

# Diagnostic Value of DNA Hybridization Test for *Gardnerella vaginalis*, *Candida* spp., and *Trichomonas vaginalis* in Women with Vaginal Discharge

## Vajinal Akıntılı Kadınlarda *Gardnerella vaginalis*, *Candida* spp. ve *Trichomonas vaginalis* İçin DNA Hibridizasyon Testinin Tanısal Önemi

Gonca İmir YENİCESU, MD,<sup>a</sup>  
Aynur ENGİN, MD,<sup>b</sup>  
Ali ÇETİN, MD,<sup>a</sup>  
Zeki ÖZSOY, MD<sup>a</sup>

Departments of  
<sup>a</sup>Obstetrics and Gynecology,  
<sup>b</sup>Infectious Diseases and  
Clinical Microbiology,  
Cumhuriyet University  
Faculty of Medicine, Sivas

Geliş Tarihi/Received: 05.08.2008  
Kabul Tarihi/Accepted: 05.02.2009

The study was presented as "Clinical evaluation and identification of *Trichomonas vaginalis*, *Gardnerella vaginalis*, and *Candida* species by conventional methods and PCR in patient with vaginal discharge" in Middle East Fertility Society (MEFS) 14<sup>th</sup> Annual Meeting, 3<sup>rd</sup> October-3<sup>rd</sup> November, Antalya, Turkey.

Yazışma Adresi/Correspondence:  
Gonca İmir YENİCESU, MD  
Cumhuriyet University  
Faculty of Medicine,  
Department of  
Obstetrics and Gynecology, Sivas,  
TÜRKİYE/TURKEY  
imirgonca@yahoo.com

**ABSTRACT Objective:** The aim of this study was to determine the efficiency of DNA hybridization test in women with vaginal discharge evaluated by conventional laboratory methods including wet mount, whiff test, and Gram stain used in combination for the diagnosis of *Gardnerella vaginalis*, *Candida* spp., and *Trichomonas vaginalis* vaginitis. **Material and Methods:** A prospective study involving 63 women reporting with abnormal vaginal discharge from January to July 2007 in our hospital was performed. The vaginal smears were evaluated by whiff test, wet mount, Gram stain and DNA hybridization test and the results were recorded. **Results:** Thirty nine (61.9%) women were negative for all the three infections by conventional laboratory methods whereas 50 (79.4 %) were negative by DNA hybridization test. *G. vaginalis* was identified as the causative organism in 11 (17.5%) patients by conventional laboratory methods and in 5 (7.9%) patients by DNA hybridization test. Nine patients (14.3%) were diagnosed with Candidiasis by conventional laboratory methods compared to 6 (9.5%) patients by DNA hybridization test. Conventional laboratory methods detected combined *Gardnerella* and *Candida* infections in 2 (3.2%) patients, whereas 1 (1.6%) patient was positive for both infections by DNA hybridization test. Two (3.2%) patients were identified with *T. vaginalis* by conventional laboratory methods and 1 (1.6%) by DNA hybridization test who incidentally was also positive for *G. vaginalis* and *Candida*. **Conclusion:** There is not superiority of the DNA hybridization test over conventional laboratory methods in the diagnosis of vaginitis in our study. We suggest that conventional laboratory tests as used in our settings is useful in diagnosing vaginitis when used in conjunction with consistent clinical signs, instead of using an expensive alternative method, DNA hybridization test.

