

Canadian Guidelines on Sexually Transmitted Infections

Laboratory Diagnosis of Sexually Transmitted Infections

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**TO PROMOTE AND PROTECT THE HEALTH OF CANADIANS THROUGH LEADERSHIP, PARTNERSHIP,
INNOVATION AND ACTION IN PUBLIC HEALTH.**

—Public Health Agency of Canada

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This document is intended to provide information to public health and clinical professionals and does not supersede any provincial/territorial legislative, regulatory, policy and practice requirements or professional guidelines that govern the practice of health professionals in their respective jurisdictions, whose recommendations may differ due to local epidemiology or context.

This chapter provides information about the collection of specimens, their transportation, and laboratory tests for the diagnosis of sexually transmitted infections. This chapter does not include detailed information pertaining to neonates and children; refer to the [infection-specific chapters](#) for in-depth guidance as required.

General principles

- Whenever possible, laboratories should use assays which are approved by Health Canada.
 - If using non-approved commercial tests or laboratory home-derived tests, they should be validated to determine performance specifications for each infection.¹
 - If using commercially approved tests for non-approved specimens (e.g., nucleic acid amplification tests [NAATs] for pharyngeal or rectal specimens), laboratory validation is required.²
 - Collection devices, transport systems and types of tests may vary depending on the agent of interest and techniques used by the laboratory.
 - If a commercial kit is being used, ensure it is not expired, and follow manufacturer instructions.
 - Since not all diagnostic laboratories perform the same tests, clinical conditions and specimen types should be discussed with the laboratory before collecting the specimen.
 - If more information is needed about transport requirements, turn-around time and interpretation of results, contact your local laboratory.
- STIs may be diagnosed in the laboratory using:
 - culture;
 - microscopy;
 - antigen detection;
 - nucleic acid detection, which includes nucleic acid hybridization and NAAT;
 - serology.

Refer to the section on *Test sensitivity, specificity and predictive values* below for more information on the performance of these tests.

Test sensitivity, specificity and predictive values

- Sensitivity and specificity are measures of how good a test is and are not dependent upon the prevalence of the infection in the population being sampled.^a
- The sensitivities and specificities of different methods vary according to specimen type and organism assayed.
 - NAAT is the most sensitive method, and culture is the most specific.
 - Antigen detection, nucleic acid hybridization, culture and microscopy are less sensitive but may be effective for certain patients and specimen types.

^a Sensitivity equals the number of true positives identified by a test, divided by the number of true positives plus false negatives (that is, the total number in the population that are actually positive); specificity equals the number of true negatives identified by the test, divided by the number of false positives plus true negatives (that is, the total number of the population that is actually negative).

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- Surrogate markers, such as leukocyte esterase strip tests, pH or amines point-of-care tests may provide more rapid screening for some conditions, but generally have a low sensitivity and are not very specific, limiting their usefulness.
- Predictive values are dependent upon the prevalence of infection in the population, and determine how useful a test will be in that population.
- For example, if a test with a sensitivity of 95% and a specificity of 97.2% is used in a population (N=2000) with a disease prevalence of 10%,
 - the positive predictive value (PPV)^b is 79.2%—that is, about 80% of the time, positive test results are true positives and the rest are false positives;
 - the negative predictive value (NPV) is 99.4%—that is, almost all negative test results are true negatives.
 - if disease prevalence drops to 1%, the PPV would drop to 25.7%—meaning that only about ¼ of positive test results are true positives; while the NPV remains relatively unchanged and high (99.9%).
- An understanding of predictive values is important where healthy people are screened (e.g., prenatal screening) in settings of low prevalence of infection. The risk of false positives is especially important in dealing with STIs due to the possible consequences for contacts, relationships and in cases of [suspected sexual abuse](#) of children.

