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MOLECULAR NAA TESTING FOR VAGINITIS

A Better Option in the ED

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Vaginitis refers to various and highly common inflammation disorders that affect most women at least once in their lifetime.[1] Accordingly, it is the driving force behind approximately 10 million OBGYN visits each year.[2] Almost all vaginitis cases (90%) are caused by three infections: bacterial vaginosis (appx. 50%), vulvovaginal candidiasis caused by *Candida* spp. (appx. 25%), and *Trichomonas vaginalis* (appx. 15%).[2,3] These infections occur individually or in combination,[4] but taken individually, there is high incidence of these three infections in the United States. Approximately 75% of women in the US will have at least one episode of vulvovaginal candidiasis;[3] studies have estimated that there are 2.1 million cases of *T. vaginalis* (TV) in the US at any given time;[5] and over 21 million women are estimated to have bacterial vaginosis (BV).[6]

Further complicating matters, the diagnosis of specific vaginitis is challenging due to overlapping symptoms among each other, along with various sexually transmitted infections (STIs). It is estimated that 37% of women diagnosed with BV also are infected with TV and/or *Candida* spp.[7]

Frontline Preparation

Failure to properly treat these common vaginal infections can have serious consequences. If left untreated, vaginitis can lead to increased susceptibility, which in turn leads to increased rates of STIs, such as chlamydia, gonorrhea, and human immunodeficiency virus type 1 (HIV-1).[8-10] Women with vaginitis also have an elevated risk of cervical cancer, as well as the possible connection to pregnancy concerns, such as preterm birth and low birth weight.[11] While OBGYN practices are the frontline healthcare providers addressing vaginal infections, both freestanding and hospital-based emergency departments (EDs) play an important role in protecting this area of women's health. Given that EDs in the US experience almost 140 million visits each year, they may often be the first, and sometimes only, contact for women experiencing symptoms of STIs and vaginal infections, such as vaginitis.[12,13]

Accurate diagnosis of the cause of vaginitis is, of course, necessary to initiate the proper course of treatment. However, traditional diagnostic testing methods often used in the ED setting may not provide the most accurate detection of vaginitis, despite the

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rapid production of results.[14] Herein we discuss testing methods, turn-around time (TAT) considerations, and an alternate method that may improve patient care.

Testing Methods

Studies have found that Nugent scoring (microscopic evaluation of Gram-stained vaginal samples where 3 bacterial morphotypes are scored) has poor sensitivity,[15,16] and up to 22% of Nugent scoring tests result in inconclusive outcomes.[17] Another traditional testing method, Amsel's criteria (where BV is defined as meeting 3 of the following 4 criteria: thin, white/yellow vaginal discharge; presence of clue cells by vaginal wet mount [VWM] microscopy; vaginal fluid pH greater than (>) 4.5; and amine odor on KOH test of vaginal sample), has poor sensitivity when multiple causes of infection are present, with up to 40% of BV diagnoses missed when TV or *Candida* spp. are present.[7,14]

Furthermore, VWM testing—where a swab specimen is placed on a slide and evaluated by microscopy for the presence of clue cells, motile TV cells, or *Candida* spp. cells[18]—also is widely utilized for the diagnosis of vaginal discharge syndromes. The main advantages of this method are that it is rapid, inexpensive, and can be performed in a laboratory near the point of care (POC) or at the POC. In the latter case, oversight is assumed by the laboratory or a designated medical director to ensure providers remain in compliance with all testing regulations; however, this is not a common course of action. Drawbacks include a lack of quality control, which can lead to increased subjectivity in the interpretation of the results, and low sensitivity for detection of yeast (44% to 78%) and TV (25% to 82%), in particular.[19]

Furthermore, wet mount sensitivity for *T. vaginalis* detection begins to decrease within 10 minutes of collection due to the loss of motility of TV cells; sensitivity continues to decrease with time, down to 35% at 30 minutes, and 78% at 120 minutes.[20] In conversations with ED colleagues, all these data were shared in addition to the fact that fluconazole does not work for all *Candida* species infections.[21] Additionally, the 2021 the Centers for Disease Control and Prevention (CDC) STI Treatment Guidelines state that nucleic acid amplification testing (NAAT) is the gold standard for TV. The guidelines recommend back-up culture or NAAT for all wet preps negative for yeast and the use of culture and NAAT for severe or recurrent yeast infection episodes. Lastly, these guidelines recommend the use of clinical criteria to diagnose BV, not just the presence of clue cells.[11]

