Testing for sexually transmitted infections

Catriona Ooi, Director, Sexual Health Service, Hunter New England Area Sexual Health Service, Newcastle, and Conjoint Lecturer, School of Medicine and Public Health, Faculty of Health, The University of Newcastle, New South Wales

Summary

Rates of sexually transmitted infections are increasing worldwide and notifications are also increasing in Australia. As many sexually transmitted infections are asymptomatic, timely and appropriate testing is needed to avoid the long-term sequelae of infection, to halt transmission and to improve associated morbidity. Testing for sexually transmitted infections has evolved over time. Although nucleic acid amplification tests have an increasing role and may enable non-invasive testing, microscopy and culture are still useful investigations for some infections.

Key words: chlamydia, gonorrhoea, herpes, HIV, syphilis.

(Aust Prescr 2007;30:8–13)

Introduction

Effective testing for sexually transmitted infections needs to be acceptable to the patient and tailored and targeted appropriately to sexual risk. This risk is determined by factors such as the use of condoms and the number of sexual partners. As many common sexually transmitted infections, such as chlamydia, are largely asymptomatic, doctors need to be aware of local epidemiology and at-risk groups, in order to facilitate opportunistic screening.

To determine which tests to perform, consider the patients' individual needs and concerns, sexual activity, condom use, local epidemiology and any symptoms (Table 1). Sexual activity such as vaginal, anal or oral sex will direct from where to collect specimens. Pretest counselling and education are important. Serology for HIV should be considered for all patients. Testing for hepatitis B should be considered for those who have not been vaccinated. Homosexually active men should routinely have additional tests for both syphilis and hepatitis A.

With most infections there is a 'window period' (Table 2) before laboratory tests become positive. This period must be considered when interpreting results.

Chlamydia

Chlamydia trachomatis is the most commonly notified sexually transmitted infection in Australia and rates have risen four-fold between 1996 and 2005.[1] In up to 80% of women and 50% of men the infection is asymptomatic.[2] If untreated, chlamydia may have serious sequelae such as pelvic inflammatory disease, ectopic pregnancy and infertility in women, and epididymitis, chronic prostatitis and urethral strictures in men. Screening is recommended for all sexually active individuals younger than 25 years regardless of condom use.

Testing

In Australia, nucleic acid amplification tests – polymerase chain reaction (PCR) and ligase chain reaction (LCR) – are accurate and reliable. The chlamydia PCR is highly specific (99–100%) with a sensitivity of 85–90%.[3] These tests allow non-invasive and self-collected sampling. One study evaluating nucleic acid amplification tests of self-collected vulval-introital specimens, first void urine samples and clinician-collected cervical samples found the self-collected swabs and urine specimens to be acceptable alternatives to cervical sampling.[4]

A positive result from a chlamydia nucleic acid amplification test is likely to be a true positive. However, these tests have only been validated for use in urine, cervical and urethral samples. Although these tests can be used to analyse samples from other sites, such as rectum and vagina, the results should be interpreted with caution.

Chlamydia trachomatis cultures are the test of choice if the results are to be used in legal investigations as culture has a high specificity. Culture also allows for antibiotic sensitivity testing, but has the disadvantage of relatively low sensitivity and high cost. Culture is also labour intensive, technically difficult and has a long turnaround time.

Herpes

Genital herpes is the clinical manifestation of infection with either herpes simplex virus type 1 or herpes simplex virus type 2 at genital sites. Infection is common and often asymptomatic. In Australia it is estimated that up to 25–30% of people are seropositive for herpes simplex virus type 2 and 80% are seropositive for herpes simplex virus type 1.

