

Evaluation Of A Latex Agglutination Test For The Identification Of *Candida* Species In Vaginal Discharge

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Abstract: *Background:* The diagnosis of *Candida* vulvovaginitis using historical symptoms, pelvic examination findings, and results of traditional in-office laboratory tests is often inaccurate. Although *Candida* cultures can verify the diagnosis, they are not routinely used.

Methods: We prospectively compared the accuracy of a commercially available, in-office, rapid, latex agglutination test (CandidaSure™) for the identification of *Candida* species with results from *Candida* culture.

Results: The sensitivity of the latex agglutination test was 66 percent and the specificity was 63 percent when compared with *Candida* culture results in patients with vaginal symptoms. When compared with a microscopic evaluation using potassium hydroxide (KOH), the CandidaSure™ test had greater sensitivity but a lower specificity. In 74 percent of cases with a positive KOH preparation, yeast forms were present on culture, and little was gained by adding the CandidaSure™ test in this situation. The addition of the CandidaSure™ test in those cases with a negative KOH preparation resulted in increased sensitivity but also increased the number of false-positive diagnoses.

Conclusions: The CandidaSure™ test has greater sensitivity than the KOH preparation, but it is less predictive of a positive culture. Because of this limitation, *Candida* should be documented by culture for any patient who has recurrent or persistent disease and a negative KOH slide. (J Am Board Fam Pract 1992;5:375-80.)

Vulvovaginitis is commonly caused by one of the *Candida* species. Approximately 70 percent of all women experience this infection at least once,¹ and 40 percent have recurring infections.² Differentiating this infection from other causes of vaginal symptoms is required if the treatment offered is to be appropriate. The accuracy of the clinical evaluation in the office, however, is less than 50 percent.^{3,4} Although the reliability of using a potassium hydroxide (KOH) preparation to find budding yeast or hyphal components is better than using clinical criteria alone, even this test has been shown to have a low sensitivity in most studies.⁴⁻⁸ During the first visit, therefore, the

diagnosis is unclear in more than one-half of the patients with *Candida* vulvovaginitis.^{1,3-5,9}

Although the diagnosis of *Candida* vulvovaginitis could be confirmed using appropriate cultures, these cultures are not performed routinely in primary care offices, and they require 24 to 48 hours for the organism to grow sufficiently for identification. A rapid, reliable, in-office test to identify *Candida* in vaginal secretions would benefit clinicians and patients by enhancing diagnostic accuracy and hence appropriate treatments.

We therefore evaluated the accuracy (sensitivity, specificity, and positive and negative predictive values) of the CandidaSure™ latex agglutination test (Medical Technology Corporation, Somerset, NJ) and compared it with the KOH preparation, *Candida* culture, and historical and physical examination findings in patients with symptoms of vaginitis and in patients who were asymptomatic.

Methods

Patients in this study were enrolled during the 1st year of a 5-year case-control study of recurrent

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Candida vulvovaginitis. Patients who were entered into the case group were eligible for participation if they had vaginal symptoms of itching, discharge, burning, or pain. Women patients who came in for a routine vaginal examination and denied symptoms of vulvovaginitis were eligible for the control group. All participants were between 18 and 50 years of age, had a male sexual partner who might be willing to participate in the ongoing study, and had not received antifungal therapy in the previous 2 weeks. All participants signed an informed consent document and completed a self-administered questionnaire, which inquired about their medical history, emphasizing past vaginal or pelvic infections and potential risk factors for *Candida* vulvovaginitis.

The women had a pelvic examination by the participating physician, who recorded findings on the appearance of the labia, vagina, and cervix, as well as on the presence and quality of vaginal and cervical discharge. Specimens of vaginal discharge were collected on individual swabs for KOH and normal saline preparations, a "whiff" test for aromatic amines volatilized on addition of KOH, a pH test, CandidaSure™ latex agglutination testing, and a fungal culture for *Candida* species. Additional vaginal discharge samples were cultured for *Gardnerella vaginalis*, group B β-hemolytic streptococcus, and *Staphylococcus aureus*. Cervical specimens were cultured for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*.

The KOH preparation was positive if any budding yeast forms or hyphal components were seen during a 1- to 2-minute microscopic examination by low (100 ×) and high (400 ×) power.

The principal investigator or her research assistant performed the CandidaSure™ latex agglutination tests according to the manufacturer's instructions. The test identifies specimens containing *Candida* antigen (regardless of viability) by means of an antibody-antigen reaction between the *Candida* organism and the *Candida*-specific antibody coated on latex particles. This test was done within 10 minutes of collection of the specimen but without the knowledge of the KOH preparation interpretation. Any agglutination of the latex particles within the sample was considered positive.

