

Demystifying Multiple *Neisseria gonorrhoeae* Testing Methods and Results

For many years, culture has been the standard test for the identification of *Neisseria gonorrhoeae*. The development of nonculture methods, such as nucleic acid amplification tests (NAATs), allows for direct molecular detection with greater sensitivity, specificity and ease of specimen transport. The CDC [Recommendations for Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*](#)¹ outlines the recommendation for the use of NAATs to detect *N. gonorrhoeae* based on the improvements to overall sensitivity, specificity and specimen transport conditions compared to other methods.

With additional monitoring for [N. gonorrhoeae antimicrobial resistance](#), there is an increased chance that a specimen submitted for *N. gonorrhoeae* screening or diagnosis may be subjected to testing by multiple methods. For example, specimens submitted for *N. gonorrhoeae* screening may receive a NAAT to diagnose the infection and may be sent for culture and antimicrobial susceptibility testing for surveillance. If the NAAT and culture have discrepant results, it can be confusing to interpret. However, as with all testing, discrepancies between test methods can occur, and it is important for laboratorians and clinicians to understand test limitations.

Misdiagnosis based on misinterpretation of laboratory results could lead to untreated disease progression, transmission of disease due to unknown diagnosis, or unnecessary and inappropriate treatment. While there are additional diagnostic methods for *N. gonorrhoeae*, they are not covered in this document as they are used rarely in the US. The table and scenarios contained in this document have been designed to improve understanding and to help troubleshoot discrepant results between culture and NAAT testing for *N. gonorrhoeae*.

Table 1: Important Aspects of the Common *N. gonorrhoeae* Testing Methodologies

Category	Culture	NAAT
Reference Test	Historically, still required for antibiotic susceptibility testing and sequencing	Recommended by CDC and APHL for laboratory diagnosis of urogenital and extragenital <i>N. gonorrhoeae</i> infections ¹
Sensitivity*	Less sensitive than nucleic acid amplification testing (NAAT) ¹	Most sensitive diagnostic method for <i>N. gonorrhoeae</i>
Specificity*	Other organisms (such as <i>Neisseria cinerea</i>) may be misidentified as <i>N. gonorrhoeae</i> unless additional testing is performed ¹	Some commercial NAATs might detect nongonococcal <i>Neisseria</i> species and other commensals ¹
Specimen Collection and Transport	<ul style="list-style-type: none"> Specimen collection technique, storage conditions, and transportation environment may impact viability⁷ Specimen collection swabs, such as those with wood shafts and cotton tips, may be inhibitory to growth¹ Consult with laboratory for appropriate specimen collection method and transport conditions 	<ul style="list-style-type: none"> Specimen collection kit typically contains nucleic acid stabilizers resulting in longer stability PCR can be inhibited by certain substances which could be in the specimen collection swab⁸ Specimen collection (swabs) and transport conditions vary by manufacturer-refer to package insert for recommended practices

Category	Culture	NAAT
Viable Organisms (<i>N. gonorrhoeae</i>)	<ul style="list-style-type: none"> • Viable organisms are required for successful culture. • <i>N. gonorrhoeae</i> has demanding nutritional and environmental growth requirements, e.g., CO₂-candle jar • For maximum recovery of <i>N. gonorrhoeae</i>, both selective and nonselective media should be used 	Viable organisms not required for detection of pathogen. Method detects nucleic acids which may persist following appropriate antimicrobial therapy
Specimen Types	<ul style="list-style-type: none"> • Culture can be performed on all specimen types • <i>Consult the laboratory that is performing testing to confirm specimen types that are accepted</i> 	<ul style="list-style-type: none"> • Limited to specimen types that are FDA-cleared: urine, endocervical, urethral and vaginal swabs. • First catch urine specimen in females may detect up to 10% fewer infections as compared to vaginal and endocervical swab samples • <i>Consult the laboratory that is performing testing to confirm specimen types that are accepted. Not all laboratories are validated and able to test extra-genital specimens.</i>
Testing For Sexual Assault	Preferred method for diagnosing boys, detecting infection in extragenital specimens, as well as from vaginal swabs in girls and both urine and urethral swabs in boys. ^{1,9}	NAAT can be used to test vaginal and urine specimens from girls but should be done after consultation with an expert to ensure proper testing and test interpretation. Data are insufficient to recommend use of NAAT in boys and extragenital sites from either gender. ^{1,9}
Antimicrobial Susceptibility Testing	Critical for detection and monitoring antimicrobial resistance	No FDA-cleared NAAT for detection of antibiotic resistance
Test of Cure- Pharyngeal Gonorrhea	If treated with an alternative regimen, the patient should return 14 days after treatment for a test of cure using either culture or NAAT. ⁹ If the NAAT is positive, efforts should be made to perform a confirmatory culture before retreatment. All positive cultures for test-of-cure should undergo antimicrobial susceptibility testing. ⁹	

*Sensitivity and Specificity vary by method, consult manufacturer package insert for direct comparisons of each assay with culture.

