EVALUATION OF THE AFFIRM VP III MICROBIAL IDENTIFICATION TEST FOR THE DIAGNOSIS OF VAGINITIS AND BACTERIAL VAGINOSIS

R. Master, E. Wolf, J. Master and R. Jones
The Reading Hospital and Medical Center, Reading, PA

REVISED ABSTRACT

Vaginitis and bacterial vaginosis (BV) are two of the most common problems in women's health care. Together they account for more than ten million office visits per year in the U.S. Diagnosis has been based on clinical signs and symptoms, point-of-care tests such as microscopy, pH and the "whiff" test, and laboratory-performed tests. The Affirm VP III Microbial Identification Test (Becton Dickinson and Company) is a test incorporating non-amplified DNA probes for *Candida* species, *Trichomonas vaginalis*, and *Gardnerella vaginalis* as an indicator of BV. Four different transport systems were evaluated. The Culturette II, Culturette EZ, and Gen-Probe systems were all unable to provide consistent positive results for *Trichomonas vaginalis*. The MML transport was the only acceptable transport for *Trichomonas* and was used for this study. One hundred seventeen patients were evaluated with the Affirm VP III, clinical diagnosis (appearance of discharge, microscopy, pH and the "whiff" test) and tests performed in the laboratory (graded Gram stain for BV, *Trichomonas* culture, and yeast culture). A bacterial culture was also performed on all vaginal specimens. There was excellent correlation between the Affirm VP III and the graded Gram stain for BV and between the Affirm VP III and culture for *G. vaginalis*. The sensitivity of the Affirm VP III was 100% and specificity was 97% compared with the Gram stain and 100% and 93% compared to culture. The sensitivity and specificity of clinical diagnosis for BV were only 62% and 91%. When the Affirm VPIII for *Candida* was compared to culture, the sensitivity was 55% and specificity 100%. However, many of the specimens that were probe-negative and culture-positive for *Candida* were considered not clinically significant. When clinical evaluation was considered, the adjusted sensitivity and specificity were 84% and 99% respectively. The Affirm VP III for *Candida* compared with Gram stain yielded a sensitivity of 88% and a specificity of 98%. The sensitivity and specificity of the wet prep in diagnosing candidiasis were 74% and 91%. There were several instances of false-positive wet preps for yeast resulting in the predictive value of a positive wet prep for yeast being only 61%. There was 100% correlation between the Affirm VP III and culture for *Trichomonas vaginalis*, however, only seven specimens were positive. The MML transport worked well for *Trichomonas*, *Candida*, and the *Gardnerella*. Overall, the Affirm VP III performed significantly better than clinical diagnosis (95% vs 65% sensitivity, 99% vs. 94% specificity).

INTRODUCTION

Bacterial vaginosis is the most common vaginal infection and accounts for 15 to 50% of the cases of vaginitis/vaginosis depending on the patient population. Vaginal candidiasis is the second most common vaginal infection and approximately 75% of adult women will experience this infection in their lifetime. Vaginal infections are usually diagnosed by clinical signs and symptoms alone or clinical signs and symptoms plus microscopic examination. Bacterial vaginosis is no longer regarded as harmless. Complications such as PID, premature rupture of membranes, pre-term delivery, and post-partum endometriosis have been reported.

There are concerns about the sensitivity of diagnosis by wet mount. The sensitivity of wet mounts is 68% for BV, 55% for *Trichomonas*, and 34% for *Candida*. The sensitivity and specificity of wet mounts are dependent on proper training and experience. Also, use of over-the-counter antifungal agents and the presence of mixed infections hampers examination of wet mounts. The pH of vaginal fluid can be affected by the presence of blood, semen, and mixed infections.

The Affirm VP III Microbial Identification System detects *Candida* species, *Gardnerella vaginalis*, and *Trichomonas vaginalis* in less than one hour using DNA probe technology. The test uses two distinct single-stranded nucleic acid probes for each organism. One probe is a capture probe attached to a bead embedded in a plastic card. The second probe, the color development probe, is provided in a multi-well reagent cassette.

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MATERIALS AND METHODS

The patient population tested consisted of women seen in obstetric and gynecology clinics and a private obstetrics and gynecology practice. Patients were selected on the basis of signs or symptoms consistent with vaginitis or vaginosis including vaginal discharge, pruritis, irritation, and dyspareunia.