Table 1

Screening guidelines for different populations *

Who	Routine tests (regardless of condom use)	Other tests to consider	When
Heterosexual men and women	Chlamydia (cervix/urine) Hepatitis B (consider vaccination†)	Depending on sexual practice: – gonorrhoea (cervix/urine/throat/anal) – chlamydia (anal) Depending on local epidemiology: – Trichomonas – syphilis – baseline serology for HIV	Consider annual screening for those who have changed partner, more frequently depending on risk
Men who have sex with men	Gonorrhoea (throat/anal) Chlamydia (urine/anal) Hepatitis A (consider vaccination†) Hepatitis B (consider vaccination†) Syphilis HIV	Anal test indications: – any anal sex with casual partners – any unprotected anal sex – any anal symptoms – HIV positive – past history of gonorrhoea – contact with any sexually transmitted infection – request	Annually if asymptomatic, more frequently depending on sexual risk
Young people (<25 years)	Chlamydia (cervix/urine) Hepatitis B (consider vaccination†)	Gonorrhoea Baseline serology for HIV	Annually for those who have changed partner, more frequently depending on risk
Sex workers	Gonorrhoea (cervix/urine) Chlamydia (cervix/urine) Syphilis Hepatitis B (consider vaccination†) HIV Gonorrhoea (throat/anus)	Depending on sexual practice: – chlamydia (anal) – hepatitis A (consider vaccination)	Every 3–6 months
People who inject drugs	Chlamydia (cervix/urine) Hepatitis B (consider vaccination†) Hepatitis C Syphilis HIV	Hepatitis A (consider vaccination)	Annually if asymptomatic, more frequently after particular risk episode

* Adapted from: Clinical guidelines for the management of sexually transmitted infections among priority populations. The Royal Australasian College of Physicians, Australian Chapter of Sexual Health Medicine, 2004

† Once patient immunised against hepatitis A/hepatitis B, further serology is unnecessary

Table 2

Tests for sexually transmitted infections

	Test	Specimen	Window period	Indication	Comments
Chlamydia	PCR/LCR	Urine, swab (urethra/cervix)	2–7 days	Screening and diagnosis	PCR at high vaginal and rectal sites not validated Retesting at one month post-treatment if indicated
	Culture	Swab – any site			Highly specific, use in legal situations
Herpes	PCR	Lesion	Lesion	Diagnosis	Negative PCR or viral culture does not exclude infection
	Viral cultures	Lesion	Lesion	Diagnosis	
	EIA/ELISA	Blood	3–12 weeks	Screening	Type-specific or herpes simplex virus type 2 serology most useful. Beware false results
	Western blot	Blood	3–12 weeks	Screening	
Gonorrhoea	PCR/LCR	Urine, swab (urethra/cervix)	24 hours	Screening and diagnosis	PCR at high vaginal, throat and rectal sites not validated
	Culture	Swab (urethra/cervix/throat/rectum)		Screening/diagnosis Confirmation of PCR	Culture allows antibiotic sensitivity and specificity testing
Syphilis	Dark ground microscopy	Lesion	3–30 days, if chancre	Diagnosis early syphilis	Only with symptoms
	PCR/LCR	Lesion, tissue, CSF, blood	3–30 days, if chancre	Diagnosis early syphilis	Not widely available
	EIA	Blood	2–12 weeks	Screening	Repeat serology for those with suspected exposure
	RPR/VDRL	Blood VDRL-CSF	3–12 weeks	Screening, diagnosis/staging, treatment response, reinfection	
	FTA-abs TPPA/TPHA	Blood	3–12 weeks	Confirmation of diagnosis	
HIV	HIV antibody: – EIA – Western blot	Blood	6–12 weeks	Screening/diagnosis	Gold standard test
	p24 antigen		earliest 2 weeks		Transient, may be absent after 2 weeks
	Qualitative PCR HIV DNA (proviral DNA)				Useful for early diagnosis
	Quantitative HIV RNA (viral load)				Beware false positives

PCR	polymerase chain reaction	RPR	rapid plasma reagin	TPPA	treponema pallidum particle agglutination
LCR	ligase chain reaction	VDRL	venereal disease research laboratory	TPHA	treponema pallidum haemagglutination test
EIA	enzyme immunoassay	CSF	cerebrospinal fluid		
ELISA	enzyme-linked immunosorbent assay	FTA-abs	fluorescent treponemal antibody absorption		

Testing

The clinical diagnosis is unreliable and must be confirmed. Whether tests are done depends on the presence of symptoms, however patients who are asymptomatic still shed the virus. Type-specific testing should be undertaken to identify herpes simplex virus type 1 or type 2, as knowing the type gives important prognostic information and may direct education and counselling. Direct detection tests (PCR, viral culture, immunofluorescence) can detect herpes simplex virus in swabs of lesions or infected secretions. However, viral cultures, immunofluorescence and, to a lesser extent, PCR swabs may all produce false negative results. A negative test therefore does not rule out genital herpes.