The Sabouraud CG agar medium was used for *Candida* culture at the time of specimen collection. The inoculated plate was streaked for colony isolation with a sterile loop and was then transported to the central laboratory (University of Michigan clinical laboratories) within 4 hours.

The inoculated plates were incubated at 30°C in a room-air incubator and examined daily for evidence of growth. Plates showing no growth of yeast-like colonies after 7 days of incubation were considered negative. Plates with fewer than 5 colonies present were graded as "rare," 5 to 20 colonies as "few," 21 to 99 colonies as "moderate," and 100 or more colonies as "numerous." Yeast-like colonies were used to prepare a saline suspension that was examined microscopically for morphologic characteristics. Yeasts yielding a positive germ tube test were identified as *Candida albicans*. Yeasts having small blastoconidia and testing positive by the rapid trehalose test were identified as *Torulopsis glabrata*. Yeasts that were negative for these tests were cultured onto cornmeal agar and were used to inoculate API 20C™

Table 1. Characteristics of Patients Enrolled.

Characteristics	Patients with Vaginitis* No. (%)	Controls No. (%)
Age		
18 to 28 years	29 (36)	9 (28)
29-38 years	37 (46)	14 (44)
39 years or older	14 (18)	9 (28)
White non-Hispanic	71 (89)	31 (91)
Education		
< 12 years	28 (35)	9 (26)
> 16 years	16 (20)	4 (12)
Married	54 (68)	28 (82)
Received antibiotics during previous month	25 (31)	3 (9)†
Current smoker	14 (17)	5 (15)
Oral contraception regimen	25 (31)	7 (21)
Vaginal symptoms		
Itch	62 (78)	3 (9)‡
Vaginal discharge	69 (89)	9 (27)‡
Vaginal swelling	31 (41)	0 (0)‡
Odor	45 (60)	4 (12)‡
Six or more episodes of <i>Candida</i> vaginal infections by history	31 (38)	5 (15)†

*Because of missing data, n = 75 to 81 for patients with vaginal symptoms, and 32 to 35 for the controls.

†P < 0.05.

‡P < 0.001.

carbohydrate-assimilation strips (Analytab Products, Plainview, NY). The plates and strips were incubated for 2 to 3 days at 30°C and examined for growth structure and assimilation pattern, respectively. Based on the outcome of the latter tests, the yeast was identified to the species level.

The remaining specimens for culture were placed in transport media and were transported to the appropriate laboratory for analysis by techniques approved by the College of American Pathologists.¹⁰ All cultures were performed at the University of Michigan clinical laboratories except the *Chlamydia* cultures, which were performed by the clinical laboratory at Catherine McAuley Health Center, Ann Arbor, MI.

Analysis of the data consisted of frequency distributions of demographic, historical, physical examination, and laboratory data. The outcome measurement consisted of the *Candida* culture results (positive or negative), because this test has been considered the reference standard for diagnosing *Candida* vulvovaginitis in symptomatic women, and because evidence of viable organisms as opposed to antigen alone was considered necessary for the diagnosis. The results of the CandidaSure™ test and other diagnostic tools were compared with the *Candida* culture results, and sensitivities, specificities, and positive and negative predictive values were calculated. Analysis of subpopulations was performed using stratified analysis.

Results

Between 1 April 1990 and 31 March 1991, vaginal samples from 81 patients with vaginitis and 34 control women were evaluated at the patients' initial visit with the CandidaSure™ latex agglutination test (Table 1). These patient groups differed significantly only by the presence of vaginal symptoms and their reported number of previous episodes of *Candida* vulvovaginitis. The prevalence of positive *Candida* cultures was 50.6 percent (41 of 81) in those with symptoms of vaginitis, and 8.8 percent (3 of 34) in the control group.

The accuracy of the CandidaSure™ test compared with culture results in those patients with vaginal complaints is shown in Table 2. Overall, the CandidaSure™ test results were in agreement with the culture results in 64 percent of patients. The sensitivity of the test varied, however, depending on the number of organisms present; the

Table 2. Accuracy of CandidaSure™ Latex Agglutination Test Compared with *Candida* Culture in 81 Patients with Vaginal Symptoms.*

CandidaSure™ Test	<i>Candida</i> Culture		
	Positive	Negative	Total
Positive	27	15	42 (52%)
Negative	14	25	39 (48%)
Total	41 (51%)	40 (49%)	81 (100%)

*Sensitivity = 66%, specificity = 63%, positive predictive value = 64%, negative predictive value = 64%.

false-positive rate was 37 percent when no organisms were present on culture and the sensitivity was 76 percent when numerous organisms were present (Figure 1). In addition, of those patients without vaginal symptoms (n = 34), 9 percent had a positive CandidaSure™ test, and 9 percent had a positive culture for *Candida* species. In no case were both tests positive for the same patient, resulting in a classification accuracy of 82 percent in this group (percentage of agreement between the CandidaSure™ test results and the culture results).