Standard collection and transportation recommendations

- Sexually transmitted pathogens are usually fastidious and fragile. Cultures and NAATs may give negative results unless stored and transported under optimal conditions.
 - Cultures should be sent within 24 hours of collection.
 - Specimen stability for NAATs depends on the type of commercial assay used.
 - Consult with your local laboratory concerning storage and transport conditions.
- Wear appropriate personal protective equipment and follow recommended routine practices and additional precautions when collecting and handling specimens.
- Avoid contamination from indigenous commensal flora to ensure a representative sampling of organisms involved in the infectious process.
 - First, use a clean swab to remove mucus from the sampling site; then, when taking the sample, avoid touching non-targeted body surfaces.
- Collect adequate volumes of each liquid specimen in the appropriate collection container(s).
 - Ensure the specimen collection kit is not expired.
- Label each specimen container with the patient's name and/or unique identifier, the source of the specimen and the date and time of collection.
 - Complete the appropriate requisition and ensure that the specimen and requisition labels match.
- All specimen containers should be leak-proof and transported within a biohazard specimen bag that has a separate compartment for paperwork.
- For guidance on specimen collection, collection devices and transportation requirements, contact your local laboratory.

^b Positive and negative predictive values are the proportion of positive and negative results that are true positives and true negatives.

Medico-legal considerations

- Culture is the preferred method for medico-legal purposes. However, a NAAT positive for *C. trachomatis* or *N. gonorrhoeae* may be used for medico-legal purposes if it is confirmed by another NAAT using a different set of primers.
 - Consult with your local laboratory regarding the availability of such testing.
- Assessment and follow-up of children who are suspected of being victims of sexual abuse or victims of sexual assault should be carried out with great sensitivity and ideally with the direct involvement of experienced teams or services.
 - All specimens for forensic evidence should be collected by professionals experienced in these procedures, following established regional/local protocols, to ensure chain of custody tracking requirements are met.
 - When direct referral cannot be made (e.g., in remote areas), every effort should be made to consult with a pediatrician and/or STI expert for guidance on optimal STI specimen collection from victims of sexual abuse/assault.
 - In addition, where available, local and provincial/territorial guidelines should be consulted.
 - Health care professionals have a legal, professional and ethical obligation to report suspected or confirmed cases of child sexual abuse.
 - Refer to your provincial or territorial legislation and guidelines as well as your professional college for guidance.
- Refer to the [Supplementary Statement for the Management and Follow-up of Sexual Abuse in Peripubertal and Prepubertal Children](#) for more information on the management of suspected child sexual abuse.
- Refer to the [Supplementary Statement for the Management and Follow-up of Sexual Assault in Postpubertal Adolescents and Adults](#) for more information on the management of victims of sexual assault.

Genital and extra-genital specimen collection

This section is meant to provide an overview of the possible specimens and tests that can be used for the diagnosis of various STIs. It does not include sensitivity and specificity information. Preferred and acceptable uses for the various tests are detailed in the Diagnosis of specific infections section below.

- Specimen collection from genital and extra-genital sites may be appropriate.
 - Consult your laboratory to determine which specimens have been validated for the various tests.
- Most will be collected and then packaged for delivery to diagnostic laboratories by clinicians. However, some will be self-collected.
 - Self-collected specimens require [appropriate collection](#) and may require laboratory validation.
 - Consult your local laboratory.
- For [medico-legal considerations](#) related to specimen collection refer to the section above.

Bubo aspirates

- Bubo aspirates (e.g., suspected LGV or chancroid) are obtained using a needle and syringe to aspirate pustular material, which should then be placed in a sterile tube and be stored and shipped refrigerated.
 - Consult your laboratory ahead of specimen collection, for shipping instructions.

Cervical specimens

- Depending on the organism of concern, endo- or exocervical samples may be preferred.
 - Using a speculum to view the cervix, remove overlying vaginal secretions and cervical exudate.
- For Pap testing, cells from the squamo-columnar junction of the cervix can be collected using cervical brushes, brooms, spatulae or swabs, and then smeared onto a slide or placed into a liquid-based (LB) container.
- For [HPV testing](#), samples may be tested out of the LB container or an approved commercial transport tube.
 - Consult with your laboratory to determine HPV tests used and approved samples.

