Diagnostic tests for sexually transmitted infections

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Abstract

The laboratory plays an essential role in the diagnosis of sexually transmitted infections (STIs). The ability to make an accurate diagnosis is essential because of the individual and public health implications associated with an STI diagnosis. This is achieved through a combination of direct microscopy, bacterial culture, molecular detection and serological testing. The choice of test is guided by the method with the highest sensitivity and specificity; in most cases, this is nucleic acid amplification testing. With antimicrobial resistance a significant global concern, monitoring resistance trends is crucial in guiding antimicrobial prescribing. STI testing must be both accessible and acceptable to patients, with testing now available in a variety of community and healthcare settings. Self-sampling for STIs offers a suitable alternative to clinician-taken swabs for those who do not want or need an examination. This paper summarizes the current methods available in the diagnosis of STIs and genital infections.

Keywords *Chlamydia trachomatis*; diagnostic testing; *Haemophilus ducreyi*; herpes simplex virus; human immunodeficiency virus; *Klebsiella granulomatis*; MRCP; *Neisseria gonorrhoeae*; sexually transmitted infections; *Treponema pallidum*; *Trichomonas vaginalis*

Introduction

The laboratory plays an essential role in the diagnosis of sexually transmitted infections (STIs) and other genital infections. A combination of direct microscopy, often available within the sexual health clinic, bacterial culture, molecular detection and serological testing is used.[1] Owing to the individual and public health implications of an STI diagnosis, choice of test is guided by the method with the highest sensitivity and specificity; in most cases, this is nucleic acid amplification testing (NAAT). Antimicrobial resistance, particularly with *Neisseria gonorrhoeae* and *Mycoplasma genitalium*, is now of significant global concern, and laboratory surveillance is essential in monitoring resistance to guide antimicrobial prescribing.

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Key points

- All patients presenting with symptoms consistent with a sexually transmitted infection (STI) should be offered screening for chlamydia, gonorrhoea, HIV and syphilis
- A combination of laboratory techniques including direct microscopy, culture, molecular detection and serology is used in the diagnosis of STIs
- Self-sampling for bacterial STIs is acceptable to patients and has comparable sensitivity to a clinician-taken swab. This is a popular alternative for individuals not requiring examination
- Development and availability of near-patient and rapid diagnostics will allow for quicker detection of infection and treatment of patients to reduce morbidity and onward transmission

In 2016, Public Health England recorded 280,622 new diagnoses of STIs in the over-25s alone, in addition to 128,098 cases of chlamydia in those aged 15–24 years. Recent reports showing a steep rise in rates of syphilis and gonorrhoea infection, highest among men who have sex with men (MSM), are worrying. Although the rate of new human immunodeficiency virus (HIV) diagnoses is decreasing, the rate of late diagnosis, with its associated increased morbidity and mortality, remains static.[2]

STI testing is now available in a variety of community and healthcare settings, making access easier. Wherever patients choose to test, they should be offered screening for chlamydia, gonorrhoea, HIV and syphilis as a minimum. Self-sampling for STIs is acceptable to many patients who do not wish to or do not require clinical examination.[3]

What tests to offer?

The choice of tests will be influenced by the history and examination, as well as by the setting in which the patient is seen, particularly regarding the availability of microscopy (Table 1). Where patients present to primary care with recurrent, refractory or unusual symptoms presentations, clinicians should have a low threshold for referral to level three sexual health services to access a broader range of investigations.

Chlamydia

Chlamydia trachomatis is an obligate intracellular bacterium. Serovars D–K cause urogenital infection, while serovars L1–L3 cause lymphogranuloma venereum (LGV). Asymptomatic urogenital infection is seen in up to 70% of women and 50% of men. A low threshold for screening is essential.

Molecular detection: NAAT is the gold standard for detection of *C. trachomatis* as it is highly sensitive and specific. For female patients, a vulvovaginal specimen is optimal; this can be taken by either the patient or the clinician. An endocervical swab can be used as an alternative but is slightly less sensitive and requires a speculum examination. First-void urine has lower sensitivity

Sampling for the diagnosis of STIs[1]