**Key Words:** Vaginal discharge; vaginosis, bacterial; nucleic acid hybridization;  
*Trichomonas* vaginitis; candidiasis

**ÖZET Amaç:** Çalışmamızın amacı Whiff testi, direkt preparat ve Gram boyamayı içeren geleneksel tanı yöntemleri ile incelenen vajinal akıntılı kadınlarda *Gardnerella vaginalis*, *Candida* spp., and *Trichomonas vaginalis* için DNA hibridizasyon yönteminin tanıdaki etkinliğini değerlendirmektir. **Gereç ve Yöntemler:** Hastanemizde Ocak-Temmuz 2007 tarihleri arasında vajinal akıntı şikâyeti ile başvuran 63 kadını kapsayan bu prospektif çalışma uygulandı. Vajinal smearler Gram boyama, direkt preparat, Whiff testi ve DNA hibridizasyon yöntemi ile incelendi ve sonuçlar kaydedildi. **Bulgular:** Konvansiyonel metot ile 39 (%61.9) kadın her üç etken için negatif iken, DNA hibridizasyon yöntemi ile 50 (%79.4) kadın negatif idi. Konvansiyonel metot ile hastaların 11 (%17.5)'inde, DNA hibridizasyon yöntemi ile 5 (%7.9)'inde etken olarak *G. vaginalis* saptandı. Konvansiyonel metot ile 9 (%14.3) hastada *Candida* bulunurken DNA hibridizasyon yöntemi ile 6 (%9.5) hastada saptandı. *Gardnerella* ve *Candida* birlikteliği konvansiyonel metotla 2 (3.2%) hastada saptanırken DNA hibridizasyon yöntemi ile 1 (%1.6) hastada bulundu. *T. vaginalis* konvansiyonel metot ile 2 (%3.2) hastada görüldü ve DNA hibridizasyon yöntemi ile sadece 1 (%1.6) hastada saptandı ki bu hastada aynı zamanda diğer iki etken de saptandı. **Sonuç:** Bizim çalışmamızda vajinitin tanısında DNA hibridizasyon yönteminin konvansiyonel laboratuvar metoduna üstünlüğü saptanmadı. Pahalı bir alternatif olan DNA hibridizasyon yöntemi yerine konvansiyonel laboratuvar testlerinin uyumlu klinik bulgu varlığında vajinitin tanısında tercih edilebileceğini düşünmekteyiz.

**Anahtar Kelimeler:** Vajinal akıntı; vajinosis, bakteriyel; nükleik asid hibridizasyon;  
*Trichomonas* vajinitis; kandidiyazis

Turkiye Klinikleri J Med Sci 2009;29(5):1242-6

Vaginal infections are among the most common problems in clinical medicine.<sup>1</sup> *Trichomonas vaginalis*, *Candida albicans* and *Gardnerella vaginalis* are three major agents of vaginal infection in reproductive years.<sup>2</sup> Accurate diagnosis of vaginitis is important as it is associated with pelvic inflammatory disease, infertility, chorioamnionitis, and endometritis.<sup>3</sup>

Vaginal discharge is one the most common symptom of vaginitis.<sup>4</sup> Diagnosis and treatment can be elusive, if based on clinical symptoms and the characterization of vaginal discharge alone.<sup>5</sup> The misdiagnosis leads to a lack of relief from symptoms and, because of inadequate treatment, to increased costs and the rate of complications.<sup>6</sup> In this report, we refer *G. vaginalis* vaginitis by using the term "bacterial vaginosis".

Several methods are available for diagnosis of vaginitis.<sup>7</sup> The culture of the vaginal discharge is both labor intensive and time consuming. The conventional laboratory methods including potassium hydroxide (KOH) amine odor test, microscopic exam (wet mount), pH test of vaginal secretion, and laboratory diagnosis via Gram stain are widely available and are practical for the diagnosis of vaginitis. In recent years, it has been reported that the Affirm VPIII assay, DNA hybridization test, is more sensitive in the detection and identification of vaginitis than conventional laboratory methods.<sup>4,8</sup>

Lately, demand for expeditious and accurate diagnosis is increasing in women with vaginitis. For the diagnosis of *G. vaginalis*, *Candida spp.*, and *T. vaginalis* vaginitis, the aim of this study was to assess the efficiency of DNA hybridization test in women with vaginal discharge evaluated by conventional laboratory methods including whiff test, wet mount, and Gram stain used in combination.