Note:

- Cervical specimens should not be taken from prepubertal girls, since in this age group, STIs involve the vagina, not the cervix. For more information, refer to the [Supplementary Statement for the Management and Follow-up of Sexual Abuse in Peripubertal and Prepubertal Children](#).

Endocervical samples

- Collect endocervical samples for the diagnosis of [C. trachomatis](#) and [N. gonorrhoeae](#)^c. Cervical swabs are appropriate for the detection of [M. genitalium](#).
 - Insert a sterile swab 1–2 cm into the endocervical canal, rotate 180° and withdraw for collection of columnar epithelial cells.
 - Gram stain smears from cervical swabs have little value in detecting *N. gonorrhoea* by microscopy and are not routinely recommended.
 - If a culture is to be performed for *N. gonorrhoeae*, directly inoculate the culture tube or plate, or place the swab into the transport medium.
 - Place a separate non-culture swab into a nucleic acid amplification transport tube.
- In women who have had a hysterectomy, collect [first-void urine](#) (FVU) for NAAT or a [vaginal swab](#) for culture or NAAT.

Exocervical samples

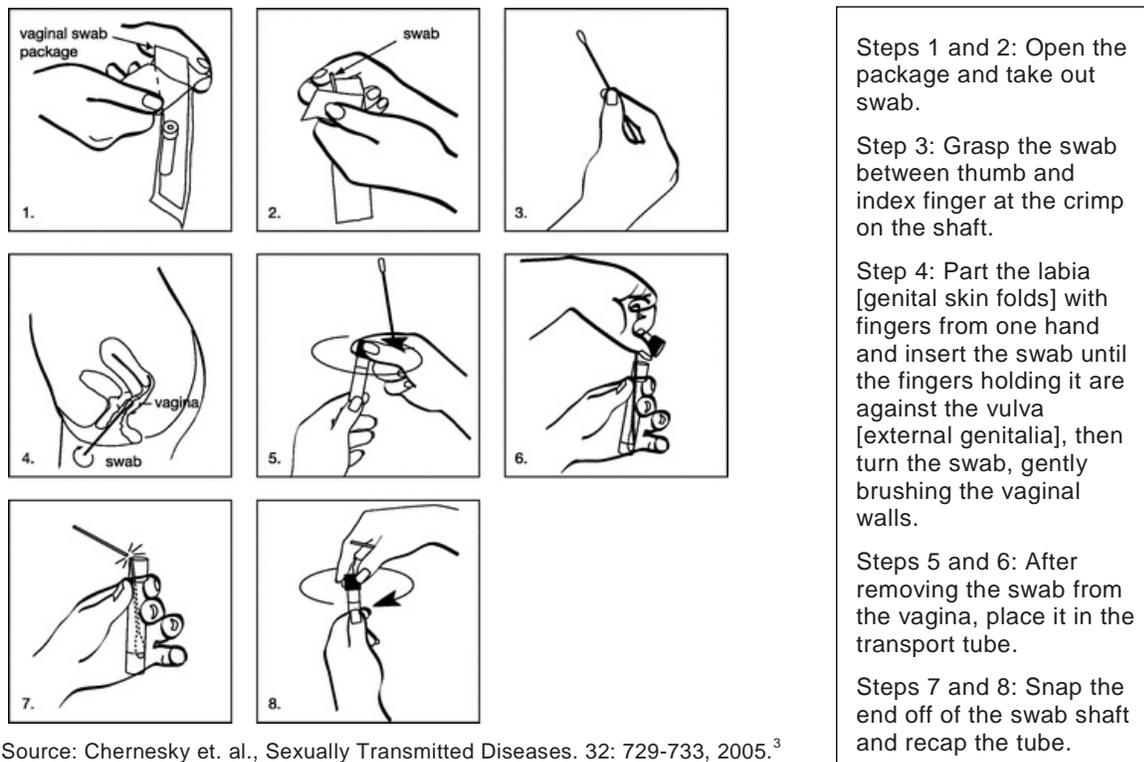
- Collect exocervical samples for the diagnosis of [herpes simplex virus](#) (HSV) and [human papillomavirus](#) (HPV), using approved collection devices and related transportation systems.