	Female patients[a]	Male patients[a]
Asymptomatic	Vulvovaginal[b] NAAT for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* + HIV and syphilis serology ± serology for hepatitis B	First-pass urine NAAT for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* + HIV and syphilis serology ± rectal NAAT for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* ± pharyngeal NAAT for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* ± serology for hepatitis A, B and C
Symptomatic	As for asymptomatic women + lateral vaginal wall sample for pH, Gram stain and microscopy, *Candida* ± routine culture ± gonorrhoea culture + posterior fornix wet slide for microscopy + endocervical sample for Gram stain and microscopy ± midstream urine ± pregnancy test	As for asymptomatic men + urethral sample for Gram stain and microscopy, culture for *Neisseria gonorrhoeae* ± rectal wall sample for Gram stain and microscopy, culture for *Neisseria gonorrhoeae* ± pharyngeal sample for culture for *Neisseria gonorrhoeae* ± midstream urine
Genital ulcer present	As per symptomatic male/female patient + HSV PCR + syphilis PCR + repeat syphilis serology at 6 and 12 weeks after high-risk exposure ± swab of ulcer base for culture, Gram stain and ± PCR for *Haemophilus ducreyi* (chancroid) ± biopsy, scraping, swab or impression smear for microscopy + culture for *Klebsiella granulomatis* (Donovanosis)	
Proctitis present	As per symptomatic male/female patient + LGV serovar testing on rectal chlamydia NAAT + rectal HSV and syphilis PCR + repeat syphilis serology at 6 and 12 weeks after high-risk exposure	

HSV, herpes simplex virus; LGV, lymphogranuloma venereum; NAAT, nucleic acid amplification testing; PCR, polymerase chain reaction.
[a] Transgender patients should have a sensitive history taken to establish the type of sexual contact they are having, which will guide testing.
[b] Rectal ± pharyngeal NAATs for chlamydia and gonorrhoea can also be considered depending on the type of sexual activity reported.

Table 1

and specificity in women and is not the optimal specimen for detection. In men, a first-pass urine sample has an equivalent or higher sensitivity than a urethral swab and is therefore favoured because of patient tolerability. Although not licensed to be used at non-genital sites, NAATs have demonstrated good performance for pharyngeal and rectal samples. MSM presenting with proctitis should have serovar testing for LGV as a longer course of antibiotics may be needed.

Bacterial culture: *Chlamydia trachomatis* can be identified from cell culture; however, because this is slow and labour-intensive, and suffers from poor sensitivity, it is no longer widely used.

Gonorrhoea

Neisseria gonorrhoeae is a Gram-negative diplococcus that infects mucus membranes via direct inoculation. The presence of symptoms is variable depending on the infected site(s). Male urethral infection causes symptoms in >80% of patients and female endocervical infection symptoms in approximately 50%, but only 10% of patients with pharyngeal infection report symptoms.

Direct microscopy: *N. gonorrhoeae* can be identified by light microscopy of Gram-stained samples, with the organism seen as Gram-negative diplococci inside polymorphonuclear leucocytes. This method is most sensitive when urethral discharge is present. It is helpful for early diagnosis and treatment but must be confirmed by culture. Gram stain microscopy can also be used to make provisional diagnoses of gonorrohoea in rectal samples in symptomatic men and in endocervical samples in symptomatic women; however, sensitivity and specificity are lower with these sample types.

Bacterial culture: *N. gonorrhoeae* is a fastidious organism so requires culture medium with nutrient supplementation such as chocolate agar with carbon dioxide. Culture of *N. gonorrhoeae* is necessary to undertake antimicrobial sensitivity testing and should be performed where a diagnosis has been made or the index of suspicion is high. As treatment options for gonorrhoea are very limited, positive cultures allow for resistance testing to guide therapy. This is vital to safeguard first-line antibiotic treatment.

Molecular detection: NAAT for *N. gonorrhoeae* is more sensitive and easier to perform than culture in both symptomatic and asymptomatic patients. A vulvovaginal or endocervical swab can be used in women. In men, a first-pass urine sample can be used. NAAT testing for *N. gonorrhoeae* also has high sensitivity with rectal and pharyngeal samples, although clinicians should be aware of the potential for cross-reactivity with commensal *Neisseria* spp., particularly in the pharynx. A positive result at extragenital sites should always be confirmed using a NAAT with a different target.

Mycoplasma genitalium

M. genitalium is an established sexually transmitted pathogen in the aetiology of urethritis, cervicitis and pelvic inflammatory disease. It is extremely small and not visible by light microscopy; furthermore, it has no cell wall so cannot be Gram-stained. As in gonorrhoea, treatment options for this organism are severely limited because of high rates of circulating antimicrobial resistance, particularly to tetracyclines and macrolides.

Molecular detection: NAATs offer the best method of detection but laboratories have so far relied on in-house assays, few of

which have been properly validated. Newer commercial assays are now available, but uptake of these has been slow. Some testing platforms combine detection with antimicrobial resistance testing, making treatment choices easier in the face of a growing global problem with antibiotic resistance.

Bacterial culture: *M. genitalium* replicates very slowly. Therefore growth in culture takes weeks and is not useful in clinical situations to decide on treatment.

HIV

Undiagnosed infection with the HIV can result in significant morbidity and mortality. Diagnosing HIV and starting antiretroviral therapy early allows for a good quality of life and reduces the risk of onward transmission.

