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Original Research

Comparison of BD Affirm VPIII with Gram and liquid-based cytology for diagnosis of bacterial vaginoses, candidiasis and Trichomonas

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Summary

Background: Vaginitis require complementary methods for adequate diagnosis. New technologies have been developed and need to be compared with those already established. *Aims:* The objective of this study was to compare the results of BD Affirm VPIII with Gram smear and liquid-based cytology in diagnosis of bacterial vaginosis (BV), *Candida sp,* and *Trichomonas vaginalis*. *Materials and Methods:* A cross-sectional study was performed in 90 women from March to November 2016 in a gynecology clinic in a Brazilian city, Fortaleza. The Affirm test, Gram, and Surepath were performed in vaginal samples. Categorical data was evaluated for sensibility, specificity, and predictive values. To quantify the agreement, the Kappa index was utilized. *Results:* Comparing Affirm test with Gram stain for BV and Surepath, the sensitivity was 83.3% and 27.8%, and the specificity was 62.2% and 95.5%, respectively. The Kappa index was 0.204 to Gram and 0.247 to Surepath. For *Candida sp* comparing with Gram and Surepath, the sensitivity was 61.1% and 56.3%, and the specificity was 93.3% and 100%, respectively. The Kappa index was 0.585 to Gram and 0.677 to Surepahth. *Conclusion:* The Affirm test had a fair concordance with Gram and Surepath in the diagnosis of BV, and substantial agreement in the diagnosis of Candida.

Key words: Molecular diagnostic techniques; Cytodiagnosis; Vaginitis; Vaginosis; Vulvovaginal candidiases; Trichomonas infection.

Introduction

In gynecological clinics, the most frequently reported complaint by women is associated with vaginitis and vaginosis. Patients anxious for a quick and correct diagnosis report genital discharge, pruritus, and odor. The three most common etiologies for vaginal symptoms are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis. A medical history is insufficient for accurate diagnosis of vaginitis and can lead to an inappropriate treatment. Therefore, a careful history, examination, and laboratory testing to determine the etiology of vaginal symptoms are warranted [1].

Routinely used exams are predominantly morphological and, therefore, individual-dependent. Identification of pathogens is most often done by examination of the vaginal contents, by bacterioscopy and even by Pap smear. For *Candida* and *Trichomonas* sensitivity of microscopy is approximately 50% [2]. Gram is considered the gold standard for the diagnosis of BV through the Nugent score [3]. Pap smear was already widely used for the diagnosis of infections[4], and more recently liquid cytology has also been used for this [5].

Recently, a DNA probe test, BD Affirm VPIII microbial identification test system was presented as a biomolecular test for the detection of *Gardnerella vaginalis*, *Candida albicans*, and *Trichomonas vaginalis* in vaginal discharge. Although the sensibility and specificity of test was studied in others countries [6-9], its results in Brazilian women is not known.

The aim of this study is compare the results of BD Affirm VPIII test with Gram smear and liquid-based cytology (Surepath) to diagnosis BV, *Candida sp,* and *Trichomonas*.

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Materials and Methods

A cross-sectional study was performed in 90 women (mean age 32 ± 9.7 years) from March to November 2016 in a private gynecology clinic in a Brazilian city (Fortaleza), all of them with a complaint of vaginal discharge.

After speculum placement, the authors obtained material from the vaginal wall with a swab and stored in tubes with Ambient Temperature Transport System (ATTS). At the same time, another swab was used to collect material for the Gram test and another for liquid-based cytology (Surepath).

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Table 1. — *Identification of pathogens in Affirm test compared with Gram smear and liquid-based cytology (Surepath).*

	Gram (73)					Surepath (90)				
Affirm	Neg N (%)	Gv N (%)	Ca N (%)	Gv+Ca N (%)	T N (%)	Neg N (%)	Gv N (%)	Ca N (%)	Gv+Ca N (%)	T N (%)
Neg	28 (66.7)	1 (25)	7 (28)	0 (0)	0 (0)	38 (100)	21 (67.7)	2 (20)	5 (62.5)	2 (66.7)
Gv	11 (26.2)	3 (75)	8 (32)	1 (50)	0 (0)	0 (0)	9 (29)	1 (10)	1 (12.5)	1 (33.3)
Ca	0 (0)	0 (0)	3 (12)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)
Gv + Ca	3 (7.1)	0 (0)	7 (28)	1 (50)	0 (0)	0 (0)	1 (3.2)	6 (60)	2 (25) (0)	0 (0)
Tricho	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	42 (100)	4 (100)	25 (100)	2 (100)	0 (0)	38 (100)	31 (100)	10 (100)	8 (100)	3 (100)

Neg: negative; Gv: Gardnerella vaginalis; Ca: Candida sp; T: Trichomonas vaginalis.