^c Depending on the [clinical situation](#), consideration should be given to using both culture and NAAT, especially in symptomatic patients.

Vaginal specimens

- Vaginal swabs can be collected with or without a speculum examination and are acceptable samples for the detection of [C. trachomatis](#), [N. gonorrhoeae](#)^d, [T. vaginalis](#) and [M. genitalium](#) using NAATs.
- Wet-mount and Gram-stain smears may be useful in the diagnosis of vaginitis, [candidiasis](#), [bacterial vaginosis](#), and [trichomoniasis](#).
- Refer to the [Diagnosis of specific infections](#) section for information on interpretation of results.
- Self-collected vaginal swabs collected in a clinical setting are an option when a pelvic exam is not warranted or is declined.³⁻⁶
 - Instructions for self-collection should be provided to the patient as indicated in *Figure 1* below.
 - Diagnostic tests that have not been approved by Health Canada for self-collected vaginal swabs collected in a clinical setting should be validated in the laboratory to determine performance specifications.¹

Figure 1 – Diagram for self-collected vaginal sampling



Source: Chernesky et. al., Sexually Transmitted Diseases. 32: 729-733, 2005.³

^d Depending on the [clinical situation](#), consideration should be given to using both culture and NAAT, especially in symptomatic patients.

Urine specimens (first-void)

- First-void urine (FVU, also called first-catch urine or FCU) to test for [C. trachomatis](#) and [N. gonorrhoeae](#)^e using NAAT is preferred over a urethral sample in asymptomatic men.
 - FVU specimens can also be tested for [M. genitalium](#) and [T. vaginalis](#) using NAAT.
- Urine NAATs are also acceptable specimens for:
 - women without a cervix;
 - women when a pelvic exam is not warranted or is declined;
 - [children](#).
- Ask adult patients to collect *only* the first 10–20 mL of urine (not mid-stream) into an approved leak-proof container and to cap it tightly.⁷
 - Collecting a void in excess of 10-20 mL dilutes the sample and may decrease the ability to detect analyte.
 - Ideally, the patient should not have voided for at least 2 hours prior to specimen collection; however, having voided more recently does not preclude testing.^{8,9}

Urethral specimens

- A urethral swab (in symptomatic men only) may be collected for culture and/or Gram stain for *N. gonorrhoeae*^f, or a NAAT for [N. gonorrhoeae](#) and [C. trachomatis](#).
 - Ideally, the patient should not have voided for at least 2 hours, as voiding may reduce the concentration of analyte in the collected sample.
 - Cultures obtained less than 48 hours after exposure may give negative results.
 - If a NAAT is used, follow manufacturer instructions.
 - Post-exposure NAAT can be done at the time of presentation without waiting for 48 hours. This is based on expert opinion, which assumes that NAATs are able to detect inoculum containing nucleic acid.
 - [T. vaginalis](#) and [M. genitalium](#) can also be detected from urethral swabs in symptomatic men, if clinically indicated.
- Use a thin swab with a flexible shaft. Moistening the swab with sterile water before insertion may help reduce discomfort.
 - Introduce the swab slowly according to the manufacturer's directions regarding depth of insertion and rotate slowly and withdraw gently.
 - The swab from symptomatic males can be used to prepare a smear for *N. gonorrhoeae* Gram staining by rolling the secretions onto a slide. Then directly inoculate the appropriate culture medium or place it in a transport medium.

