

# Will NAAT replace microscopy for the identification of organisms causing vaginitis?

Molecular testing is more sensitive and specific than microscopy for the identification of organisms causing disease. There will come a time when molecular tests will replace office microscopy for the identification of the organisms causing vaginitis. That time may be just over the horizon.



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Over the past 200 years, identification of the specific organism causing an infection has evolved from a reliance on patient history and physical examination to the use of microscopic examination of relevant biological samples to the rise of microbial culture and immunological testing as the gold standards for diagnosis. More recently, advances in nucleic acid testing have made nucleic acid amplification testing (NAAT) a primary method for identifying the specific organism causing an infection.

The evolution of the diagnosis of gonorrhea in clinical practice is a good example of the inexorable evolution of diagnostic techniques from physical examination to microscopic analysis to culture and finally to NAAT. Neisser discovered *Neisseria gonorrhoea* in 1879.<sup>1</sup> In 19th century general medical practice gonorrhea was often diagnosed based on history and physical examination and

sometimes microscopy was also utilized.<sup>2</sup> In the mid-20th century, it was realized that culture was a superior approach to diagnosis of gonorrhea, and it became the gold standard for diagnosis in general practice.<sup>3</sup> NAAT has now replaced culture as the gold standard for the diagnosis of gonorrhea because of its superior performance in clinical practice.<sup>4</sup> **It may now be time to consider using NAAT rather than microscopy and culture in general practice for the identification of specific microorganisms causing vaginitis.**

## Trichomoniasis

Vaginitis caused by *Trichomonas vaginalis* is characterized by a discharge that is foamy and green-yellow in color, with a vaginal pH that is >4.5. Microscopy of a vaginal specimen has low sensitivity, in the range of 50%, for detecting *T vaginalis*.<sup>5-7</sup> There are many factors that make microscopy a poor approach to the diagnosis of *T vaginalis*, including the rapid decrease in protozoan motility once

a vaginal specimen is placed on a glass slide and the similar size of non-motile *T vaginalis* and other cells in the vagina.

Given the low sensitivity of microscopy for the diagnosis of trichomoniasis, the American College of Obstetricians and Gynecologists (ACOG) recommends NAAT as a primary approach to test for *T vaginalis*, with culture or NAAT testing as alternative approaches.<sup>8</sup> The Centers for Disease Control and Prevention (CDC) recommends that if a wet mount is negative for *T vaginalis* that NAAT should be utilized.<sup>9</sup>

In this 2-step testing process, the first step is to test the vaginal pH and perform a microscopic examination of a vaginal specimen for *T vaginalis*. If *T vaginalis* organisms are detected, the diagnosis of trichomoniasis is confirmed. If organisms are not detected the second step would be to send a vaginal or urine specimen for NAAT for *T vaginalis* or for culture. An advantage of NAAT over culture is that urine specimens can be used for diagnosis of *T vaginalis* while urine

doi: 10.12788/obgm.0177

specimens are not suitable for culture because of low sensitivity. For patients diagnosed with trichomoniasis, the CDC recommends that testing be repeated in 3 months because of high rates of reinfection. NAAT would be an optimal test to use in this situation.

## Bacterial vaginosis and candidiasis

ACOG recommends using Amsel criteria or Nugent scoring of a specimen colorized with a Gram stain for the diagnosis of bacterial vaginosis and microscopy or culture for the diagnosis of candidiasis.<sup>8</sup> Recent research reports that NAAT testing for bacterial vaginosis and candidiasis may be more sensitive than standard office-based approaches for detecting these two causes of vaginitis. In a study of approximately 1,740 patients with symptoms of vaginitis, vaginal specimens were analyzed using NAAT or standard office approaches to diagnosis.<sup>10</sup> In this study the diagnostic gold standards were Nugent scoring with Amsel criteria to resolve intermediate Nugent scores for bacterial vaginosis and culture for *Candida*. The study demonstrated the superiority of NAAT testing over standard office approaches for the identification of the cause of the vaginitis. NAAT testing was reported to have superior sensitivity for diagnosing bacterial vaginosis compared with the original Amsel criteria (93% vs 76%, respectively ( $P < .0001$ ), with similar respective specificities of 92% and 94%.<sup>10</sup> NAAT testing also had superior sensitivity for diagnosing candidiasis compared with microscopy after potassium hydroxide treatment of a vaginal specimen (91% vs 58%, respectively ( $P < .0001$ )).<sup>10</sup> NAAT testing also had superior specificity compared with microscopy after

potassium hydroxide treatment of a vaginal specimen (94% vs 89%, respectively ( $P < .0005$ )).<sup>10</sup>