Clinical Diagnosis

The clinical diagnosis of trichomoniasis was based on the observation of motile trichomonads on microscopic evaluation of a wet prep prepared with normal saline. The clinical diagnosis of candidiasis was based on the observation of hyphae or budding yeast cells on a wet prep prepared with 10% KOH. The clinical diagnosis of bacterial vaginosis (BV) was based on the criteria established b Amsel et al. (Am. J. Med. 1983.74:14-22). The diagnosis of BV was established by finding three of the following four signs :

1. A thin, homogeneous discharge that adheres to the vaginal walls
2. An elevated pH $\geq$ 4.5
3. A positive KOH "whiff" test
4. The presence of clue cells on microscopic examination. The pH was determined using nitrazine paper.

Graded Gram Stain

Specimens were collected using a Culturette II (Becton Dickinson Microbiology Systems, Cockeysville, MD). They were transported to the laboratory where a smear was made and a Gram stain performed. Smears were interpreted using the criteria of Nugent et al. (J. Clin. Microbiol. 1991.29:297)

Trichomonas Culture

Specimens were obtained with a swab and inoculated directly into the InPouch TV *Trichomonas vaginalis* Test culture system (Biomed Diagnostics, San Jose, CA). The InPouch system was incubated at 35°C. The pouches were examined microscopically once a day for three days.

Yeast Culture

Specimens were collected using a Culturette II. They were transported to the laboratory where they were inoculated onto Sabouraud dextrose agar and incubated for three days at 30°C. Yeasts were identified using the Vitek YBC card.

Bacterial Culture

Specimens were collected using a Culturette II. They were transported to the laboratory where they were inoculated onto Columbia CNA agar, chocolate agar, and Levine eosin methylene blue agar and incubated for three days at 35°C.

Affirm VP III

Specimens were obtained with a swab and inoculated directly into a tube of MML transport medium – Amies (MML Diagnostics Packaging, Troutdale, OR). Specimens were transported to the laboratory and stored at 2 to 8°C prior to testing.

Sample Preparation

1. Add the swab to the sample collection tube.
2. Add 12 drops of Lysis Solution and mix for at least 10 seconds.
3. Place the swab with cap back into the tube.
4. Incubate the tube in the Lysis Block for 10 minutes.
5. Add 12 drops of Buffer Solution and mix.
6. Remove the swab and replace the cap. Mix by flicking the tube briskly 10 times.
7. 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 the swab. Press a Filter Tip firmly onto each Sample Collection Tube.
8. Specimens may be stored at room temperature at this point for up to 24 hours.

Automated processing

1. Select one Reagent Cassette for each sample to be tested and place the Cassette into the caddy. Carefully pull the foil covering off the Cassette, lifting from the end without the upward bent flap.
2. Open the top of the pouch containing the Probe Analysis Card (PAC), but do not remove the PAC from the pouch. Avoid touching the beads.
3. Press the (RUN) key. The system will prompt for the addition of each reagent.

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Add 4 drops of Substrate Solution to well No 7 of the Reagent Cassette.

4. Invert the Sample Collection tube and firmly squeeze the entire contents of each tube through the Filter Tip into the reservoir of well No 1 of the appropriate Reagent Cassette. Dispose of the patient sample tube.
5. Place a labelled PAC into Well I of each Reagent Cassette.
6. Carefully replace the Cassette Caddy on the processor, taking care not to splash reagents.
7. The processor will automatically pick up and move the PACs through the test procedure. The instrument will begin the processing sequence and will indicate the time remaining. At the end of the processing time, the instrument will beep and present the PAC for removal.
8. 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.

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

Statistical Methods

Statistical calculations were performed using the SigmaStat version 2.0 (SPSS, Inc., Chicago, IL) statistical software.

RESULTS

One hundred seventeen patients were tested. Thirty-eight (33%) had bacterial vaginosis, nineteen (16%) and candidiasis, and seven (6%) had trichomoniasis. Ten (9%) patients had mixed infections with seven cases of BV and candidiasis and three cases of BV and trichomoniasis.