Although slow and labour-intensive, viral culture is type specific and has long been regarded as the gold standard due to its specificity of nearly 100%. Sensitivity varies greatly as it depends on viral shedding, transport conditions, specimen quality and the timing of specimen collection. Indeed, virus isolation may range from 52–90% for vesicles to 19–27% for crusted lesions.

Type-specific PCR is both sensitive and specific. Studies have shown that tests using PCR may increase the rate of virus detection by 24–71%.[5,6] Herpes simplex virus immunofluorescence is rarely performed despite its high specificity as it has low sensitivity (80%). The results may depend upon specimen quality and the experience of the laboratory technician.

Serological tests which are not type specific have little diagnostic value and are not recommended. Herpes simplex virus type-specific antibody tests are widely available, however they vary in sensitivity and specificity. Only those based on glycoprotein G have acceptable accuracy with good sensitivity and specificity in high prevalence populations. The positive predictive value (the proportion of positive results that are true positives) is lower in groups with a low prevalence of infection.[7] With some tests for herpes simplex virus type 2, clinicians should be aware of the possibility of cross-reactivity between herpes simplex virus type 1 and type 2 antibodies. The gold standard for serological tests is the western blot. This test is highly sensitive and specific for both herpes simplex virus types 1 and 2, but it is expensive and not widely available. The window period for serological tests ranges from 2 to 12 weeks.

Screening serology may be useful in epidemiological studies, but is of limited benefit for asymptomatic patients. A positive serology test in those with no symptoms is unlikely to change treatment decisions or behaviour, and may lead to significant psychological distress. Herpes simplex virus serological tests may be useful in pregnancy, partners of herpes simplex virus-infected individuals and patients with HIV.

Gonorrhoea

Notification rates for gonococcal infections are increasing. In Australia, men who have sex with men, those who have had sexual contact abroad and rural and remote indigenous communities have the highest rates of gonorrhoea. Most urethral infections are symptomatic, however the majority of rectal, pharyngeal and cervical infections will be silent, only becoming symptomatic when complications such as pelvic inflammatory disease occur.

Testing

Microscopy and culture are the mainstay of testing. Culture is highly specific and allows for antibiotic sensitivity testing, but the sensitivity of the test may drop with lengthy delays between the collection site and the laboratory. Nucleic acid amplification tests are more robust. These newer tests have a high sensitivity (90–95%) and specificity (98–100%) for swab samples.[3] Non-invasive testing with first void urine samples and self-collected anal swabs are an option, however in women endocervical swabs are more sensitive than urine samples (94.2% vs 55.6%). Like nucleic acid amplification tests for chlamydia, those for gonorrhoea have only been validated for use with urine, cervical and urethral samples. The positive predictive value of nucleic acid amplification tests for gonorrhoea decreases in a low prevalence population resulting in higher rates of false positive results. Where possible, positive results should be confirmed with culture for antibiotic sensitivity testing and to exclude false positives particularly in low-risk individuals.

Syphilis

The rates of syphilis in Australia are about 10/100 000, nearly double that in New South Wales, and up to 140/100 000 in the Northern Territory, with a national indigenous rate of 300/100 000.[8] Despite remaining fairly stable in the heterosexual community, syphilis rates continue to rise in homosexually active men.[8] Other groups in Australia at risk of syphilis include rural and remote indigenous communities and those from overseas. Most infections are detected in the late latent phase, when the patient is asymptomatic, having passed the early infectious stages unrecognised and undiagnosed.

Testing

National antenatal screening includes syphilis testing. Diagnostic serological tests are widely available, cheap and accurate. For most patients, diagnosis and staging of infection depends upon interpretation of a combination of treponemal and nontreponemal tests.

