

Office Laboratory Diagnosis of Vaginitis

Clinician-Performed Tests Compared with a Rapid Nucleic Acid Hybridization Test

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Background. The traditional diagnosis of vaginitis incorporates patient symptoms, clinical findings observed during vaginal examination, and laboratory analysis of vaginal fluid. The purpose of this study was to evaluate routine clinician-performed office laboratory diagnostic techniques for women with abnormal vaginal symptoms, and to compare these results with those obtained by a DNA hybridization test for *Trichomonas vaginalis*, *Gardnerella vaginalis*, and *Candida* species.

Methods. The study included 501 symptomatic women who were between the ages of 14 and 67 years. Three vaginal specimens were obtained for saline wet mount, potassium hydroxide (KOH) prep, amine "sniff," pH, and nucleic acid hybridization (*T vaginalis*, *G vaginalis*, and *Candida* sp) tests. Clinicians and medical technicians independently evaluated the wet mount, KOH prep, amine, and pH tests. A medical technician processed the DNA tests according to manufacturer's protocol.

Results. Of 499 subjects for whom complete data were available, vulvovaginal candidiasis was diagnosed in 20.0%, vaginal trichomoniasis in 7.4%, and bacterial vaginosis in 52.1%. Fourteen percent of subjects had multiple vaginal infections. The sensitivity and specificity of clinician microscopically diagnosed vulvovaginal candidiasis, vaginal trichomoniasis, and bacterial vaginosis were 39.6% and 90.4%, 75.0% and 96.6%, and 76.5%

and 70.8%, respectively. The sensitivity and specificity of the DNA probe diagnosis of the same types of vaginitis were 75.0% and 95.7%, 86.5% and 98.5%, and 95.4% and 60.7%, respectively. When only women with multiple vaginal infections were considered, the percentages of correct clinician diagnoses for vulvovaginal candidiasis, vaginal trichomoniasis, and bacterial vaginosis were 49.3%, 83.6%, and 59.7%, respectively. For the DNA probe test, the percentages of correct diagnoses were 72.9%, 92.9%, and 90.0%, respectively.

Conclusions. Primary care clinicians demonstrated a high specificity but low sensitivity when identifying vaginal trichomoniasis and vulvovaginal candidiasis by microscopic techniques. Correct microscopic diagnosis of bacterial vaginosis was even more difficult for clinicians, as was the diagnosis of multiple vaginal infections. Clinicians were not as accurate as the DNA probe test in diagnosing vaginal infections. Clinicians need more education in the laboratory diagnosis of vaginitis. Clinicians should carefully scrutinize each microscopic slide, systematically examine the slide for each type of vaginitis, and consider specimen pH and the presence of leukocytes, *Lactobacillus* organisms, or amine odor as additional clues to infection.

Key words. Vaginosis, bacterial; trichomonas vaginitis; vulvovaginal candidiasis; DNA tests; office laboratory; diagnostic test. (*J Fam Pract* 1995; 41:575-581)

The traditional clinical diagnosis of vaginal infection is based on information expressed verbally by the patient, clinical findings observed during the vaginal examination,

and laboratory analysis of vaginal specimens. The office laboratory analysis of a vaginal specimen provides the most objective information. A microscopic saline wet-mount examination of the specimen permits detection of the motile protozoa *Trichomonas vaginalis* and of squamous epithelial cells coated by adherent bacteria known as clue cells, which are one of several important criteria for the diagnosis of bacterial vaginosis. A microscopic potassium hydroxide (KOH) examination allows recognition of pseudohyphae and buds indicative of vulvovaginal can-

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didiasis. The KOH examination also enables the amine "sniff" test, which, when an odor is present, suggests the diagnosis of bacterial vaginosis. An easily performed vaginal specimen pH test helps to further differentiate specific types of vaginitis when present. A vaginal pH less than 4.5 indicates the normal vagina or vulvovaginal candidiasis; a pH greater than 4.5 suggests bacterial vaginosis or vaginal trichomoniasis. These tests comprise the standard for the office laboratory diagnosis of vaginitis.