In contrast, compared with the *Candida* culture, the KOH preparation, which was positive for budding yeast or hyphae in 19 (24 percent) of 79 symptomatic patients, was less sensitive; although when positive, it was more predictive of infection (Table 3). Of those 27 patients who were missed by the KOH preparation, 15 (56 percent) were detected by the CandidaSure™ test, resulting in the detection of an additional 15 (37 percent) of the 41 patients with *Candida* present by culture, at

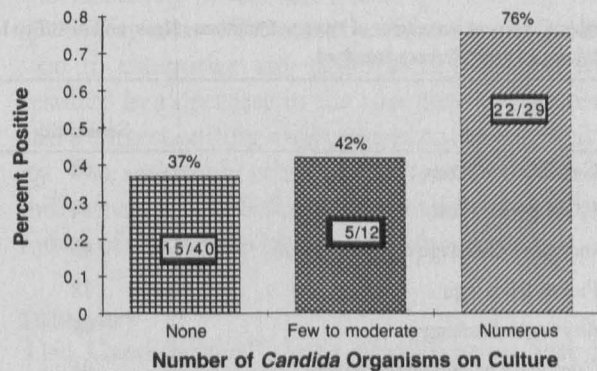


Figure 1. Percent of positive CandidaSure™ tests compared with the number of *Candida* colonies present on the culture ($\chi^2 = 10.5, P < 0.01$).

Table 3. Accuracy of Potassium Hydroxide (KOH) Latex Agglutination Test Compared with *Candida* Culture in 79 Patients with Vaginal Symptoms.*

KOH Test	<i>Candida</i> Culture		
	Positive	Negative	Total
Positive	14	5	19 (24%)
Negative	27	33	60 (76%)
Total	41 (52%)	38 (48%)	79 (100%)

*Sensitivity = 34%, specificity = 87%, positive predictive value = 74%, negative predictive value = 55%.

the expense of 11 (18 percent) of the 60 patients with a negative KOH preparation having *Candida* diagnosed erroneously.

The accuracy of the CandidaSure™ latex agglutination test was evaluated in various subpopulations of the symptomatic group, including those with and without a positive KOH preparation, with an elevated vaginal pH (pH ≥ 5.0), with a positive culture for other cervical or vaginal pathogens, and with a positive picket-fence sign (severe itch with the perceived desire to scratch)¹¹; current smokers; those on oral contraceptives; those who had taken antibiotics in the past month; and those who had a history of more than five episodes of *Candida* vulvovaginitis. No difference in the accuracy of the test was noted in these subpopulations with the following exceptions: a marked difference was noted in the accuracy of the CandidaSure™ test when stratified by the presence (n = 19) or absence (n = 60) of a positive KOH preparation, with respective sensitivities of 86 percent versus 56 percent and specificities of 40 percent versus 67 percent. Also, within the group of patients who had a positive *Gardnerella vaginalis* culture (n = 11), the

CandidaSure™ test was not associated with the *Candida* culture results (odds ratio = 1.0, sensitivity = 33 percent, positive predictive value = 25 percent).

The accuracies of other factors potentially associated with *Candida* vulvovaginitis were also calculated to compare with those of the CandidaSure™ test. These factors included results of the KOH preparation, a combination of symptoms, the picket-fence sign, and whether the patient had had *Candida* vulvovaginitis six or more times before (by her history). The accuracies of each factor as a test for *Candida* vulvovaginitis were calculated from our data, assuming the prevalence of *Candida* vaginitis was 50.6 percent (Table 4). The symptom of vaginal itching was the most sensitive indicator of *Candida* vulvovaginitis, but none of the factors or tests had a positive predictive value as great as that of the KOH preparation. In a population with a prevalence of infection of less than 50.6 percent, the predictive values would be considerably lower (Figure 2).

Discussion

The accurate diagnosis of the cause of vaginal symptoms is crucial if appropriate and effective treatment is to be prescribed. Unfortunately, the routine diagnostic evaluation of vaginitis in the outpatient setting results in uncertainty about the diagnosis in more than one-half of the cases — both in underdiagnosis because of false-negative KOH preparations, and in overdiagnosis when the physician assumes the infection to be *Candida* because of a negative microscopic evaluation for bacterial vaginosis or *Trichomonas*

Table 4. Percent Accuracies of Various Symptoms, Signs, and In-Office Laboratory Tests in Patients with Vaginal Symptoms, Using *Candida* Culture as the Reference Standard.

