beads when handling PAC.
9. Press the [RUN] key. You will be prompted to “Place Caddy.” 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 again. 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.

10.0 Interpretation of Results

10.1 Interpretation

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 *Trichomonas 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.

10.2 Results Input

Results, including the internal controls, should first be documented on the BD Affirm™ Test Log. The results recorded for each test should be visually verified against the PAC prior to discarding the test device to ensure no clerical errors occurred. Report the results based on the test that was ordered.

- **VPDNAP**: report each of the three analytes as positive or negative by DNA probe.

  Example: **Negative for Trichomonas vaginalis by DNA Probe**
  Positive for Gardnerella vaginalis by DNA Probe
  Positive for Candida by DNA Probe
- **VAGPAN**: report only the *Gardnerella* and *Candida* results. The *Trichomonas* result is determined by nucleic acid amplification testing performed and reported at PAML.

The following comment should automatically attach when the report is finalized:

The results for this test are only valid if the specimen was submitted using the Affirm VPIII Ambient Temperature Transport System, which includes the addition of the ATTS Reagent to the specimen tube. This reagent stabilizes the sample for up to 72 hours. [VPC]

11.0 Quality Control & Quality Assurance

11.1 Internal Controls

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, 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 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. Document the internal controls on the BD Affirm™ Test Log.

11.2 External Controls

1. Quality control testing using external QC material should be performed on each new lot or shipment and subsequently once per calendar week. Weekly QC testing should alternate between instruments in order to check the instruments against each other for correlation.
2. One PAC of each new lot or shipment should be tested using trivalent control material, which contains a mixture of *Candida albicans* ATCC 90028, *Gardnerella vaginalis* ATCC 14018, and *Trichomonas vaginalis*. Another PAC should be tested using *E.* coli ATCC 25922 as a negative control. Control materials should be prepared using 50 μL of bacterial suspensions equivalent to a 0.5 McFarland and 50 μL of actively growing *Trichomonas* culture. Clinical isolates of *Trichomonas vaginalis* may be used. While the concentration of *Trichomonas* cannot be effectively quantitated, the final concentration of *C. albicans* and *G. vaginalis* will be approximately 1 X 10^4 and 1 X 10^6 CFU/swab, respectively. This is close to the manufacturer's published limit of detection of 1 X 10^4 for *Candida* and 2 X 10^5 CFU/swab for *G. vaginalis.* The weak positive control material helps to verify the lower limit of detection for each new lot/shipment. If *Trichomonas* is not available, trivalent commercial controls may be purchased from Gibson Laboratories at 1-800-477-4763 (catalogue number TVS-01). Controls should be prepared and processed using the same procedure as for patient samples. This should result in blue beads for all three analytes and the internal controls should yield acceptable results.
3. Record QC results in LIS. Quality control failures should be verified and addressed with the Microbiology supervisor. Refer to CLIA Equivalent Quality Control guidelines for further information.

11.3 Quality Assurance

All test results should be documented on the BD Affirm™ Test Log and resulted in LIS. Results entered into LIS should be reviewed by a second technologist on the same shift or the beginning of the next shift. The review should be documented on the BD Affirm™ Test Log.

12.0 Limitations

1. The assay is intended to be used with the Affirm™ VPIII Ambient Temperature Transport System. Other methods of collection have not been evaluated. Specimens held longer than 72 h in the transport system at ambient (15 to 30°C) or refrigerated (2 to 8°C) conditions may cause false results.
2. 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.
3. When performing this test, the temperature of the testing environment must be 22 to 28°C.

4. A negative result for *Candida, Gardnerella* and/or *Trichomonas* indicates nucleic acid from less that 1 x 10^4 *Candida* cells, 2 x 10^5 CFU of *G. vaginalis* or 5 x 10^3 *T. vaginalis*, respectively, may be present in the patient sample.
5. The Affirm™ VPIII Microbial Identification Test detects the presence of *G. vaginalis* at concentrations of greater than 2 x 10^5 CFU per patient sample. The diagnostic value of this level of detection is not definitive.
6. 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 laboratory data available to the clinician such as pH, amine odor, clue cells and vaginal discharge characteristics.
7. Vaginitis/Vaginosis is most frequently caused by *G. vaginalis*, *Candida* species, and *T. vaginalis*. 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.*
8. *Cryptococcus neoformans* at concentrations greater than 1 x 10^8 yeast/mL react with the Affirm™ test for *Candida* species. *C. neoformans* is only rarely encountered in the vagina.
9. *M. mulieris* at concentrations greater than 4 x 10^6 bacteria/mL and *Bifidobacterium dentium* at concentrations greater than 8 x 10^5 bacteria/mL may react non-specifically with the Affirm™ test for *G. vaginalis*. *B. dentium* is rarely encountered in the vagina.
10. The performance of this test on patient specimens collected during or immediately following 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.
11. Adulteration of reagents or failure to follow instructions exactly as set forth in the directions for use may adversely affect performance as described.

13.0 Validation Information

A total of 44 specimens were tested using the Affirm™ VPIII Microbial Identification Test System. These specimens were submitted on swabs in BBL CultureSwab™ Plus with Amies gel, not in the Ambient Temperature Transport System. Results from the Affirm™ test were compared with results obtained by wet mount (WM) and scored gram stain smears (GS). Overall, 20 samples tested negative for all 3 analytes with 100% correlation to both WM and GS. A total of 17 samples tested positive for *Gardnerella*. Clue cells were not seen on the WM of 2 of these samples. However, both samples were suggestive of bacterial vaginosis when scored GS was performed. A total of 9 samples were positive for *Candida* by the Affirm™ test. Yeast were not seen in the WM of one of these samples but were seen on the GS. A total of 9 samples were positive for *Trichomonas* by the Affirm™ test as well as by WM. Overall, there were no false positive or false negative results detected with the Affirm™ test.

14.0 References

1. BD Affirm™ VPIII Microbial Identification Test CLSI Procedure Rev. 02/2006.
2. BD Affirm™ VPIII Ambient Temperature Transport System CLSI Procedure Rev. 02/2002.

15.0 Document Control History

Adopted/Approved by director (AR) 11/03/2008

Approved by J. Schappert 03/10/2010

Reviewed by supervisor (JC) 11/04/2008, 11/25/2009, 04/2011, 06/18/2013, Jason Ammons 07/2015

06/14/2012 Added clerical review protocol and reporting section.

06/18/2013 Updated processing and reporting for new test codes (VPDNAP and VAGPAN). Added sections for Safety, Interfering Substances, and Warnings and Precautions for PPM

format. 05/13/2014 Updated information regarding positive external control concentration. The control material is prepared to be weak and close to the LOD.