In another study comparing NAAT with clinical diagnosis for 466 patients with symptoms of vaginitis, standard office approaches to the diagnosis of vaginitis resulted in the failure to identify the correct infection in a large number of cases. For the diagnosis of bacterial vaginosis, clinicians missed 42% of the cases identified by NAAT. For the diagnosis of *Candida*, clinicians missed 46% of the cases identified by NAAT. For *T vaginalis* diagnosis, clinicians missed 72% of the cases identified by NAAT. Clearly, this resulted in clinicians not treating many infections detected by NAAT.<sup>11</sup>

## One in 5 patients with symptoms of vaginitis have 2 causes of vaginitis

In a recent study, 1,471 patients with a symptom of vaginitis (abnormal vaginal discharge, itching or irritation, or odor) self-collected a vaginal swab and had a vaginal swab collected by a clinician.<sup>12</sup> The swabs were placed in buffer and the samples were tested by NAAT using the BD Max system (Franklin Lakes, New Jersey) for the presence of nucleic acid sequences of the microorganisms responsible for the most common causes of vaginitis. In this cohort, using the clinician collected vaginal swabs for NAAT, the investigators reported the following pattern of detection of nucleic acid sequences: 36.1%, bacterial vaginosis pattern; 16.2%, *Candida* spp.; 1.6%, *T vaginalis*; 0.7%, *Candida glabrata*; and 0.1%, *Candida krusei*. Nucleic acid sequences of multiple organisms were detected in 21.7% of patients, including 13.9% with bacterial vaginosis pattern plus *Candida*

spp., 4.9% with bacterial vaginosis pattern plus *T vaginalis*, 0.3% with *Candida* spp. plus *T vaginalis*, 0.2% with *Candida* spp. plus *Candida glabrata*, 0.2% with bacterial vaginosis pattern plus *Candida glabrata*, and 2.2% with all 3 organisms. A total of 23.8% of the women had no detectable nucleic acid sequences associated with organisms known to cause vaginitis.

In another study of 1,491 patients with a symptom of vaginitis, clinician-collected vaginal swabs were tested by NAAT using the Aptima BV and Aptima *Candida/Trichomonas* systems (Hologic, Marlborough, Massachusetts) for the presence of nucleic acid sequences of microorganisms responsible for most cases of vaginitis.<sup>13</sup> The investigators reported the following pattern of detection of nucleic acid sequences: 28.6%, bacterial vaginosis pattern; 14.2%, *Candida* spp.; 3%, *T vaginalis*; 1.9%, *Candida glabrata*.<sup>13</sup> Nucleic acid sequences from multiple organisms were detected in 23.3% of patients. Nucleic acid sequences suggesting the presence of two different causes of vaginitis were detected among 20.8% of patients, including bacterial vaginosis plus *Candida* spp., 11.1%; bacterial vaginosis plus *T vaginalis*, 7.2%; *Candida* spp. plus *T vaginalis*, 1.0%; *Candida* spp. plus *Candida glabrata*, 0.9%; bacterial vaginosis plus *Candida* spp., 0.5%; *Candida glabrata* plus *T vaginalis*, 0.1%. Nucleic acid sequences suggesting the presence of 3 different causes of vaginitis were detected in 2.4% of patients, the most common being the combination of bacterial vaginosis plus *Candida* spp. plus *T vaginalis*, 1.7% and bacterial vaginosis plus *Candida* spp. plus *Candida glabrata*, 0.5%. Nucleic acid sequences suggesting the presence of 4 different causes of vaginitis

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were detected in 0.1% of patients. A total of 28.8% of the women had no detectable nucleic acid sequences associated with organisms known to cause vaginitis.<sup>13</sup>

In clinical practice it is uncommon to see the diagnosis of multiple causes of vaginitis recorded in the medical record of a patient. This suggests that we are not effectively identifying the 20% of patients with multiple causes of vaginitis.

