

Affirm Probe for the Laboratory Diagnosis of Infectious Vaginitis

Background

Vaginitis, an inflammation of the vagina presenting with a vaginal discharge, can be caused by the yeast *Candida* sp. (usually *Candida albicans*), the parasite *Trichomonas vaginalis*, and/or the syndrome bacterial vaginosis (BV). Infectious vaginitis is one of the most common women's health problems in the US, resulting in health care costs of more than \$500 million annually.⁴ Approximately 40 to 45 percent of the infections are caused by bacterial vaginosis, about 25 percent by *Candida*, and another 20 percent by *Trichomonas vaginalis*.⁴ Diagnosis of these entities has historically been done by examination of wet mount and/or stained preparations of the vaginal discharge in the physician's office or clinical laboratory. However, recent studies suggest the sensitivity of these traditional methods is low and up to 14 percent or more of infectious vaginitis may be a mixed infection.² A study evaluating the usual methods of direct examination of the discharge for the diagnosis of vaginitis was conducted among a cohort of 220 women presenting with vaginal complaints. Results indicated that two thirds of the cases of *Candida* and BV were misdiagnosed by general practitioners and gynecologists.¹⁰

Both trichomoniasis and bacterial vaginosis have been linked with other sequelae apart from the asymptomatic or symptomatic vaginitis they cause. This includes enhanced HIV transmission, increased pelvic inflammatory disease, tubal infertility and possible associations with preterm birth and prematurity.¹¹ Therefore, appropriate diagnosis of BV and its etiology is important.

Clinical Information

Vaginitis, the presence of a vaginal discharge, can be due to infection or non-infectious conditions. The latter includes allergic vaginitis or chemical vaginitis. The etiology of infectious vaginitis can be one or more of the following: *Candida*

albicans, *Trichomonas vaginalis* or the syndrome bacterial vaginosis (BV). Treatment of infectious vaginitis depends on the etiology; if more than one entity is responsible, treatment for both should be given. Yeast vaginitis (candidiasis) often presents with a white vaginal discharge with a "cottage cheese" consistency. The wet mount examination for detecting budding yeast cells has a sensitivity of < 60%; culture for *Candida* sp. is a more sensitive method and usually considered to be the gold standard for diagnosis, but it is more time-consuming. *Trichomonas vaginalis* usually presents with a frothy, sometimes green-tinged discharge that has an acidic pH; the usual wet mount for the detection of *Trichomonas vaginalis* can be a sensitive and specific method, provided the examination is performed immediately after the discharge has been collected and is read by experienced personnel. However, when vaginal specimens are transported to clinical laboratories, often the parasite is non-viable by the time wet mounts are prepared and read, reducing the ability of the observer to detect it, hence considerably reducing the sensitivity of detection. Even a 10-minute delay may reduce the sensitivity to 20%.⁵ Culture for *Trichomonas vaginalis* is > 95% sensitive, however it requires a specialized medium and incubation up to two to three days.⁴ Many clinical laboratories do not offer *Trichomonas* cultures.

BV usually presents with a thin homogeneous, malodorous discharge. BV is a syndrome, not a monomicrobial disease, resulting from a disturbance of the usual vaginal flora. When disease occurs, there is a decrease in the usual predominance of gram positive *Lactobacillus* sp. and an increase or predominance in gram negative bacteria including *Gardnerella vaginalis*, *Mobiluncus*, spp. and *Atopobium* spp., among others.³ Many females carry *Gardnerella* routinely as part of the vaginal flora and its mere presence in culture does not signify the syndrome. Culture would hence be quite sensitive, but not specific and should not be used for the

diagnosis of BV. Culture of the other involved organisms that could be responsible is very time-consuming and methods are not routinely available. The traditional physician's office method of detection is to look for "clue cells" on a wet mount preparation, in conjunction with determination of the pH (should be higher than 4.5 in BV) of the discharge and appearance of a fishy amine odor after application of 40% KOH. These clue cells are vaginal squamous epithelial cells covered with coccobacilli and curved rods in place of the usual gram positive *Lactobacillus* sp. The borders of these epithelial cells are studded with bacteria and the margins of the epithelial cells are masked. Although a long-used method for diagnosis, the detection of clue cells via wet mount has been shown to lack sensitivity and specificity, although the latter can be increased if > 20% of the epithelial cells are classified as clue cells.⁹ The scored Gram stain has become the more standardized and sensitive method for detection of BV. In this test, the vaginal discharge is Gram stained and read for the presence and quantity of *Lactobacillus*, gram variable bacilli, gram negative bacilli, and curved gram negative bacilli. The more *Lactobacillus*, the lesser the score; the less *Lactobacillus* and greater numbers of gram variable and gram negative bacilli, the more likely the interpretation will be BV. Although shown to have a very good correlation with the clinically recognized Amsel criteria for diagnosis of BV, the scored Gram stain can lack reproducibility among readers.^{1,8,9}