However seemingly simple these office laboratory tests appear, the accurate diagnosis of vaginitis is subject to many variables. Patient factors, clinician and laboratory skill, specimen sampling, processing and interpretation each affect the accuracy of diagnosis. Because these tests for vaginal infection have been recently classified as "physician-performed microscopy" tests under the Clinical Laboratory Improvement Amendments of 1988, a new and greater diagnostic burden has been placed on many clinicians in small offices choosing to maintain a laboratory.

Other more complex laboratory tests are available for use to diagnose vaginitis. Cultures for *T vaginalis* and *Candida* sp are more sensitive than wet-mount examination,¹ but they are also more expensive and labor intensive, require more time for confirmation, and have limited routine clinical utility.² Cultures for *Gardnerella vaginalis*, only one microorganism of the polymicrobial infection bacterial vaginosis, demonstrate no clinical value³ since a high percentage (58%) of healthy women are asymptomatic carriers.⁴ A Gram's stain of vaginal secretions may be used to diagnose bacterial vaginosis and vulvovaginal candidiasis⁵; however, because nonmotile trichomonads are difficult to distinguish from leukocytes, the Gram's stain is not useful for the detection of *T vaginalis*. The Papanicolaou (Pap) smear is able to identify all three types of vaginal infection but is limited in sensitivity.¹ Furthermore, because of the potential for an obscuring inflammatory process, most clinicians actually refrain from obtaining a Pap smear when an active vaginal infection is clinically suspected. Tests for proline aminopeptidase,⁵ sialidases,⁶ and various amine and acid byproducts^{7,8} have demonstrated value in identifying women with bacterial vaginosis. Unfortunately, these tests are complex, limited to research facilities, not available to most clinicians, and impractical for general clinical use.⁹

Contemporary nucleic acid hybridization or DNA probe tests are commonly used in medicine to detect a variety of pathogens. A rapid, easy, and accurate test to identify the three common types of vaginitis using a single swab for specimen collection may be clinically valuable. The purpose of this study was to evaluate primary care clinicians' abilities to use routine office laboratory and clinical examination diagnostic techniques to establish the

presence of vaginitis in symptomatic women. The accuracy of a nucleic acid hybridization test designed to simultaneously identify *T vaginalis*, *Candida* sp, and *G vaginalis* in women with symptoms of vaginitis was also compared with the results from the routine office laboratory tests.

Methods

Consenting women who were 14 years of age or older and had symptoms of vaginitis were enrolled in the study at five clinics in the Augusta, Georgia, area: the Family Medicine Center, Obstetrics and Gynecology Clinic, Adolescent Medicine Clinic and Student Health Center, Medical College of Georgia, and the Family Planning Clinic, Richmond County Health Department. Symptoms of vaginitis were defined as itching, irritation, burning, an odor, abnormal discharge, or increased vaginal discharge. The exclusion criteria were excessive menses, recent use of antifungal or certain antibiotic medications (less than 2 weeks since the initiation of treatment), or douching or use of non-oxynyl 9 spermicide within 24 hours of examination.

Patients with vaginal symptoms were asked to participate in the study. Following visual examination of the cervix and vagina, specimens of vaginal discharge were obtained from the lateral side walls or anterior fornix using three Dacron swabs. The order of swab sampling was consecutively alternated. Care was taken to avoid sampling cervical mucus and the posterior vaginal fornix pool so as to obtain a reliable pH determination. The first swab was placed in a test tube containing 0.2 mL of normal saline for wet-mount microscopic examination. The second swab was rolled across pH paper for pH determination. The third swab was placed in the collection tube for DNA probe analysis. Shortly thereafter, the tube was placed into a refrigerator set at 2°C to 8°C until transported to the Family Medicine Center Laboratory. The clinicians independently prepared and examined a portion of the first specimen for saline wet mount, KOH prep, sniff test, and pH determination. The clinician then indicated the appropriate diagnosis. Vulvovaginal candidiasis was defined as the presence of pseudohyphae or buds in the saline or KOH exam. Vaginal trichomoniasis was defined as the presence of the motile protozoan in the saline wet-prep examination. Bacterial vaginosis was defined as the presence of clue cells on saline wet-prep examination. Clinicians also considered an adherent off-white vaginal discharge, a positive amine sniff test, and a pH greater than 4.5 as suggestive of bacterial vaginosis. Clue cells were defined as the presence of squamous epithelial cells coated by adherent bacteria.