## MATERIAL AND METHODS

We performed a prospective study involving 63 women with the complaint of vaginal discharge from January to July 2007 in the gynecology outpatient clinic of Cumhuriyet University Hospital. The study was approved by the local ethics committee of Cumhuriyet University and informed consent

was obtained from all of the subjects. Inclusion criteria were sexually active women with complaints of vaginal discharge. Women who had received antibiotic or antifungal therapy within the last two weeks, who had douched within 24 hours, or who were menstruating and pregnant women were excluded.

Whiff test, wet mount, and Gram staining used in combination as conventional laboratory tests for the diagnosis of vaginitis. Vaginal examination was performed to evaluate the physical characteristics of vaginal discharge. Vaginal pH level was determined by placing the pH paper directly on the vaginal wall or in the vaginal discharge. Four vaginal samples from the vaginal discharge were obtained from the lateral and the posterior fornices. The three vaginal samples for each woman were sent to the Laboratory for Infectious Diseases for whiff test, wet mount, and Gram stain and evaluated by a single physician (A.E.) without knowledge of the clinical findings or polymerase chain reaction (PCR) results. The fourth sample for each woman was sent to microbiology laboratory for PCR.

The whiff test was performed by placing a drop of vaginal discharge on a glass slide and adding a drop of 10% KOH and the presence of fishy odor was defined as a positive test. For the wet mount, second swab was placed in a drop of sterile normal saline on a glass slide and immediately examined microscopically for the presence of motile trichomonads, clue cells, and yeast or hyphae. The Gram-stained smear of vaginal discharge was examined for the presence of hyphae, buds, polymorphonuclear cells, and clue cells (squamous epithelial cells studded with Gram-variable coccobacile).<sup>7</sup> Diagnostic criteria were as follows; *G. vaginalis* was diagnosed by an abnormal grayish discharge, elevated pH value, a wet preparation showing clue cells, and a positive amine test. Vulvovaginal candidiasis was diagnosed by potassium hydroxide preparation or Gram stained smear showing typical yeast and hyphae. The diagnosis of Trichomoniasis was based on the presence of motile *T. vaginalis* organisms in wet preparations.

The last swab was inserted in the Affirm sample testing vessel and immediately transported to the testing area at room temperature. The swab was then tested using the Affirm VPIII assay on the BD MicroProbe Processor according to manufacturer's recommendations. The Affirm VPIII test (Becton Dickinson and Company, Sparks, MD, USA) is based on the principles of nucleic acid hybridization and uses two distinct single-stranded probes for *T. vaginalis*, *G. vaginalis*, and *Candida* spp., a capture probe and a color development probe. After completion of the test, the results of the assay were visually observed and the results were recorded.

### STATISTICAL ANALYSIS

Frequencies and percentages were calculated for demographic and laboratory data. The sensitivity, specificity, positive and negative predictive values were calculated for conventional and DNA hybridization tests. Sperman correlation test were used for association of the diagnostic methods. A value of  $p < 0.05$  was considered as statistically significant.

## RESULTS

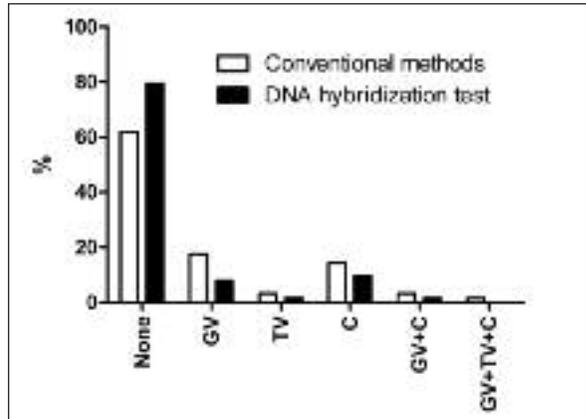
During the study period from January to July 2007, 72 patients with the complaint of vaginal discharge were recruited. Four refused to participate in the study and 5 were missed because of the high work load. Therefore, 63 patients completed the study and were included in the final data analysis.