Affirm VP III vs. Culture for *Trichomonas vaginalis*

	Culture Positive	Culture Negative
Affirm VP III *Trichomonas* Positive	7	0
Affirm VP III *Trichomonas* Negative	0	110

*1 wet prep + for trichomonas, Affirm+ for trichomonas, trichomonas culture not performed
Sensitivity:100%; Specificity:100%; Predictive Value Positive:100%; Predictive Value Negative:100%

Affirm VP III vs. Graded Gram Stain for Bacterial Vaginosis

	Gram Stain Interpretation		
	Bacterial Vaginosis	Intermediate Vaginal Flora	Normal Vaginal Flora
Affirm VP III *Gardnerella* Positive	38	8	2
Affirm VP III *Gardnerella* Negative	0	6	62

Sensitivity:100%; Specificity:97%; Predictive Value Positive:95%; Predictive Value Negative:100%

Affirm VP III vs. Culture for *Gardnerella vaginalis*

	Culture Positive for *G. vaginalis*	Culture Negative for *G. vaginalis*
Affirm VP III *Gardnerella* Positive	39	5
Affirm VP III *Gardnerella* Negative	0	68

Sensitivity:100%; Specificity:93%; Predictive Value Positive:89%; Predictive Value Negative:100%

Affirm VP III vs. Culture for *Candida* species with Clinical Review

	Final Diagnosis Candidiasis	Final Diagnosis Not Candidiasis
Affirm VP III *Candida* Positive	16	1
Affirm VP III *Candida* Negative	3	96

Sensitivity:84%; Specificity:99%; Predictive Value Positive:94%; Predictive Value Negative:97%

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Affirm VP III vs Gram Stain for Yeast

	Gram Stain Interpretation	
	Yeast Seen	No Yeast Seen
Affirm VP III *Candida* Positive	15	2
Affirm VP III *Candida* Negative	2	97

Sensitivity:88%; Specificity:99%; Predictive Value Positive:88%; Predictive Value Negative:98%

Wet Prep vs Culture for *Trichomonas vaginalis*

	Culture Positive	Culture Negative
Wet Prep Positive for *Trichomonas*	4	0
Wet Prep Negative for *Trichomonas*	3	110

Sensitivity:57%; Specificity:100%; Predictive Value Positive:100%; Predictive Value Negative:97%

Clinical Diagnosis vs. Graded Gram Stain for Bacterial Vaginosis

	Gram Stain Interpretation		
	Bacterial Vaginosis	Intermediate Vaginal Flora	Normal Vaginal Flora
Clinical Diagnosis Positive for BV	23	3	6
Clinical Diagnosis Negative for BV	14	11	59

Sensitivity:62%; Specificity:91%; Predictive Value Positive:79%; Predictive Value Negative:81%

Wet Prep for Yeast vs. Culture for *Candida* species with Clinical Review

	Final Diagnosis Candidiasis	Final Diagnosis Not Candidiasis
Wet Prep Positive for Yeast	14	9
Wet Prep Negative for Yeast	5	88

Sensitivity:74%; Specificity:91%; Predictive Value Positive:61%; Predictive Value Negative:97%

Wet Prep for Yeast vs. Gram Stain for Yeast

	Gram Stain Interpretation	
	Yeast Seen	No Yeast Seen
Wet Prep Positive for Yeast	13	10
Wet Prep Negative for Yeast	5	88

Sensitivity:72%; Specificity:90%; Predictive Value Positive:56%; Predictive Value Negative:95%

Culture vs. Graded Gram Stain for Bacterial Vaginosis

	Gram Stain Interpretation		
	Bacterial Vaginosis	Intermediate Vaginal Flora	Normal Vaginal Flora
Culture Positive for *G. vaginalis*	32	5	2
Clinical Diagnosis *G. vaginalis*	2	7	64

Sensitivity:94%; Specificity:97%; Predictive Value Positive:94%; Predictive Value Negative:97%

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Summary

- The MML transports were effective holding specimens for 24 hours prior to testing for *Trichomonas*, *Candida*, and *Gardnerella.*
- The sensitivity and specificity of the Affirm VP III for *Trichomonas*, *Candida* and *Gardnerella* were 100%/100%, 100%/97%, and 84%/99% respectively.
- False-positive wet preps for yeast (predictive value of a positive 61%) and clue cells were more common than expected and indicates that training in microscopy needs improvement.
- The sensitivity of microscopy for *Trichomonas vaginalis* was only 57%, the sensitivity of clinical diagnosis for bacterial vaginosis was only 62%, and the sensitivity of the wet prep for yeast was 74%. The overall sensitivity for bedside diagnosis of vaginitis and vaginosis was 65%. One-third of the patients with vaginal infections would be missed if additional testing is not performed.
- Overall, the Affirm VP III performed significantly better than clinical diagnosis (95% vs 65% sensitivity, 99% vs 94% specificity) and can be recommended for use as a screening test for vaginitis and bacterial vaginosis.

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