Twenty-three family physicians or residents in training, several obstetrician-gynecologists, an adolescent medicine specialist, and approximately six nurse clinicians participated. The proportions of clinicians' interpretations of vaginal specimens were reported as follows: nurse clinician 38%, resident 33%, and faculty 29%. Experienced and proficient medical technicians independently performed the same tests as the clinicians. The medical technicians' diagnoses served as the criterion standard for comparison with the clinician evaluations and nucleic acid hybridization test results.

The saline wet mount was prepared by combining a small amount of vaginal discharge specimen with one drop of normal saline, covered with a cover slip, and examined by light microscopy for the presence of clue cells, trichomonads, pseudohyphae, leukocytes, and *Lactobacillus* sp.

The KOH test was performed by combining a small vaginal specimen with 10% potassium hydroxide on a glass slide. The fluid was immediately evaluated for the presence of a fishy odor indicative of a positive amine or sniff test result. A cover slip was positioned and the specimen was then examined for fungal elements under high power of the light microscope.

The pH determination was made following the application of the vaginal discharge specimen on pH paper (MicroEssential Laboratory, Inc, Brooklyn, NY) with a pH range of 3.0 to 5.5. The resulting colormetric reaction was compared with the corresponding pH reference scale to determine the vaginal pH.

Following successful completion of a preinvestigation proficiency test, a medical technician processed the nucleic acid hybridization tests (Affirm VP III, MicroProbe Corporation, Bothell, Wash) submitted during the study. Test methodology has been previously described.¹⁰ The semiautomated test was designed to detect specific sequences of nucleic acid found in *T vaginalis*, *G vaginalis*, and *Candida* sp. The test contained complementary unique sequences of nucleic acid or probes bound to a probe analysis card. The bound probe hybridized to the nucleic acid of the target microorganism forming a double strand. An additional color probe in solution bound to the target nucleic acid, and with exposure to an enzyme conjugate, produced a blue color indicative of a positive test result. Any blue bead color was reported as a positive result; a colorless bead was reported as negative. A blue positive control bead and a colorless negative control bead were necessary for each test to be considered valid. A positive test for bacterial vaginosis was also defined as a positive blue bead color for *G vaginalis* with a pH of greater than 4.5. The DNA assay required approximately 40 minutes for processing.

Sensitivity and specificity were calculated for each

diagnostic method, using the medical technologist's wet mount or KOH prep diagnoses as the diagnostic standard. Confidence intervals for sensitivity and specificity were calculated, based on the F distribution. Comparisons between the sensitivity and specificity of the clinician diagnosis and the DNA probe test in diagnosing vulvovaginal candidiasis and vaginal trichomoniasis were made using McNemar's chi-square statistic. Comparisons between clinician diagnosis, DNA probe test, and DNA probe plus pH greater than 4.5 in the diagnosis of bacterial vaginosis were made by Cochran's Q test. When significant overall differences were found, this was followed by McNemar's tests to identify the individual differences in performance. Demographic variables were compared between diagnostic groups using chi-square tests for categorical variables and Wilcoxon's rank-sum test for continuous variables.

Results

A total of 501 women were enrolled in the study, and complete data were available for 499 subjects. The mean age of subjects was 28.96 years (standard deviation [SD]=9.83) with a range of 14 to 67 years. A history of previous vaginal infections was reported by subjects as follows: bacterial vaginosis, 21.6%; vulvovaginal candidiasis, 28.9%; and vaginal trichomoniasis, 10.8%. Few subjects were pregnant (5.2%) or menstruating (6.6%) at the initial visit. Only 13.6% of subjects had ever previously used over-the-counter antimycotic agents for treatment of self-diagnosed vulvovaginal candidiasis.

Of the 499 subjects, 20.0% had vulvovaginal candidiasis, 7.4% vaginal trichomoniasis, and 52.1% bacterial vaginosis. Multiple vaginal infections were identified in 70 (14.0%) women. In 6.5% of women, no vaginal infection was identified. All women with more than one simultaneous vaginal infection received a diagnosis of bacterial vaginosis. Sixty percent had vulvovaginal candidiasis, while 50% also had vaginal trichomoniasis.