Table 2. — *Sensibility, specificity, positive and negative predictive values, and Kappa index to Affirm test against Gram smear and liquid-based cytology (Surepath) for bacterial vaginosis.*

Affirm	Gram	Surepath	Both (Gram and Surepth)
Sensitivity	83.3	27.8	32.3
Specificity	62.2	95.5	71.9
PPV	20.8	83.3	47.6
NPV	97.1	61.8	57.1
Kappa	0.204 (0.011-0.396)	0.247 (0.078-0.417)	0.042 (-0.182-0.266)

PPV: positive predictive value; NPV: negative predictive value. Kappa index: < 0 less than chance agreement; 0.01–0.20 slight agreement; 0.21– 0.40 fair agreement; 0.41–0.60 moderate agreement; 0.61–0.80 substantial agreement; 0.81–0.99 almost perfect agreement. (Viera A.J., Garrett J.M.: "Understanding Interobserver Agreement: The Kappa Statistic". Fam. Med., 2005, 37, 360.

Table 3. — *Sensibility, specificity, positive and negative predictive values, and Kappa index to Affirm test against Gram smear and liquid-based cytology (Surepath) for Candida sp.*

Affirm	Gram	Surepath	Both (Gram and Surepth)
Sensitivity	61.1	56.3	44.4
Specificity	93.3	100	98
PPV	78.6	100	92.3
NPV	86	91	76.6
Kappa	0.585 (0.358-0.813)	0.677 (0.460-0.894)	0.482 (0.283-0.681)

PPV: positive predictive value; NPV: negative predictive value. Kappa index: < 0 less than chance agreement; 0.01–0.20 slight agreement; 0.21– 0.40 fair agreement; 0.41–0.60 moderate agreement; 0.61–0.80 substantial agreement; 0.81–0.99 almost perfect agreement. (Viera A.J., Garrett J.M.: "Understanding Interobserver Agreement: The Kappa Statistic". Fam. Med., 2005, 37, 360).

The Affirm VPIII test was used to identify the pathogen. The test has three steps: denaturation of the materials to release the nucleic acids specific for each pathogen, automatic processing. The experiment includes negative and positive controls.

The Gram was performed as classically described and the Surepath slide was performed complying the manufacturer's recommendations (Surepath).

Categorical data was evaluated for sensibility, specificity, and predictive values. To quantify the agreement between the tests was used the Kappa index. The GraphPad Prism version 5.00 was utilized. The study was approved by the Ethics and Research Committee of the Federal University of Ceará.

Results

The authors performed the test in 90 patients, of which 90 patients underwent liquid-based cytology, but in 17 cases, they encountered problems with the air-dried smears and Gram was performed only in 73 patients simultaneously with Affirm VPIII test. The results are shown in Table 1.

Comparing Affirm test with Gram stain for bacterial vaginosis (BV) the sensitivity was 83.3%, the specificity was 62.2%, the positive predictive value was 20.8%, and the negative predictive value was 97.1%. The Kappa index between the tests was 0.204, considered as fair agreement. When Affirm was compared with Surepath for BV the sensitivity was 27.8%, the specificity was 95.5%, the positive predictive value was 83.3%, and the negative predictive value was 61.8%. The Kappa index between the tests was 0.247, considered as fair agreement. When Affirm result was compared with the positivity of the both tests at the same time, the results became worse (Table 2).

To identify *Candida sp* the results comparing with Gram were, sensitivity 61.1%, the specificity 93.3%, the positive predictive value was 78.6%, and the negative predictive value was 86%. The Kappa index between the tests was 0.585, considered as moderate agreement. In turn comparing the Affirm VPII with Surepath for identification of *Candida sp*, the sensitivity was 56.3%, the specificity was 100%, the positive predictive value was 100%, and the neg-

ative predictive value was 91%. The Kappa index between the tests was 0.677, considered as substantial agreement. When Affirm result was compared with the positivity of the both tests in the same time, the results became a little worse, but no significant (Table 3). There were three cases of *Trichomonas* in Surepath, but not in Affirm test (Table 1).

Discussion

The more frequent causes of vaginal discharge is BV, candidiasis, and trichomoníasis. However, the identification of a pathogen sometimes is not associated with the symptom; it could be a commensal, as occurs with *Gardnerella vaginalis* and *Candida sp*. Because of this, the present decided that the agents' research would be done only in women with symptoms of vaginal discharge.

To diagnosis of BV, the Nugent score is a gold standard independent of the reference of the discharge or other symptom [3]. Confronting the Gram test, the present authors observed a very good sensitivity (83.3%) and good specificity (62.2%) to Affirm test, however, a fair concordance index (Kappa). Also comparing Affirm with Gram other researchers [6] observed a sensitivity of 75% and a specificity of 89% for BV. On the other hand, the sensitivity of Affirm compared to liquid based cytology (more than 20% of clue cells) [10] was low (27.8%), but the specificity was high (95.5%), but also with a fair agreement ($\kappa = 0.25$). Levi *et al.* [11], comparing the test with liquid-based cytology, a poor agreement for the diagnosis of BV ($\kappa = 0.32$) was observed. This low concordance may occur because the biomolecular assay specifically identifies *Gardnerella vaginalis,* while the morphological one diagnoses the complex condition of bacterial vaginosis.

If both tests were utilized, the results were worse, but the authors do not have studies evaluating the results of Affirm and Gram /liquid-based cytology.