### **When multiple organisms that cause vaginitis are present, NAAT is superior to clinical evaluation for diagnosis**

In a study of 1,264 patients with symptoms of vaginitis who had an identified microbial cause, more than 20% had multiple organisms

detected by NAAT.<sup>10</sup> The reference methods for diagnosis in this study were Nugent scoring with Amsel criteria to resolve intermediate Nugent scores for bacterial vaginosis, culture for *Candida*, and culture for *T vaginalis*. Compared with the reference method for diagnosis, the sensitivities for NAAT and clinician detection of cases of bacterial vaginosis plus *Candida* were 74% and 18%, respectively ( $P < .0001$ ). Compared with the reference method for diagnosis, the sensitivities for NAAT and clinician detection of cases of bacterial vaginosis plus *T vaginalis* were 72% and 21%, respectively ( $P < .0001$ ). Compared with the reference method for diagnosis, the sensitivities for NAAT and clinician detection of cases of bacterial vaginosis plus *Candida* plus *T vaginalis* were 80% and 10%, respectively

( $P < .0005$ ).<sup>10</sup> Based on this one study, it appears that clinicians are not very effective at diagnosing a case of vaginitis caused by multiple different microorganisms.

### **Patient collection of a vaginal swab for NAAT**

Multiple studies have reported that collection of a vaginal swab for NAAT by the patient or a clinician results in similar excellent test performance.<sup>4,12,13</sup> This observation might catalyze the development of clinical protocols where patients with vaginitis could collect the swab for NAAT analysis, without needing to have a speculum examination by a clinician.

When collecting a vaginal specimen for NAAT it is important that no vaginal lubricants or creams contaminate the collection swab. Vaginal

lubricants and creams may inhibit the polymerase chain reaction enzymes resulting in a false negative. The swab may be directly inserted into the vagina to collect the specimen or a speculum without a lubricant, except water can be used to facilitate specimen collection. To collect a specimen without a speculum the swab is inserted 2 inches into the vagina and rotated for 10 to 15 seconds.

### What should clinicians do while waiting for a NAAT result?

A major problem with NAAT testing for vaginitis is that the results are not available at the initial patient visit, impacting the ability to make an immediate diagnosis and provide targeted antibiotic treatment. Given that bacterial vaginosis and *Candida* species are the most common causes of infectious vaginitis in many

populations of gynecology patients, one approach is to initiate treatment with one dose of an oral antifungal agent and a multiday course of vaginal metronidazole. Once the NAAT test results are available, the treatment can be refined to specific infectious agents identified by the test, or the antibiotics can be discontinued if no relevant microorganisms are detected. Another approach would be to wait until the NAAT test is completed and then prescribe the appropriate antibiotic. My sense is that most patients would not favor this wait and see approach.

### Barriers to the use of NAAT for vaginitis

A barrier to the use of NAAT for the diagnosis of vaginitis is that leading organizations do not currently recommend NAAT as a primary approach to diagnosis, favoring microscopy and measurement of

vaginal pH.<sup>9</sup> In addition, clinicians and patients may be rightfully concerned about the cost of NAAT, which can be substantial.

Vaginitis, especially when it is recurrent, can be stressful<sup>14</sup> and have an impact on a patient's quality of life<sup>15,16</sup> and sexual health.<sup>17</sup> Arguably, our current practice algorithms for diagnosing the cause of vaginitis are not optimized.<sup>18</sup> Our failure to accurately diagnose the cause of vaginitis contributes to inappropriate antibiotic treatment and return visits because of inadequate initial treatment.<sup>18</sup> We can improve and simplify our approach to the diagnosis of vaginitis by prioritizing the use of NAAT.<sup>19</sup> In turn, reliably making the right diagnosis will result in the optimization of treatment. ●