The results from the clinician-performed diagnostic tests and DNA probe tests for vaginitis were compared with the medical technologist's test results (Table 1). In general, the accuracy of the DNA probe test was superior to that of the clinician-performed test result. The sensitivity of the DNA probe test was greater than that of the clinicians for all three types of vaginitis. These differences were statistically significant for the diagnosis of vulvovaginal candidiasis ($P<.001$) and for bacterial vaginosis ($P<.001$). The same was true for the specificity of the DNA probe test, except in the case of bacterial vaginosis where the specificity of the clinician diagnosis exceeded that of the DNA probe test ($P=.02$). The predictive val-

Table 1. Clinician and DNA Probe Diagnoses of Vaginitis, by Type of Vaginitis and Basis for Diagnosis

Variables	Vulvovaginal Candidiasis		Vaginal Trichomoniasis		Bacterial Vaginosis		DNA Test + pH>4.5
	Clinician	DNA Test*	Clinician	DNA Test	Clinician	DNA Test	
Specimens examined, n	480	499	480	499	481	499	482
Correct diagnoses, %	80.2	91.6	95.0	97.6	73.8	78.8	81.1
Sensitivity, %	39.6	75.0†	75.0	86.5‡	76.5	95.4	93.3§
Specificity, %	90.4	95.7	96.6	98.5¶	70.8	60.7	67.8#
Positive predictive value %	50.7	81.5	64.3	82.1	74.7	72.5	76.1
Negative predictive value %	85.7	93.9	98.0	98.9	72.7	92.4	90.2

*DNA Probe test for Gardnerella vaginalis; † $\chi^2m=28.66$, $P<.001$; ‡ $\chi^2m=10.03$, $P=.002$; § $\chi^2m=1.125$, $P=NS$; ¶ $\chi^2m=3.063$, $P=NS$; §Clinician vs DNA probe, $\chi^2m=35.629$, $P<.001$, clinician vs DNA probe+pH, $\chi^2m=24.53$, $P<.001$; #Clinician vs DNA probe, $\chi^2m=5.319$, $P=.02$, clinician vs DNA probe+pH, $\chi^2m=0.424$, $P=NS$.
Note: χ^2m denotes McNemar's chi-square statistic.

ues, positive and negative, were also greater for the DNA probe test for all three infections except for an equivalent predictive value positive for bacterial vaginosis.

To determine the clinicians' diagnostic accuracy, they were stratified to training levels: nurse clinician, resident, and faculty (Table 2). The results were similar except for significantly better specificity by the nurse clinicians and faculty in diagnosing vaginal trichomoniasis compared with that of the residents. A significantly better specificity was demonstrated by the physicians in diagnosing bacterial vaginosis when compared with that of the nurse clinicians ($P=.002$). The sensitivity for the microscopic diagnosis of vulvovaginal candidiasis by all clinicians was less than 50%.

Diagnostic results were further analyzed for women who were found simultaneously to have more than one vaginal infection (Table 3). In all cases, the DNA probe test was more accurate than the clinician

diagnosis. The greatest discrepancies were noted in the differences of sensitivity for the diagnosis of vulvovaginal candidiasis and bacterial vaginosis. Only 7 of 40 subjects with vulvovaginal candidiasis and a second infection were correctly diagnosed by clinicians. A comparison of results from Table 1 and Table 3 show that clinicians performed much worse when multiple infections were present.

Discussion

Vaginitis is a common condition encountered in the ambulatory care setting. The saline wet-mount prep and KOH examination are the tests most frequently performed in the physician office laboratory to evaluate women for a vaginal infection. Moreover, these two tests are the most frequently performed and taught tests in

Table 2. Sensitivity and Specificity of Clinicians' Diagnoses of Vaginitis

Type of Vaginal Infection	Diagnosis by			Comparison*
	Nurse Clinician (n=187)	Resident (n=164)	Faculty (n=140)	
Vulvovaginal candidiasis				
Sensitivity, %	30.0	37.9	50.0	$\chi^2=2.558$ $P=NS$
Specificity, %	88.4	93.1	90.1	$\chi^2=1.802$ $P=NS$
Vaginal trichomoniasis				
Sensitivity, %	81.8	73.3	66.7	$\chi^2=.445$ $P=NS$
Specificity, %	98.3	93.8	98.4	$\chi^2=6.352$ $P=.042$
Bacterial vaginosis				
Sensitivity, %	75.0	82.0	72.2	$\chi^2=2.199$ $P=NS$
Specificity, %	57.0	76.1	81.9	$\chi^2=12.672$ $P=.002$

*Clinicians' diagnoses vs medical technologist interpretations.