To *Candida albicans* the authors observed that the sensitivity and specificity comparing Affirm to Gram was 61.1% and 93.3%, respectively. The agreement was good ($\kappa = 0.59$). Byun *et al.* [6] using culture for Candida as a gold standard observed that the sensitivity and specificity of the Affirm test were 82.76% and 98.80%, respectively. With Surepath the sensitivity was lower (56.3%), but the specificity was higher (100%) and the agreement was substantial ($\kappa = 0.68$).The results comparing both were worse. Other researchers also observed an index of agreement between Affirm and liquid-based cytology substantial ($\kappa = 0.66$) [7].

The present authors did not identify Trichomonas infection with Affirm, although in the Surepath they observed three cases suggesting the parasite. Levi *et al.* [7] observed only one case of Trichomonas, but the corresponding molecular test was negative. On the other hand ten (2.3%) were positive for *T. vaginalis* on Affirm, but not with liquid-based cytology. They considered the risk of false positive in cytology. Others studies observed very few *T. vaginalis* in Affirm test [6, 8, 12].

The studies have different results between Affirm and cytology because BV is more likely to be better represented by vaginal samples used in the Affirm VPIII assay than in cervical samples used for cytology [7]. The present authors eliminated this bias, because they used vaginal samples for all tests. Although they assume limitations of the study, due to a low number of cases, a less of correlation with symptoms besides discharge, and a lack of research of others pathogens with molecular biology methods. The main limitation may have been comparing the test with morphological exams by themselves. With the exception of bacterial vaginosis, they are not gold standard.

The Affirm test has a good sensitivity, but not sufficient specificity for BV and good specificity and not sufficient sensitivity for candidiasis. Thus, there is a fair concordance with Gram and Surepath in the diagnosis of BV, but substantial agreement in the diagnosis of Candida.

References

[1] (Centers for Disease Control and Prevention (CDC): "2015 Sexually Transmitted Diseases Treatment Guidelines". https://www.cdc.gov/std/tg2015/vaginal-discharge.htm

[2] Nye M.B., Schwebke J.R., Body B.A.: "Comparison of APTIMA Trichomonas vaginalis transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women". *Am. J. Obstet. Gynecol.*, 2009, *200*, e181.

[3] Schwebke J.R., Hillier S.L., Sobel J.D., McGregor J.A., Sweet R.L.: "Validity of the vaginal gram stain for the diagnosis of bacterial vaginosis". *Obstet. Gynecol.*, 1996, *88*, 573.

[4] Güdücü N., Gönenç G., Işçi H., Yiğiter A.B., Başsüllü N., Dünder I.: "Clinical importance of detection of bacterial vaginosis, trichomonas vaginalis, candida albicans and actinomyces in Papanicolaou smears". *Clin. Exp. Obstet. Gynecol.*, 2012, *39*, 333.

[5] Noël J.C., Engohan-Aloghe C.: "Morphologic criteria associated with Trichomonas vaginalis in liquid-based cytology". *Acta Cytol.*, 2010, *54*, 582.

[6] Byun S.W., Park Y.J., Hur S.Y.: "Affirm VPIII microbial identification test can be used to detect gardnerella vaginalis, Candida albicans and trichomonas vaginalis microbial infections in Korean women". *J. Obstet. Gynaecol. Res.*, 2016, *42*, 422.

[7] Levi A.W., Harigopal M., Hui P., Schofield K., Chhieng D.C.: "Comparison of Affirm VPIII and Papanicolaou tests in the detection of infectious vaginitis". *Am. J. Clin. Pathol.*, 2011, *135*, 442.

[8] Andrea S.B., Chapin K.C.: "Comparison of Aptima Trichomonas vaginalis transcription-mediated amplification assay and BD affirm VPIII for detection of T. vaginalis in symptomatic women: performance parameters and epidemiological implications". *J. Clin. Microbiol.*, 2011, *49*, 866.

[9] Brown H.L., Fuller D.D., Jasper L.T., Davis T.E., Wright J.D.: "Clinical evaluation of affirm VPIII in the detection and identification of Trichomonas vaginalis, Gardnerella vaginalis, and Candida species in vaginitis/vaginosis". *Infect. Dis. Obstet. Gynecol.*, 2004, *12*, 17.

[10] Discacciati M.G., Simoes J.A., Amaral R.G., Brolazo E., Rabelo-Santos S.H., Westin M.C., Montemor E.B.: "Presence of 20% or more clue cells: an accurate criterion for the diagnosis of bacterial vaginosis in Papanicolaou cervical smears". *Diagn. Cytopathol.*, 2006, *34*, 272.

[11] Levi A.W., Harigopal M., Hui P., Schofield K., Chhieng D.C.: "Comparison of Affirm VPIII and Papanicolaou tests in the detection of infectious vaginitis". *Am. J. Clin. Pathol.*, 2011, *135*, 442.

[12] Eleutério J. Jr., Oliveira A.K.G., Giraldo P.C., Gordiano A.B., Queiroz F., Eleutério R.M.N., Linhares I.M.: "Co-occurrence of Gardnerella vaginalis and Candida sp. in women with and without vulvovaginitis". *Int. J. Med. Res. Health Sci.*, 2016, *5*, 32.

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