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#### References

1. Jose PP, Vivekanandan V, Sobhanakumari K. Gonorrhoea: Historical outlook. *J Skin Sex Transm Dis.* 2020;2:110-114.
2. Bayly HW. The diagnosis and treatment of chronic gonorrhoea and its local complications. *Br Med J.* 1914;14:584-587.
3. Stuart RD. The diagnosis and control of gonorrhoea by bacteriological cultures: with a preliminary report on a new method for transporting clinical material. *Glasgow Med J.* 1946;27:131-142.
4. Wilson JD, Wallace HE, Loftus-Keeling M, et al. Swab-yourself trial with economic monitoring and testing for infections collectively (SYSTEM-ATIC): Part 2. A diagnostic accuracy and cost-effectiveness study comparing rectal, pharyngeal and urogenital samples analyzed individually, versus as a pooled specimen, for the diagnosis of gonorrhoea and chlamydia. *Clin Infect Dis.* 2021;73:e3183-3193.
5. Hollman D, Coupey SM, Fox AS, et al. Screening for *Trichomonas vaginalis* in high-risk adolescent females with a new NAAT: association with ethnicity, symptoms and prior and current STIs. *J Pediatr Adolesc Gynecol.* 2010;23:312-316.
6. Roth AM, Williams JA, Ly R, et al. Changing sexually transmitted infection screening protocol will result in improved case finding for *Trichomonas vaginalis* among high-risk female populations. *Sex Transm Dis.* 2011;38:398-400.
7. Hobbs MM, Sena Ac. Modern diagnosis of *Trichomonas vaginalis* infection. *Sex Transm Infection.* 2013;89:434-438.
8. Vaginitis in nonpregnant patients. ACOG Practice Bulletin No 215. American College of Obstetricians and Gynecologists. *Obstet Gynecol.* 2020;135:e1-e17.
9. Workowski KA, Bachmann LH, Chan PA, et al. Sexually transmitted infections treatment guidelines 2021. *MMWR.* 2021;70:1-187.
10. Schwebke JR, Gaydos CA, Hyirjesy P, et al. Diagnostic performance of a molecular test versus clinician assessment of vaginitis. *J Clin Microbiol.* 2018;56:e00252-e00218.
11. Broache M, Cammarata CL, Stonebraker E, et al. Performance of vaginal panel assay compared with clinical diagnosis of vaginitis. *Obstet Gynecol.* 2021;138:853-859.
12. Gaydos CA, Beqaj S, Schwebke JR, et al. Clinical validation of a test for the diagnosis of vaginitis. *Obstet Gynecol.* 2017;130:181-189.
13. Schwebke JR, Taylor SN, Ackerman N, et al. Clinical validation of the Aptima bacterial vaginosis and Aptima *Candida/Trichomonas vaginalis* assays: results from a prospective multi-center study. *J Clin Microbiol.* 2020;58:e01643-e01619.
14. Ehrstrom S, Kornfeld D, Rylander E. Perceived stress in women with recurrent vulvovaginal candidiasis. *J Psychosomatic Obstet Gynecol.* 2007;28:169-176.
15. Abellea S, Guelfucci F, Wagner J, et al. Subjective health status and health-related quality of life among women with recurrent vulvovaginal candidosis in Europe and the USA. *Health Quality Life Outcomes.* 2013;11:169.
16. Fukazawa EI, Witkin SS, Robial R, et al. Influence of recurrent vulvovaginal candidiasis on quality of life issues. *Arch Gynecol Obstet.* 2019;300:647-650.
17. Giraldo PC, Polpetta NC, Juliato CT, et al. Evaluation of sexual function in Brazilian women with recurrent vulvovaginal candidiasis and localized provoked vulvodynia. *J Sex Med.* 2012;9:805-811.
18. Hillier SL, Austin M, Macio I, et al. Diagnosis and treatment of vaginal discharge syndromes in community practice settings. *Clin Infect Dis.* 2021;72:1538-1543.
19. Sobel JD. Syndromic treatment of women with vulvovaginal symptoms in the United States: a call to action. *Clin Infect Dis.* 2021;72:1544-1545.