Frequently Asked Questions

1. A physician requested testing for *N. gonorrhoeae* and the culture came back negative but the NAAT was positive—why would this be the case?

Answer: While NAAT is more sensitive than culture, there are several factors that could account for the discordant test results in this case. Before we address the differences in how the different methods detect *N. gonorrhoeae*, it is important to know that in most cases in order to perform both culture and NAAT two different samples (swabs and/or urine) are collected. Many of the explanations of discordance could be traced back to this detail. For example, even if the exact same anatomic location is swabbed, there could be inadequate sampling with one of the swabs that could impact the testing. Alternatively, it is possible that the NAAT was performed on a urine specimen and the culture was performed on a swab, leading to discordant results merely due to the specimen type used. In the bullets below and in the subsequent examples, we provide other potential explanations for discordance.

Please review the list and consider using it in future cases of discordance you may encounter.

- The most probable explanation is that **culture is less sensitive than NAAT**.¹ The patient could have been infected with *N. gonorrhoeae* which was detected by the NAAT but it was not detected by culture due to the lower sensitivity.

- The **site of collection** may impact the results. For example, pharyngeal samples may not grow as well in culture but there was enough nucleic acid present for the NAAT method to detect the *N. gonorrhoeae*.
- The **specimen collection swab** itself might not have been appropriate for the method (either it was inhibitory to the growth¹ and/or there was not enough of the organism on the collection swab).
- The organism may **no longer be viable** but the nucleic acid from the organisms was still detected by the NAAT. Some reasons the organism may no longer be viable include:
 - **ineffective transport media**⁷
 - **transport delays**
 - **inappropriate specimen transport conditions**
 - the patient having received **antibiotic therapy prior to sample collection**
 - ◆ If the NAAT is performed within a certain time frame (refer to manufacturer's package insert for exact time frame) of receiving antibiotic therapy it can still detect residual nucleic acid despite successful treatment. This means that the patient is no longer infected and no viable organism can be cultured but the NAAT is positive because nucleic acid is still present. To prevent this outcome refer to the manufacturer's package insert for the intended use and limitations of the assay.
- The person could have been truly negative for *N. gonorrhoeae* but the **NAAT detected a nongonococcal *Neisseria* species**. This is considered a false-positive result because the person was not infected with *N. gonorrhoeae*, but the NAAT result indicates that the patient is positive for *N. gonorrhoeae*. This is a limitation of the assay design. See the manufacturer's package insert for assay-specific limitations.

Being aware of the potential reasons for the discordant results and exploring whether any of these scenarios could have contributed to the result is an important next step in determining whether the patient may have gonorrhea. The clinician's decision to initiate treatment is dependent on many factors including laboratory findings, any potential follow-up testing, clinical examination and risk factors, public health department case definitions and the CDC STD Treatment Guidelines.⁹

2. We just received test results for a patient suspected of having gonorrhea—the NAAT was negative but the culture was positive, should we be concerned?

Answer: Discordant results are not uncommon. While we normally think of the NAAT assay as being more sensitive, there are potential explanations for these results. As in the example above, let us examine some of the reasons that could contribute to the discordant test results from this patient.

- The **site of collection** may impact the results. The optimal specimen type for detection of *N. gonorrhoeae* infection by NAAT are vaginal swabs from women and first catch urine from men. The use of other specimen types can result in decreased sensitivity in the NAAT resulting in a negative NAAT result from a patient that has a positive culture. Also, there are some substances present in patient specimens that may be inhibitory to NAAT^{8,10} but not to growth in culture.
- The **specimen collection swab** itself might not have been appropriate for the method (either it was inhibitory to the PCR⁸ and/or there was not enough of the organism on the collection swab) resulting in a negative NAAT despite growth in culture.
- NAAT assays are designed to be very specific to the organism being tested, therefore any **mutations** in the pathogen that occur within the target area of the assay could result in the NAAT being negative despite the pathogen being present and being able to be cultured. While this is likely to be rare, it is possible.
- Technical error, specimen mix-up, instrumentation error, or target levels below the assay's limit of detection may result in a negative NAAT despite growth in culture.

Neisseria meningitidis

There has been an increase in cases of *N. meningitidis*, a normal colonizer of the nasopharynx identified in persons presenting with signs and symptoms indicative of gonorrhea.²⁻⁵ Due to the potential biosafety concerns,⁶ laboratories working up genital and extragenital cultures, including presumptive isolates of *N. gonorrhoeae*, should be aware of the potential presence of *N. meningitidis* in these cultures. Laboratories should consult with a biosafety expert and/or perform a risk assessment of their testing procedures to ensure safe testing practices.