Serology

The nontreponemal tests are the venereal disease research laboratory test and the rapid plasma reagin test. They detect non-specific antibodies. These tests are simple and cheap with sensitivity of 78–86% in primary syphilis, virtually 100% in secondary syphilis and 95–98% in late latent infection. They may cross-react with other treponemal infections and false positive

results may occur in 1–2% of the population in association with pregnancy, HIV and other medical conditions. False negative results may occur in patients with very high titres – the prozone phenomenon. The titre is both a marker of infectivity and reinfection, and is used to monitor response to treatment.

Treponemal tests detect antibodies that are specific for treponemes. They include the treponema pallidum particle agglutination tests, treponema pallidum haemagglutination test and fluorescent treponemal antibody absorption test. These tests are mostly used to confirm the diagnosis. The treponeme-specific tests have a sensitivity of about 80% in primary syphilis and nearly 100% thereafter. The syphilis enzyme immunoassay may be used for screening sera. It has a sensitivity of 82–100% and specificity of 97–100%.[9]

The nontreponemal tests may become negative after treatment, however they may remain positive at a low titre for life. Similarly, most of those with reactive treponemal specific tests will remain positive for life regardless of treatment or disease activity, with 15–25% of those treated in primary syphilis reverting to negative serology after several years.[10]

Other tests

For symptomatic patients with lesions suggestive of primary or secondary syphilis, direct detection methods, such as dark ground microscopy, may be used, however these are not widely available. Performed correctly, dark ground microscopy has a sensitivity of up to 74–86% and is 97% specific. However, accuracy may vary depending on the age and condition of the lesion. Microscopy also requires trained laboratory staff, specialised equipment and rigorous conditions for the storage and transport of the sample. In primary syphilis (that is, before the production of syphilis antibodies) this method is highly sensitive and specific compared to serological testing.

The nucleic acid amplification tests such as syphilis PCR have sensitivity of 91% and specificity approaching 100%. They have the ability to detect as few as 10 treponemes per lesion. The tests are useful for the diagnosis of congenital syphilis, however they require serological confirmation once the child reaches a certain age.[9] A reactive treponemal test at 18 months is diagnostic of congenital syphilis. These tests are not widely available in Australia and are not routinely used for screening.

HIV

In Australia, the highest risk of HIV exposure occurs in homosexually active men and those from, or those who have had sexual contact in, high prevalence countries. Given the serious sequelae of untreated infection, testing should be offered to everyone presenting for sexually transmitted infection screening, those specifically asking for HIV testing and pregnant women. Pre- and post-test counselling are essential and should cover associated legal aspects and test limitations including window periods.

Testing

HIV antibody testing is used for screening. Typically, sera are first tested with an enzyme immunoassay or enzyme-linked immunosorbent assay. If either test is positive, a confirmatory western blot, the gold standard, is performed. The window period for HIV antibody tests to become positive is three months, but symptomatic patients may have positive antibody tests three weeks after the onset of clinical signs and symptoms.

HIV may be detected earlier with HIV antigen tests. These tests are costly and specialised, usually requiring a reference laboratory. Direct viral detection should be undertaken only if clinically indicated. Viral protein tests such as the p24 antigen may become positive within a few days of symptoms, however this will be absent after two weeks. Detection of viral nucleic acid can be qualitative, PCR for HIV DNA (proviral DNA testing), or quantitative, HIV RNA (viral load). These tests may become positive within days and will remain positive as the antibody develops. For immediate diagnosis, qualitative proviral DNA is recommended. Quantitative HIV RNA testing is not generally recommended as it has a 3% false positive rate in the acute setting.[11]

Human papillomavirus

Anogenital human papillomaviruses are sexually transmitted and extremely common, with up to 75% of sexually active individuals having evidence of current or past infection.[12] Patients presenting with genital warts may have concurrent sexually transmitted infections, and appropriate screening is recommended.

While most infections are subclinical and transient, others may cause a spectrum of disease from genital warts to cervical cancer. Although cervical cancer is a rare outcome of human papillomavirus infection, over 99.7% of cervical cancers are positive for human papillomavirus DNA. Cervical screening programs and guidelines capture many cases of cervical change related to high-risk human papillomavirus types, however the diagnosis of genital warts remains largely clinical.[13]

Conclusion

Accurate and appropriate screening for sexually transmitted infections is essential to prevent significant individual morbidity and mortality and is highly important for public health. As well as the diagnosis and management of each individual, opportunistic testing for other infections, safe sex advice, education and contact tracing of partners is often required.