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
CandidaSure™ test	66	63	64	64
KOH preparation	34	87	74	55
Increased discharge on examination	46	72	63	56
Picket-fence sign	38	83	68	57
Any vaginal itching	95	40	62	89
Complaint of vaginal discharge	41	56	49	48
Previous <i>Candida</i> vaginitis episodes by history ≥ 6	32	55	42	44

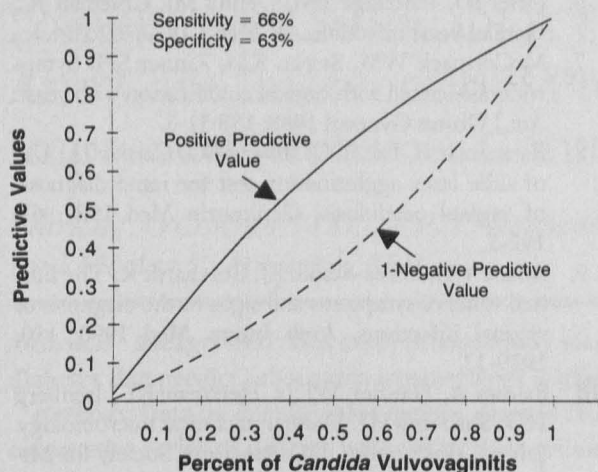


Figure 2. Effect of changing prevalence of disease on the positive and negative predictive values of the CandidaSure™ test.

vaginalis. This study again affirms that in this community-based population, the KOH preparation is not a sensitive indicator of the presence of *Candida* organisms but, when positive, is one of the more predictive tests of a positive culture result.

The accuracy of the latex agglutination test for the detection of *Candida* species in vaginal discharge has been controversial. Previous studies on several latex agglutination tests for *Candida* species reported sensitivities that were 65 to 81 percent and specificities that were 95 to 98.5 percent for this type of test.^{8,12-14} These studies, however, enrolled patients from selected populations (a *Candida* vaginitis specialty clinic,¹² a genitourinary clinic,^{8,13} and sexually transmitted disease and family planning clinics¹⁴) and did not use commercially available latex agglutination tests. Our evaluation of a commercially available latex agglutination test for the identification of *Candida* species in primary care patients indicates that the accuracy of the CandidaSure™ test is 64 percent compared with the cultures of patients seen in the community.

Our data, however, also suggest that the sensitivity and specificity of the test are not stable, and that they differ depending on such factors as the number of organisms present and the presence of other infections. The increasing sensitivity of the latex agglutination test as the number of organisms increases has been previously reported.¹⁴ Although the number of organisms present in the vaginal discharge can be associated with itching

and quantity of discharge,¹⁵ or with symptoms in general,¹⁶ this association remains controversial,¹⁷ and there is considerable overlap between the clinical diagnosis of vaginitis and the quantities of *Candida* species present on culture. Consequently, negative test results with the CandidaSure™ test when colony counts by culture are low can still be missing clinically important disease. Furthermore, we found that the CandidaSure™ test was not helpful in the diagnosis of *Candida* species in the vaginal discharge of patients who also had *Gardnerella vaginalis* present. This finding has not been reported previously and requires further study.

One limitation of this study is the question of commensal carriage of *Candida* in the vagina. Others have considered *Candida* in the vagina without signs or symptoms of vaginitis to be commensal,⁸ and we similarly limited the population on whom we defined sensitivity and specificity of the test to those with vaginal symptoms. In only 19 percent of the positive cultures (8 of 41) was the colony count low (rare or few colonies per plate). If these cases were assumed to be those with commensal carriage and were reclassified into the "negative culture" category, no significant changes in the accuracy of the test are observed. A further limitation of the evaluation of this test arises from the degree of subjectivity inherent in the classification of the result as positive or negative. The agglutination can result in coarse granular particles or in a finely speckled appearance, which can be difficult to differentiate from the control sample. Early in our experience with this test, before this study, we had classified the test as positive only when coarse particles were present. In these preliminary cases ($n = 20$), the sensitivity of the test was 25 percent and the specificity was 100 percent. Clearly the decision to categorize any agglutination as positive resulted in a decrease in the specificity of the test and a corresponding improvement of the sensitivity. The sensitivity of the test as read by different individuals can therefore differ, making interpretation of the results by others problematic.

Summary

The CandidaSure™ latex agglutination test is more sensitive but less specific than the KOH preparation when compared with culture results in the symptomatic population studied. In pa-

tients with a positive KOH preparation, little is gained by adding the latex agglutination test. In those with a negative microscopic examination, however, approximately one-half of those missed might be detected by including this test. Because of the low sensitivity of the KOH preparation in nonreferred populations, this additional sensitivity could be useful in the acute setting. Because the predictive value of the CandidaSure™ test is only 64 percent when this test is positive, however, a definitive diagnosis in recurrent or resistant cases requires culture confirmation for maximum accuracy.

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