Serological testing: fourth-generation tests looking for the presence of both HIV antibodies and p24 antigen are recommended for HIV screening. Although they are highly sensitive and specific, additional confirmatory testing using a different platform or format is required to exclude a false-positive result. Confirmatory testing also distinguishes between infection with HIV-1 and HIV-2. As a positive result requires development of an immune response to the virus, it is essential to be aware of the window period for testing and to offer repeat testing where appropriate (Table 2).[4]

Point-of-care tests (POCTs): these allow for rapid HIV screening, with a result available within minutes. They can be particularly useful where knowledge of a patient's HIV status is time-critical, for example a pregnant woman presenting in labour; however, they can also help improve acceptability for patients and retention in care. Although POCTs have good sensitivity in established HIV infection, all reactive results require laboratory confirmation to exclude false-positive results. They also lack sensitivity in acute HIV, in comparison with fourth-generation serological testing.

Syphilis

Treponema pallidum subsp. *pallidum* is a spirochaete bacterium that can be transmitted via direct contact during sex, vertically from mother to child, via blood transfusion or use of contaminated injecting paraphernalia.

Direct microscopy: syphilitic treponemes from lesions of primary or secondary syphilis can be visualized by dark-ground (or field) microscopy. Identification by their characteristic watchspring, corkscrew and annular movements confirms a diagnosis of syphilis.

Molecular detection: polymerase chain reaction (PCR) testing is increasingly available for the direct detection of *T. pallidum* from the lesions of primary or secondary syphilis. This, and the use of dark-ground microscopy, can aid in the earlier detection of syphilis, as these tests do not require the patient to develop the immune response needed for serological testing.

Serological testing: serology plays an essential role in diagnosing syphilis and is useful in monitoring treatment. A combination of specific (treponemal) tests and non-specific tests is used for diagnosis (Table 3). As serological testing requires the development of an immune response, early testing may miss a diagnosis, so serology should be repeated at 6 and 12 weeks (see Table 2).

Genital herpes

Infection with herpes simplex virus (HSV) is often asymptomatic but can cause ulceration either of either the oro-labia (HSV-1) or genitals (HSV-1, HSV-2). Following initial infection, the virus becomes latent in the sensory ganglia, from where recurrences can emanate. Confirmation of the diagnosis at initial presentation to the health services is useful to counsel a patient and guide management. Type 1 HSV on the genitals tends to be less recurrent than type 2.

Molecular detection: a swab from a genital lesion or mucosa can detect HSV DNA using PCR. This also allows for viral typing (type 1, type 2). A combined HSV/*T. pallidum* PCR test is commercially available.

Culture: viral culture for HSV is highly specific but lacks sensitivity compared with PCR. Challenges associated with sample processing means that this technique is no longer widely used.

Serological testing: antibody testing for HSV is available but often not clinically useful. The presence of immunoglobulin (Ig) G does not distinguish between a recent or long-standing

Window periods

- In the diagnosis of most STIs, there is a 'window period' between possible contact with an infection and the time a test can reliably exclude that infection
- It is important to inform patients of these window periods and arrange for repeat testing where required
- Patients with continuing high-risk exposure should be encouraged to attend for screening on a 3-monthly basis
- HIV — a negative result on a fourth-generation test performed at 4 weeks is highly likely to exclude HIV infection.[4] A further test at 8 weeks should be considered in those at high risk
- Syphilis — patients presenting with genital ulceration should have serology repeated at 2 weeks. Those with a high-risk exposure should be offered repeat testing at 6 and 12 weeks
- Chlamydia — to exclude infection, patients should undergo testing at 2 weeks after potential exposure
- Gonorrhoea — while often detectable earlier than 2 weeks, patients are asked to return at 2 weeks after potential exposure to allow for simultaneous chlamydia screening

Table 2

Syphilis serology

Specific (treponemal) tests	EIA — treponemal enzyme immunoassays CLIA — treponemal chemiluminescent assay TPHA — *Treponema pallidum* haemagglutination assay TPPA — *Treponema pallidum* particle agglutination assay FTA-abs — fluorescent treponemal antibody absorption test	Confirms diagnosis at first presentation Usually positive for life Also positive in other treponemal infections, e.g. yaws, pinta and bejel
Non-specific tests	VDRL — Venereal Diseases Research Laboratory RPR — rapid plasma reagin	Reported as a titre that can give some indication of stage of infection The titre responds to treatment, allowing monitoring of outcome and detection of treatment failure or reinfection Biological false-positives have been reported in a number of conditions including herpes simplex virus, measles, mumps and some autoimmune diseases

Table 3

infection, and IgM detection is unreliable. Evolution of an antibody response can be useful in managing HSV in pregnancy but is not widely recommended outside this context.