Table 1 summarizes the demographic data including patient age, gravidity, parity, duration of sexual activity, the number of vaginal deliveries, and the number of vaginal infections in the last one year. The mean age of the patients was  $32.5 \pm 7.7$  years (range, 17-53 years). Four (6.3%) of the patients were postmenopausal. The mean duration of sexual activity of the patients was  $12.6 \pm 7.6$  years. The average number of vaginal deliveries was  $1.4 \pm 1.3$ .

Figure 1 presents number of patients diagnosed as no infection, *G. vaginalis*, *Candida* spp., *T. vaginalis*, and combined infection according to conventional laboratory tests including whiff test, wet mount, and Gram staining and DNA hybridization methods. Although case numbers were low

**TABLE 1:** Demographic data of the patients.

	Mean $\pm$ SD
Age (years)	32.5 $\pm$ 7.7
Gravidity	2.4 $\pm$ 1.3
Parity	1.9 $\pm$ 1.3
Duration of sexual activity (years)	12.6 $\pm$ 7.6
Number of vaginal deliveries	1.4 $\pm$ 1.3
Number of vaginal infections in the last one year	5.3 $\pm$ 3.6



**FIGURE 1:** Patients diagnosed as no infection (None), Gardnerella vaginalis (GV), Candida spp., Trichomonas vaginalis (TV), and combined infections according to conventional laboratory methods including whiff test, wet mount, and Gram staining and DNA hybridization tests.

**TABLE 2:** Results of the conventional laboratory methods and DNA hybridization test for diagnosis of vaginitis.

Result and test system	n (%)
Positive DNA hybridization test only	2 (3.2%)
Positive conventional laboratory methods only	13 (20.6%)
Positive DNA hybridization test and conventional laboratory methods	11 (17.5%)
Negative DNA hybridization test and conventional laboratory methods	37 (58.7%)

for each diagnosis to compare their ratios, as seen in the Figure 1, the association of conventional and DNA hybridization tests were good in cases with no infection, but low in cases with *G. vaginalis* infection. No infection was detected in 37 (58.7%) vaginal samples by either of the methods (Table 2). Thirty-nine (61.9%) patients had no infection by the conventional laboratory methods, whereas 50 (79.4%) patients had no infection in DNA hybridization test. Eleven (17.5%) patients were positive for *G. vaginalis* alone by the conventional labora-

tory methods whereas 5 (7.9%) patients were positive in DNA hybridization test ( $p > 0.05$ ). For *T. vaginalis*, 2 (3.2%) patients were positive by the conventional laboratory methods and 1 (1.6%) by DNA hybridization test in whom, in addition *G. vaginalis* and *Candida* spp. were also detected. Nine (14.3%) patients were positive for *Candida* alone by the conventional laboratory methods compared to 6 (9.5%) patients in DNA hybridization test ( $p > 0.05$ ). *G. vaginalis* and *Candida* together were positive in 2 (3.2%) patients by the conventional laboratory methods, and in one (1.6%) by DNA hybridization test. One patient was positive for all three microorganisms in only DNA hybridization test.

There was a significant moderate correlation between the conventional and DNA hybridization methods in the diagnosis of vaginitis ( $r = 0.6$ ;  $p < 0.05$ ). The sensitivity and specificity for the diagnosis of vaginitis with DNA hybridization test were 45.8% and 94.8%, respectively. With regard of diagnosing vaginitis, the positive and negative predictive values were 84.6% and 74%, respectively, by DNA hybridization test. The false positive and false negative values were 5.1%, 54%, respectively, by DNA hybridization test.

## DISCUSSION

In this study, we have found that for the overall diagnosis of vaginitis, conventional laboratory methods were more sensitive than DNA hybridization test. The results of conventional and DNA hybridization tests had low-moderate correlation in the diagnosis of vaginitis. The size of our study was not big enough to evaluate all three type of vaginal infections, especially for *Candida* spp. and *T. vaginalis*. DNA hybridization test may provide decent performance of the detecting the women with no infection but its drawback is that it is not satisfying method in the diagnosis of vaginitis.