Regardless of the testing method employed, a delay in accurate diagnosis postpones the initiation of the best treatment for the patient's condition. While the previously mentioned testing methods provide some efficacy, NAAT consistently provides accurate outcomes. Recommended by the CDC and the US Preventive Services Task Force for identifying a variety of STIs and vaginal infections,[11,22] NAAT produces consistently accurate detection and optimized clinical management of patients with BV, *Candida* spp., and TV. NAAT also has been found to detect three times more mixed infection cases than clinical diagnosis with wet mount, culture, and Amsel's criteria.[23]

A Case for NAAT in the ED Setting

Despite the high levels of sensitivity and consistency of NAAT for vaginitis diagnosis, not all EDs are utilizing this diagnostic testing method. In seeking to demonstrate how patients and EDs might benefit from a switch to NAAT, our laboratory conducted a study utilizing data obtained from the ED at Baptist Health Medical Center in Jacksonville, Florida, which recently switched to NAAT from VWM methods for suspected cases of vaginitis.

The study compared the single and combination infection rates for BV, *Candida* spp., and TV from VWM tests and NAAT in time periods pre- and post-implementation of the NAAT method (VWM, pre-implementation: August 2022 and May 30 to June 26, 2023; NAAT, post-implementation: June 28 to July 25, 2023, and August 2023). The data only included tests ordered by the ED and not from other departments, and VWM data was not reviewed for patients younger than 14 years of age because NAAT is not performed on this population. The results of this analysis were presented at the 2023 Fall Meeting of the Pan American Society for Clinical Virology.[24]

TABLE 1

Monthly Comparisons of Positive Individual Infection Rates (%) for VWM versus NAAT

	VWM (August 2022)	VWM (May 30 - June 26, 2023)	NAAT (June 28 - July 25, 2023)	NAAT (August 2023)
Bacterial vaginosis	32.8%	39.7%	50.6%	54.2%
***Candida* spp.**	9.0%	8.4%	28.4%	26.2%
Trichomonas	6.7%	5.2%	11.1%	10.7%

TABLES 1 AND 2 show the monthly comparisons for individual and co-infection rates for VWM testing versus NAAT. In this analysis, the VWM test had overall 46.4% abnormal results, which included BV (indicated by the presence of clue cells), TV, and/or *Candida* spp. Meanwhile, the BV, *Candida* spp., and TV NAATs had overall higher positivity of 68% for BV, TV, *Candida glabrata*,

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and/or *Candida* species group. Use of NAATs resulted in higher rates of single-infection detection with positivity rates of 50.6% (BV), 28.4% (*Candida* spp.), and 11.1% (TV) compared with VWM testing results of 39.7%, 8.4%, and 5.2%, respectively. This trend of higher rates of positive tests with NAAT continued in a pre- and post-implementation comparison of month 2, post implementation compared with the same month of the previous year (August 2023 versus August 2022). See **TABLE 1**.[24]

Regarding co-infection detection rates, NAAT provided higher rates of detection. Comparison between VWM testing and NAAT pre- and post-implementation demonstrated a three-fold increase in dual clue cell/BV and TV positives with NAAT versus VWM testing (7.4% vs. 2.3%), a three-fold increase in dual clue cell/BV and *Candida* spp. positives (14.1% vs. 4.3%), and a five-fold increase in dual TV and *Candida* spp. positive results (3.1% vs. 0.5%). As with the single-infection rates, this trend continued in a pre- and post-implementation comparison of month 2, post implementation compared with the same month in the previous year (August 2023 versus August 2022). See **TABLE 2**.[24]

TABLE 2

Monthly Comparisons of Positive Dual Infection Rates (%) for VWM versus NAAT

	VWM (August 2022)	WM (May 30 - June 26, 2023)	NAAT (June 28 - July 25, 2023)	NAAT (August 2023)
BV and TV	2.2%	2.3%	7.4%	8.8%
TV and *Candida* spp.	0.5%	0.5%	3.1%	4.3%
BV and *Candida* spp.	4.1%	4.3%	14.1%	9.9%
Trifecta	0.2%	0.2%	1.2%	1.9%