- The specimen could be negative for *N. gonorrhoeae*, as the NAAT result indicated but a **nongonococcal *Neisseria* species** was cultured that based on initial identification methods appeared to be *N. gonorrhoeae*.
 - As discussed in the first example, in order to perform both culture and NAAT, two different swabs are collected. Discrepant results could be the result of inadequate sampling with one of the swabs.

Being aware of the potential reasons for the discordant results and exploring whether any of these scenarios could have contributed to the result is an important next step in determining whether the patient may have gonorrhea. The determination of the diagnosis and whether to initiate treatment will be dependent on many factors including laboratory findings, any potential follow-up testing, the clinical examination and risk factors, public health department case definitions and the CDC STD Treatment Guidelines.⁹

3. I received a preliminary report from the laboratory that indicated that the patient sample was presumptive negative for gonorrhea but on the final report the NAAT was positive for *N. gonorrhoeae* and the culture was confirmed *N. gonorrhoeae* positive. How could this happen?

Answer: A presumptive result based on colony morphology growth on selective media, Gram stain and oxidase alone may be evaluated at 24 hours and considered presumptive negative if the colonies examined do not fit the biochemical profile. However, growth of *Neisseria* species may be delayed. In the laboratory, the plates are incubated for 48 hours to check for final growth. It is possible and not uncommon for a culture to be negative at 24 hours but positive at 48 hours. In addition, *N. meningitidis* resembles *N. gonorrhoeae* using initial screening tests and can only be differentiated using further confirmatory testing.

Some organisms may take longer to grow and require additional measures, such as enhanced culturing techniques, to promote growth. Testing methods require pure growth of an organism and this can be time-consuming.

REFERENCES

1. Centers for Disease Control and Prevention. Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*—2014. MMWR Recomm Rep Morb Mortal Wkly Rep Recomm Rep. 2014 Mar 14;63(RR-02):1–19.
2. Retchless AC, Kretz CB, Chang H-Y, Bazan JA, Abrams AJ, Norris Turner A, et al. Expansion of a urethritis-associated *Neisseria meningitidis* clade in the United States with concurrent acquisition of *N. gonorrhoeae* alleles. BMC Genomics. 2018 02;19(1):176.
3. Ma KC, Unemo M, Jeverica S, Kirkcaldy RD, Takahashi H, Ohnishi M, et al. Genomic Characterization of Urethritis-Associated *Neisseria meningitidis* Shows that a Wide Range of *N. meningitidis* Strains Can Cause Urethritis. J Clin Microbiol. 2017;55(12):3374–83.
4. Bazan JA, Peterson AS, Kirkcaldy RD, Briere EC, Maierhofer C, Turner AN, et al. Notes from the Field: Increase in *Neisseria meningitidis*-Associated Urethritis Among Men at Two Sentinel Clinics - Columbus, Ohio, and Oakland County, Michigan, 2015. MMWR Morb Mortal Wkly Rep. 2016 Jun 3;65(21):550–2.
5. Bazan JA, Turner AN, Kirkcaldy RD, Retchless AC, Kretz CB, Briere E, et al. Large Cluster of *Neisseria meningitidis* Urethritis in Columbus, Ohio, 2015. Clin Infect Dis Off Publ Infect Dis Soc Am. 2017 01;65(1):92–9.
6. Centers for Disease Control and Prevention (CDC). Laboratory-acquired meningococcal disease—United States, 2000. MMWR Morb Mortal Wkly Rep. 2002 Feb 22;51(7):141–4.
7. Papp JR, Henning T, Khubbar M, Kalve V, Bhattacharyya S, Travanty E, et al. Recovery of *Neisseria gonorrhoeae* from 4 commercially available transport systems. Diagn Microbiol Infect Dis. 2016 Oct;86(2):144–7.
8. Schrader C, Schielke A, Ellerbroek L, Johne R. PCR inhibitors - occurrence, properties and removal. J Appl Microbiol. 2012 Nov;113(5):1014–26.
9. Centers for Disease Control and Prevention. Sexually Transmitted Diseases Treatment Guidelines, 2015. MMWR Recomm Rep Morb Mortal Wkly Rep Recomm Rep. 2015 Jun 5;64(RR-3):1–137.

10. Ng L-K, Martin IE. The laboratory diagnosis of *Neisseria gonorrhoeae*. Can J Infect Dis Med Microbiol. 2005;16(1):15–25.

Acknowledgments

This document was developed by APHL's Sexually Transmitted Diseases Subcommittee.

Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

This project was 100% funded with federal funds from a federal program of \$485,382. This publication was supported by Cooperative Agreement #5NU600E000103 from the US Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC or the Department of Health and Human Services.



8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Phone: 240.485.2745
Fax: 240.485.2700
www.aphl.org