Bacterial vaginosis (BV) and candida vulvovaginitis were responsible for most of the infections in our study, as previously reported responsible for 90% of cases of vaginitis.<sup>9-11</sup> The incidence of bacterial vaginosis varies; the reported rates are 10-35% in patients visiting gynecological wards in the

world.<sup>12</sup> In our country, the BV incidence rates have varied from 13-31% among similar groups.<sup>13,14</sup>

Prevalences of 4.9% to 36% have been reported from European and American studies.<sup>15</sup> The condition is symptomatic in half of the women and also represents a psychological burden.<sup>10,15</sup> BV is often misdiagnosed using clinical criteria because the components are subjective and dependent on the performance of the clinician and available equipment.<sup>16</sup> Direct microscopic examination of vaginal discharge is often used in the diagnosis of bacterial infections. Gram stain method correlates well with clinical diagnosis and presents a more reliable and reproducible method in the diagnosis of bacterial vaginosis.<sup>17</sup> It is also inexpensive and widely available to many laboratories. The Gram stain method allows the interpreter to identify other associated findings, such as the presence of yeast or neutrophils seen with acute vaginitis. Early diagnosis and intervention in symptomatic women could prevent complications. The rapidity, specificity, and sensitivity of the Gram stain technique for the diagnosis of vaginitis could potentially prevent failure in treatment. All these together with a low false-negative rate make the Gram stain an excellent diagnostic method.<sup>7</sup> Other laboratory method, DNA hybridization test, is highly specialized procedure and as a result is not readily available to many laboratories.

Vaginal cultures for *G. vaginalis* are often the primary laboratory test available for the diagnosis of vaginitis; however, the study results in the literature are controversial. According to the results of the study by Tokyol et al, with a specificity of 97.7%, and a positive predictive value of 93.3%, vaginal culture seems to be an adequate diagnostic criterion when it is positive.<sup>18</sup> However, in another study by Sobel et al, it was reported that vaginal cultures for *G. vaginalis* are sensitive but not specific, as 50 to 60% of healthy asymptomatic women will be culture positive.<sup>19</sup> Although it has a sensitivity of 83-94% among the women who have clinical signs of BV, the usefulness of these cultures is doubtful.<sup>8,20,21</sup> The use of vaginal cultures for *G. vaginalis* is limited by the test's poor specificity.<sup>20</sup>

In recent years, PCR method using DNA hybridization test was frequently utilized in diagnosing vaginitis and several studies have shown that PCR test was a more sensitive diagnostic test for detection and identification of symptomatic vaginitis/vaginosis than conventional clinical examination and wet mount testing.<sup>4,8</sup> However, this diagnostic test is more expensive than the conventional methods.

The Gram stain method is a rapid and cost-effective test that is also highly reproducible and readily available in many laboratories. These features make the Gram stain method a more desirable screening procedure for BV in a clinic population.<sup>7</sup> DNA hybridization test for detecting BV is more labor-intensive and costly than conventional labo-

ratory methods for clinical diagnosis especially in developing countries.

There is not superiority of the DNA hybridization test over conventional laboratory tests used in combination in our settings in the diagnosis of vaginitis. We suggest that conventional laboratory methods appear to be a useful tool to provide presumptive diagnostic information for women with vaginitis when used in conjunction with clinical and patient information.