BV=bacterial vaginosis; **TV**=Trichomonas

Substantial Results

These results demonstrate NAAT's ability to broadly identify BV, *Candida* spp., and TV positive results, capturing infections that likely would have been missed if relying on VWM alone. That said, time from collection to test result was shorter with VWM testing than with NAAT in our study. In three free-standing EDs and six acute care hospitals, the VWM expected TAT, lab receipt to report, was 60 minutes. However, upon review of the actual TAT of collect to lab receipt and lab receipt to result, the average VWM evaluation time exceeded the 10-minute optimal result window.[25] This is important because even at optimal testing parameters (evaluation within 10 minutes), VWM is already missing identification of vaginitis infections.

Sharing the review of our internal TAT data for each facility along with literature surrounding the limitations of the VWM, including current CDC STI testing guidelines, helped demonstrate to our clinician partners that while VWM may be faster, it was not being used optimally in clinical practice, putting patients at risk of missed diagnoses. Prior to implementation, the set expectation for NAAT was 16 hours from sample collection to result and 4 hours from receipt of the sample in the lab to result. Our analysis showed that NAAT TATs were longer than VWM, but that clinician TAT and quality result expectations were met and exceeded.[24]

Converting EDs to NAAT for Vaginitis Testing

Communication and setting expectations are two important parts of negotiating change. ED leaders are likely to have reservations about switching to NAAT for vaginitis testing due to longer times from collection to result and the effect of this on the timing of treatment. Thus, communicating with clinician leaders about their expectations for result TAT is critical. If the actual NAAT times from collection to result are shorter than expected, this should go a long way to easing their concerns. If the actual times are longer, it is important that they see that up front and are not surprised. A plan must be established for how to treat (or not treat) patients in the interim prior to patient callback and how to manage patient expectations of immediate treatment from the ED.

Based upon my experience, there are likely to be several clinical leaders and groups that will need to be briefed and will have questions regarding a switch to NAAT for vaginitis testing. In Baptist Health's transition to NAAT for vaginitis testing, I advised steering committees, chief operating officers, ED leads, and ED physicians, among others, about the advantages of switching to NAAT.

During those discussions with department heads, it was helpful to highlight that the increase in the percentage of positive results with NAAT for vaginitis diagnosis was worth the increase in collection-to-result time. It was also helpful to show, with our own institution's data, that the average time for VWM testing was exceeding the window for optimal detection, putting patients at risk of missed diagnoses. Finally, it was also beneficial to note that patients could be discharged while waiting for the NAAT results, which meant fewer patients waiting onsite in the ED.

For NAAT to be widely implemented, changes must be made to ED standard operating procedures, such as how test samples

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are processed, contacting patients at home when their results come in, and sending prescriptions to the patient's personal pharmacy instead of the hospital's pharmacy. These concerns are important, but manageable, and need to be talked through to facilitate a change that can result in improved patient care.

Conclusion

Emergency departments provide important services to women with vaginal infections and utilizing accurate testing methods is necessary to initiate appropriate treatments. At Baptist Health, NAAT has demonstrated higher positivity rates for BV, *Candida* spp, and TV, as well as for dual- (and triple-) positive test results versus VWM testing. The increased collection-to-result times have been readily managed through open communication with healthcare providers and appropriate updates to clinical procedures. Moving the ED to NAAT from VWM and other traditional diagnostic testing methods will require buy-in from key stakeholders. Emphasizing the capabilities of NAAT to improve patient quality of care can help aid in successful implementation. ■

Frances Valencia-Shelton, PhD, D(ABMM), SM(ASCP)CM, is the Clinical Infectious Diagnostics Director for the Baptist Health System in Jacksonville, Florida. She serves on the American Society for Microbiology Laboratory Practices subcommittee, whose mission is to provide expert guidance on issues regulated by an external body or overseen by accrediting or standard-setting agencies, and which impact the science and technology of clinical and public health microbiology laboratory practice. Frances also is the current president of the Jacksonville area Microbiology Society (JAMS), whose mission is to promote higher standards in clinical laboratory science and microbiology research through continuing education.

Her interests include defining and utilizing clinical best-practices for testing and reporting, and optimizing current and new infectious diagnostic methodologies for patient-centered clinical testing through cost-effective measures. She is equally interested in learning with and educating others in the field of clinical microbiology.

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