Table 3. Diagnosis of Vaginitis When Mixed Infection Is Present, by Type of Vaginitis and Basis of Diagnosis

Variables	Vulvovaginal Candidiasis		Vaginal Trichomoniasis		Bacterial Vaginosis	
	Clinician	DNA Probe Test	Clinician	DNA Probe Test	Clinician	DNA Probe Test
Specimens examined, n	67	70	67	70	67	70
Correct diagnosis, %	49.3	72.9	84.0	92.9	59.7	90.0
Sensitivity, %	17.5	54.8	76.5	85.7	59.7	90.0
Specificity, %	96.3	100.0	90.9	100.0	NA†	NA†

*DNA probe test positive for *Gardnerella vaginitis*.

†All subjects with mixed vaginitis had bacterial vaginosis.

family practice and obstetrics-gynecology residency programs.¹¹ The reliance on these tests is important because of the limited utility of clinical signs and symptoms of vaginitis.¹² Unfortunately, when these two microscopic tests are compared with culture or more sophisticated microbiologic assays, lower performance is found.¹³⁻¹⁶ Because of the clinical limitations of the two microscopic tests in accurately identifying the various causes of vaginitis, a high level of proficiency and skill by the microscopist is essential. This clinical trial demonstrated that clinicians are not universally skilled in diagnosing vaginitis by microscopic methods. Diagnostic errors can occur because of the somewhat subjective nature of the diagnostic criteria, the presence of cellular mimicry, specimen inadequacy, variable clinician skill, odor that may be transient, and pH altered physiologically by menses, douching, semen, and mucus.

The microscopic performance by clinicians in this study is similar to the results reported previously for laboratorians. Our reported sensitivity and specificity of 75% and 97%, respectively, for the saline wet-mount diagnosis of vaginal trichomoniasis are similar to those reported by medical technologists: 60% to 82% and 98% to 100%, respectively.¹³⁻¹⁵ Other than marginal skill, variables that may have accounted for some of the diagnostic error include specimen desiccation, few organisms present, or delay in examination. The sensitivity and specificity of clinicians in the diagnosis of vulvovaginal candidiasis by KOH microscopic examination, 40% and 90%, respectively, are also similar to those reported previously by laboratorians (19% to 84% and 98% to 99%, respectively).^{13,14,16} The test's low sensitivity but high specificity implies that clinicians may examine specimens carelessly or too quickly, whereas they are generally precise in diagnosing once the fungi are detected. Small budding yeast are particularly difficult to visualize and appropriately identify. Finally, the sensitivity and specificity of clinicians' diagnoses of bacterial vaginosis (76% and 71%, respectively) are comparable to those reported by others.⁹ The clinical diagnosis of bacterial vaginosis by Amsel's criteria¹⁷ (presence of 3 of 4 indicators: adherent vaginal discharge, clue cells, pH >4.5, and a positive amine test)

is the reference standard to which other tests for bacterial vaginosis are usually compared.^{18,19} In many office laboratories, the complex criteria are no doubt frequently reduced to include only clue cell recognition, despite its low sensitivity. Other than the vaginal pH determination, which few clinicians actually perform, Amsel's criteria demonstrate individual test sensitivities of only 80% or less.⁹ Proper diagnosis of bacterial vaginosis is problematic primarily because the polymicrobial vaginal ecosystem disturbance is variable and complex. As such, accurate diagnoses are flawed by considering only a single finding or characteristic. This is particularly true if only clue cells are considered, since "pseudo" clue cells or normal cells with vacuoles can easily be confused with clue cells. Clinicians must evaluate the vaginal specimen for clue cells and for odor, pH, and absence of normal lactobacilli. As demonstrated by this study, patient symptoms and the appearance of the vaginal discharge did little to improve clinician accuracy in diagnosing any type of vaginitis when compared with the diagnoses of medical technicians blinded to patient history and examination findings.