References

1. HIV/AIDS, viral hepatitis and sexually transmissible infections in Australia. 2006 annual surveillance report. Sydney: National centre in HIV epidemiology and clinical research; 2006.
2. Gaydos CA, Howell MR, Pare B, Clark KL, Ellis DA, Hendrix RM, et al. Chlamydia trachomatis infections in female military recruits. N Engl J Med 1998;339:739-44.

3. Cook RL, Hutchison SL, Ostergaard L, Braithwaite RS, Ness RB. Systematic review: noninvasive testing for Chlamydia trachomatis and Neisseria gonorrhoeae. Ann Intern Med 2005;142:914-25.
4. Carder C, Robinson AJ, Broughton C, Stephenson JM, Ridgway GL. Evaluation of self-taken samples for the presence of genital Chlamydia trachomatis infection in women using the ligase chain reaction assay. Int J STD AIDS 1999;10:776-9.
5. Scoular A, Gillespie G, Carman WF. Polymerase chain reaction for diagnosis of genital herpes in a genitourinary medicine clinic. Sex Transm Infect 2002;78:21-5.
6. Ramaswamy M, McDonald C, Smith M, Thomas D, Maxwell S, Tenant-Flowers M, et al. Diagnosis of genital herpes by real time PCR in routine clinical practice. Sex Transm Infect 2004;80:406-10.
7. Strick L, Wald A. Type specific testing for herpes simplex virus. Expert Rev Mol Diagn 2004;4:443-53.
8. HIV/AIDS, viral hepatitis and sexually transmissible infections in Australia. 2005 annual surveillance report. Sydney: National centre in HIV epidemiology and clinical research; 2005.
9. Peeling RW, Ye H. Diagnostic tools for preventing and managing maternal and congenital syphilis: an overview. Bull World Health Organ 2004;82:439-46.
10. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. MMWR 2002;51:RR-6.
11. HIV/Viral hepatitis: a guide for primary care. Sydney: Australasian Society for HIV Medicine; 2002. p. 30-6.
12. Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med 1997;102(Suppl 5):3-8.
13. Screening to prevent cervical cancer: guidelines for the management of asymptomatic women with screen detected abnormalities. Canberra: National Health and Medical Research Council; 2005.

Conflict of interest: none declared

Self-test questions

The following statements are either true or false (answers on page 27)

1. A negative viral culture for herpes simplex does not exclude infection.
2. Up to 80% of women infected with chlamydia are asymptomatic.

Dental notes

Prepared by Dr M McCullough of the Australian Dental Association

Testing for sexually transmitted infections

Dentists may not realise that there is an increase in the proportion of cases of genital herpes that are caused by herpes simplex virus type 1. In developed countries there is an increase in the proportion of adults who have not been exposed to herpes simplex virus type 1 during childhood but who contract it genitally in adulthood. The recurrence rate of genital herpes due to type 1 is apparently less frequent than with type 2. Conversely, there are several reports of primary herpetic gingivostomatitis and up to 4% of recurrent herpes labialis being caused by herpes simplex virus type 2. Dentists treating these patients should be aware of this developing trend and the availability of laboratory tests to aid them in their diagnosis.

Tests for sexually transmitted diseases have shortcomings such as the window period required before the test becomes positive. This is particularly important for dentists or their staff who sustain a needle-stick injury.

Further reading

Lafferty WE. The changing epidemiology of HSV-1 and HSV-2 and implications for serological testing. Herpes 2002;9:51-5.

Olin L, Wald A. Case report: symptomatic oral herpes simplex virus type 2 and asymptomatic genital shedding. Herpes 2006;13:25-6.

Lowhagen GB, Tunback P, Bergstrom T. Proportion of herpes simplex virus (HSV) type 1 and type 2 among genital and extragenital HSV isolates. Acta Derm Venereol 2002;82:118-20.