Candida

Most *Candida* or thrush infections are caused by *Candida albicans*, with non-albicans species such as *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* and *Saccharomyces cerevisiae* diagnosed far less commonly. Candidiasis is not an STI, and many women are colonized without any symptoms. Only those with symptoms require investigation and treatment.

Microscopy: where available, a Gram-stained sample from the vaginal wall can confirm the diagnosis of candidiasis. However, this method lacks sensitivity, and many clinicians elect to treat empirically in the presence of typical symptoms and signs even where microscopy is negative.

Culture: *Candida* culture on solid fungal media can improve the sensitivity of microscopy alone and can also aid with speciation and sensitivities in complex or recurrent disease.

Bacterial vaginosis (BV)

BV is a common cause of altered vaginal discharge, resulting from an overgrowth of predominantly anaerobic organisms that overwhelm the normal lactobacilli of the vagina.

Clinical diagnosis: many women with BV present to a primary care setting where microscopy is not available. Onward referral in all cases is not practical, and a clinical diagnosis can be made based on the history, an elevation of the vaginal pH to >4.5 and the presence of a thin, white, homogenous discharge that may have a characteristically fish smell.

Direct microscopy: where microscopy is available, a definitive diagnosis of BV can be made using a Gram-stained vaginal smear to assess the vaginal flora. When BV is present, large numbers of Gram-positive and Gram-negative cocci are seen, with reduced lactobacilli (Gram-positive bacilli). The Hay/Ison criteria can be used to evaluate the vaginal smear (Table 4). Clue cells, which are vaginal epithelial cells heavily coated with bacteria, are diagnostic of BV.

Clinical diagnosis: when microscopy is not available, a clinical diagnosis can often be made based on the visual appearance of the discharge and characteristic odour associated with it. Culture is not recommended as commonly associated organisms can often be isolated from asymptomatic women.

Trichomoniasis

Trichomonas vaginalis is a flagellated protozoon that can infect the vagina, urethra and paraurethral glands in women, and the urethra, the subpreputial sac and penile lesions in men.

Direct microscopy: wet-mount microscopy of a sample of discharge collected from the posterior fornix of the vagina can be used to identify *T. vaginalis*. The motile organism can be identified by the presence of flagellar movement. Unfortunately, the sensitivity of microscopy is low, at 45–60%, particularly in clinics where *T. vaginalis* is not regularly seen.[5] Microscopy has an even lower sensitivity in men.

Hay/Ison criteria for BV

Grade 0	No bacteria	Normal
Grade 1	Lactobacilli predominate	Intermediate
Grade 2	Mixed flora with some lactobacilli but	BV
Grade 3	*Gardnerella* ± *Mobiluncus* also present	
Grade 4	Predominantly *Gardnerella* ± *Mobiluncus* Clue cells Gram-positive cocci	

Table 4

Culture: *T. vaginalis* culture was previously considered to be the gold standard method for diagnosing *T. vaginalis* because it has higher sensitivity than microscopy. A culture medium is commercially available, but this method is not widely used.

Molecular detection: NAATs now offer the highest sensitivity for identifying *T. vaginalis*. Although not currently widely available, they are likely to play a central role in the diagnosis of *T. vaginalis* in the future.

Donovanosis

Donovanosis is a cause of sexually transmitted genital ulcer disease seen in the tropics. It is caused by *Klebsiella granulomatis.*

Microscopy: donovanosis can be diagnosed by the identification of Donovan bodies from a scraping or impression smear from the genital lesion or from a tissue biopsy sample. Even in areas where donovanosis is endemic, microscopy has a sensitivity of only 60–80% so many patients are diagnosed clinically or managed as part of a syndromic approach to genital ulceration.

Chancroid

Chancroid is another cause of sexually transmitted genital ulcer disease. It is caused by *Haemophilus ducreyi.*

Microscopy: although a Gram-stained smear of material from the ulcer base or lymph node aspirate may show Gram-negative coccobacilli, the sensitivity of microscopy is too low for this to be a recommended for diagnosis.

Culture: material obtained form the ulcer base or via lymph node aspiration can be cultured for *H. ducreyi.* Sensitivity remains low, at 80%, because of the fastidious nature of the organism, but growth can be optimized by the use of a combination of culture media.

Molecular detection: although PCR for *H. ducreyi* DNA is possible, it is not widely available and is restricted to the research setting.

Serological tests for other sexually transmitted infections

In certain risk groups, such as MSM and intravenous drug users, tests for hepatitis A, B and C may be indicated (see Table 1).

Future directions

Recent advances in molecular testing mean that more sensitive assays with quicker turn-around will be available. This will significantly improve time to treatment for both patients and their partners, and reduce the transmission chain. Home sampling is growing in popularity and is likely to increase with the introduction of national online testing. ◆

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FURTHER READING

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CHRONOLAB

CHRONOLAB SYSTEMS