The Affirm VPIII probe utilizes a nucleic acid probe to detect the pathogens associated with infectious vaginitis. It can detect 10⁴ CFU/mL *Candida* cells; 10 *Trichomonas vaginalis* and > 10⁵ CFU *Gardnerella vaginalis* (GV) (numbers higher than GV carriage). Studies have shown that use of the probe can increase sensitivity over wet mounts for *Trichomonas vaginalis* (TV) and BV, and is equivalent to culture of *Candida* spp., for example.^{1a} In a recent study comparing the Affirm VPIII vs PAP smears for detection of agents of infectious vaginitis, 42.5% of 431 cases were positive for BV vs. 13.9% with PAP; 16.2% positive for *Candida* sp. vs 13.9% with

PAP; 2.3% were positive on AFFIRM for TV vs 0.7% with PAP. Coinfection, predominantly with GV and *Candida* spp. were noted in 30 cases by Affirm and one case had all three organisms. Coinfection was seen with PAP in five cases only.⁶ In a study by Lowe *et al*, the accuracy of detection of BV, TV and *Candida* clinically was compared to the Affirm probe. Sensitivity of detection was 81-85% and specificity 70-99%⁷; using the Affirm VPIII for diagnosing *Candida* spp., Petrikos *et al* reported a sensitivity of 82% and specificity of 100% when compared to the KOH examination.^{8a}

Interpretation

The Affirm method detects *Candida* species, *Trichomonas vaginalis*, and *Gardnerella vaginalis* nucleic acid in vaginal fluid specimens. A negative results indicates <1x10⁴ CFU *Candida* cells, <2 x 10⁵ CFU *G. vaginalis*, and < 5 x 10³ trichomonads.

All test results require correlation with clinical signs and symptoms. The cause of bacterial vaginosis (BV) is not fully understood, but the condition is associated with a reduction in normal *Lactobacillus* flora and an increase in other bacteria including *Gardnerella vaginalis*, *Mobiluncus*, and *Bacteroides*. This test detects >2 x 10⁵ CFU *G. vaginalis* and a positive result is suggestive, but not diagnostic for BV; results should be interpreted in conjunction with other data such as pH, amine odor, clue cells, and vaginal discharge characteristics.

Limitations of the Assay

Samples that are > 72 hours in transit to the laboratory from the time of collection will be rejected. Samples sent in other than the AFFIRM VPIII transport tube will not be processed. Samples should only be collected in patients who have clinical symptoms of vaginitis and/or a clinical diagnosis is made during examination by the physician. False positives can occur if the predictive value of the disease is low of the samples submitted.

False negatives can occur with AFFIRM in cases where the organism is in lower numbers than the threshold of the probe, ie $< 10^4$ CFU *Candida* or $< 10^3$ *Trichomonas vaginalis*.

Patients could have other sexually transmitted infections (STI), such as *Chlamydia* or gonorrhea that would not be detected by this probe.

Methodology

The Affirm probe (Becton Dickinson Microbiology Systems, Sparks, MD) is a rapid test for the detection of the nucleic acid (RNA) of *Candida albicans*, *Trichomonas vaginalis* and *Gardnerella vaginalis* (as a marker for bacterial vaginosis). Vaginal discharge should be collected on a special swab (AFFIRM) from the posterior fornix and side walls of the vagina and submitted to the laboratory via a specific transport tube. The nucleic acid in the transport tube is stable for up to 72 hours at room temperature before processing. After a lysing reagent is added to each specimen, samples are placed into the AFFIRM instrument, a series of additions of a capture and detector probe occurs, and results are read on a Probe analysis card for each pathogen. The same cards contain a positive and negative control and results can be available within hours of collection of the samples.

References

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Test Overview

Test Name	AFFIRM Vaginal Pathogens DNA Direct Probe test (VAGDNA)
Methodology	Nucleic acid probe, qualitative
Specimen Requirements	<p>Collect: Vaginal fluid; must use swab in BD Affirm VPIII Ambient Temperature Transport System (Lawson #360546).</p> <p>Specimen Preparation: Transport swab in BD Affirm VPIII Ambient Temperature Transport System (Lawson #360546).</p> <p>Stability: Ambient: 72 hours Refrigerated: 72 hours Frozen: Unacceptable</p> <p>Unacceptable conditions: Specimens received in transport system other than BD Affirm VPIII Ambient Temperature Transport System. Specimens received >72 hours after collection.</p>
Reference Range	<p><i>Candida</i> species DNA Probe: Negative <i>Gardnerella vaginalis</i> DNA Probe: Negative <i>Trichomonas vaginalis</i> DNA Probe: Negative</p>
Billing Code	88354
CPT Codes	87480, 87510, 87660

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