The diagnostic accuracy for vaginal trichomoniasis and vulvovaginal candidiasis reported by clinicians (95% and 80%, respectively) is also comparable to results documented by the 1990 to 1994 AAFP-Proficiency Testing (AAFP-PT) program: 89% to 94% and 82% to 97%, respectively. The poorest clinician diagnostic accuracy (74%) was reported for the detection of bacterial vaginosis. Unfortunately, in the past 5 years, the AAFP-PT program has not included a challenge for bacterial vaginosis. It would appear, based on the results of this study, that the inclusion of such a challenge would possibly benefit the participants of the AAFP-PT program. Office tests for vaginitis are the most commonly taught tests in primary care residency programs.¹¹ Although further government-imposed scrutiny of clinical practice appeals to few clinicians, the results of this study indicate that proficiency testing for physician-performed microscopy tests may be rightfully substantiated.

The nucleic acid probe test for vaginal infection, which was removed from the market following the study, performed better than did clinicians' microscopic tests.

Significant differences were particularly noted for the diagnosis of vulvovaginal candidiasis and bacterial vaginosis. Except in the case of specificity for bacterial vaginosis (60.7%), the DNA probe test performed extremely well. This poor specificity was expected since many asymptomatic women harbor *G vaginalis*. The addition of a vaginal pH determination to the DNA test did little to improve the combined test specificity, as the pH measurement alone is known to demonstrate high sensitivity but poor specificity for the diagnosis of bacterial vaginosis.⁴ In general, our nucleic acid probe results were similar to those previously reported by others.^{10,18,20}

The ability of the DNA probe test to accurately diagnose disease in women with more than one simultaneous infection and the inability of clinicians to do the same were particularly noteworthy. As demonstrated in this study, mixed vaginal infections are common (14%). Clinicians likely bias their diagnoses based on the history and clinical appearance of the vaginal discharge. Because vaginitis infections rarely occur with pathognomonic clinical features,¹⁹ such a bias is frequently misleading. Furthermore, there are no clinical features that reliably describe the characteristics of a mixed vaginal infection. Many clinicians probably turn off the microscope light source once the expected offending microorganism is detected. This reflex response is obviously inappropriate when a mixed infection exists. Only a complete systematic and carefully structured evaluation of the clinical specimen will prevent the error of missing a second or even third type of infection.

Clinicians must first consider whether each individual pathogen is present, in addition to then noting the presence of clue cells, leukocytes, and lactobacilli. The sniff test and pH determination also assist the clinician in selectively discriminating between the types of vaginitis. Clinicians identified *T vaginalis* to a greater extent than the two other forms of vaginitis when a mixed infection was present. It is likely that the movement of the organism improved casual recognition. Unfortunately, the pH determination may not be reliable in the case of a mixed vaginal infection. The discordant results of clinicians compared with those of nucleic acid technology can be explained by the DNA probe test's sampling for the three main causes of vaginitis.

The study may be limited by the selected criterion standards used for the diagnosis of the three types of vaginitis. Certified, experienced laboratorians' microscopic diagnoses of vaginitis have been the accepted criterion standard for practicing clinicians. The chief laboratorian in this study has extensive clinical experience, vast research background, and national prominence as an educator in office laboratory testing. Although the validity of this study's results may have been improved by includ-

ing cultures for *Candida* sp and *T vaginalis*, doing so would have limited their generalizability since such cultures are not typically performed in clinical practice.

This study may be one of the first to assess various primary care clinicians' abilities to perform microscopic testing of vaginal specimens. Primary care clinicians demonstrated a high specificity and a low sensitivity when identifying vaginal trichomoniasis and vulvovaginal candidiasis by microscopic techniques. Correct microscopic diagnosis of bacterial vaginosis was even more difficult for clinicians. The study is also one of the first to evaluate a complete DNA probe test designed to detect the three main types of vaginitis. Except for a better specificity identifying bacterial vaginosis, clinicians were not as accurate as DNA probe assays in diagnosing vaginitis. Clinicians' diagnoses were even less accurate than the DNA probe test when coexisting vaginal infections were present. Finally, clinicians need further education in the laboratory diagnosis of vaginitis. Specifically, they must scrutinize the microscopic slide more carefully, systematically examine the slide for additional types of vaginitis once a single type of vaginal infection is found, and also consider specimen pH and the presence of leukocytes, *Lactobacillus* organisms, or amine odor.

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