### Acknowledgement

*The authors thank to the Laboratory of Clinical Microbiology for testing the vaginal samples by Affirm VPIII assay and thank to Ziynet Çınar, PhD, for biostatistical analysis.*

## REFERENCES

- Kent HL. Epidemiology of vaginitis. Am J Obstet Gynecol 1991;165(4 Pt 2):1168-76.
- Ozgunen FT. [Vaginitis, cervicitis, pelvic inflammatory disease, tubo-ovarian abscess]. *Turkiye Klinikleri J Gynecol Obst* 2001;11(6):403-11.
- Myziuk L, Romanowski B, Johnson SC. BVBlue test for diagnosis of bacterial vaginosis. *J Clin Microbiol* 2003;41(5):1925-8.
- Brown HL, Fuller DD, Jasper LT, Davis TE, Wright JD. 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(1):17-21.
- Bornstein J, Lakovsky Y, Lavi I, Bar-Am A, Abramovici H. The classic approach to diagnosis of vulvovaginitis: a critical analysis. *Infect Dis Obstet Gynecol* 2001;9(2):105-11.
- Schwartz A, Taras D, Rusch K, Rusch V. Throwing the dice for the diagnosis of vaginal complaints? *Ann Clin Microbiol Antimicrob* 2006;5:4.
- Tam MT, Yungbluth M, Myles T. Gram stain method shows better sensitivity than clinical criteria for detection of bacterial vaginosis in surveillance of pregnant, low-income women in a clinical setting. *Infect Dis Obstet Gynecol* 1998;6(5):204-8.
- Briselden AM, Hillier SL. Evaluation of affirm VP Microbial Identification Test for Gardnerella vaginalis and Trichomonas vaginalis. *J Clin Microbiol* 1994;32(1):148-52.
- Fleury FJ. Adult vaginitis. *Clin Obstet Gynecol* 1981;24(2):407-38.
- Muhcu M, Müngen E, Dündar O, Tütüncü L, Atasever B, Yergök YZ. [Effect of the treatment of bacterial vaginosis in pregnancy on preterm labour]. *Turkiye Klinikleri J Gynecol Obst* 2007;17(3):181-5.
- Milanese M, Segat L, De Seta F, Pirulli D, Fabris A, Morgutti M, et al. MBL2 genetic screening in patients with recurrent vaginal infections. *Am J Reprod Immunol* 2008;59(2):146-51.
- Georgijević A, Cjukic-Ivancevic S, Bujko M. [Bacterial vaginosis. Epidemiology and risk factors]. *Srp Arh Celok Lek* 2000;128(1-2):29-33.
- Ozturk CE, Ozdemir I, Yavuz T, Kaya D, Behcet M. Etiologic agents of cervicovaginitis in Turkish women. *Saudi Med J* 2006;27(10):1503-7.
- Köksalan H, Esen N, Çağatay M, Tülek N, Mert A. [Isolation of Gardnerella vaginalis from vaginal discharge specimens]. *Mikrobiyol Bul* 1993;27(3):191-5.
- De Backer E, Verhelst R, Verstraelen H, Alkumber MA, Burton JP, Tagg JR, et al. Quantitative determination by real-time PCR of four vaginal Lactobacillus species, Gardnerella vaginalis and Atopobium vaginae indicates an inverse relationship between L. gasseri and L. iners. *BMC Microbiol* 2007;7:115.
- Mastrobattista JM, Bishop KD, Newton ER. Wet smear compared with gram stain diagnosis of bacterial vaginosis in asymptomatic pregnant women. *Obstet Gynecol* 2000;96(4):504-6.
- Joesoef MR, Hillier SL, Josodiwondo S, Linnan M. Reproducibility of a scoring system for gram stain diagnosis of bacterial vaginosis. *J Clin Microbiol* 1991;29(8):1730-1.
- Tokyo C, Aktepe OC, Cevrioğlu AS, Altındaş M, Dilek FH. Bacterial vaginosis: comparison of Pap smear and microbiological test results. *Mod Pathol* 2004;17(7):857-60.
- Sobel JD. Vaginitis. *N Engl J Med* 1997;337(26):1896-903.
- Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;74(1):14-22.
- Hillier SL. Diagnostic microbiology of bacterial vaginosis. *Am J Obstet Gynecol* 1993;169(2 Pt 2):455-9.