^e Depending on the [clinical situation](#), consideration should be given to using both culture and NAAT, especially in symptomatic patients.

^f IBID

Rectal specimens

- Although no products are currently licensed for these specimens in Canada, validated NAATs can be used to detect rectal *N. gonorrhoeae* and *C. trachomatis* infections. Confirmation of positives with culture or a second NAAT should be performed.²
- Specimens for culture or NAAT for the detection of [N. gonorrhoeae](#)^g, [C. trachomatis](#) (*non-LGV and LGV serovars*) may be obtained blindly or through an anoscope.

Anoscopy (preferred for symptomatic patients)

- With an unlubricated anoscope using only tap water (to reduce discomfort), fecal contamination can be avoided and specimens can be collected under direct visualization.
- For patients presenting with rectal lesions, refer to the [Lesions](#) section below.

Blind swabbing

- Insert swab 2–3 cm into the anal canal, press laterally to avoid fecal material and, in the case of *C. trachomatis* or *N. gonorrhoeae*, to obtain columnar epithelial cells.
- If there is visible fecal contamination, discard the swab and obtain another specimen.

HPV testing and anal Paps

- For anal warts, no specific testing is recommended to verify the presence or type of HPV as this will not alter management.
- Anal Pap and/or HPV testing may be of value to identify precancerous anal intraepithelial neoplasia (AIN) in high-risk groups.
 - Currently, there is no consensus about the use of anal Pap and high-resolution anoscopy for screening those at increased risk of anal cancer.
- For comprehensive information related to HPV risk factors and screening for anal cancer, refer to the Human Papillomavirus Infections chapter.

Pharyngeal specimens

- Although no products are currently licensed for these specimens in Canada, validated NAATs can be used to detect oropharyngeal *N. gonorrhoeae* and *C. trachomatis* infections. Confirmation of positives with culture or a second NAAT should be performed.²
- Swab the posterior pharynx and the tonsillar crypts for culture or NAAT for the detection of [N. gonorrhoeae](#)^h and [C. trachomatis](#).
 - Use the swab to directly inoculate the appropriate culture medium, or place it in a transport medium.
- Gram stain of smears from pharyngeal swabs is of no value in detecting pharyngeal *N. gonorrhoeae* by microscopy and is not recommended.
- For patients presenting with oral lesions, refer to the *Lesions* section below.

^g Depending on the [clinical situation](#), consideration should be given to using both culture and NAAT, especially in symptomatic patients.

^h IBID

Lesions (vesicles and ulcers)

Herpes

- Obtain fluid for culture or NAAT for the detection of HSV by removing or de-roofing/lifting the top of the vesicle (e.g., with a needle) and swabbing the lesion to collect epithelial cells (and liquid) onto the swab.
- For ulcers, gently scrape the base of the lesion to obtain a specimen for culture or NAAT.
- Other methods, such as antigen detection and Tzanck smear cytology lack accuracy and should not be used.
- For further information on HSV testing refer to the [Herpes simplex virus](#) section below.

Syphilis

- NAATs can be used as a non-serological method for identifying *T. pallidum* in mucosa and skin involve. They are very sensitive and specific.^{10,11}
- When genital lesions characteristic of early syphilis are present, clear serous fluid may be collected for dark-field microscopy, enabling observation of morphology and movement of the spirochetes for the detection of *T. pallidum* (not reliable for oral or rectal lesions).¹²
- Contact your laboratory to determine the availability of dark-field microscopy or NAAT testing.

If dark-field microscopy is available, collect the specimen as follows.

- Remove scabs or overlying debris.
- Cleanse the lesion with sterile saline without preservatives and dry the area.
- Abrade the lesion with a dry sterile gauze pad to provoke slight bleeding and exudation of tissue fluid.
- As oozing occurs, wipe away the first few drops and await the appearance of relatively clear serous exudate. It is sometimes necessary to apply pressure at the base of the lesion to express tissue fluid.
- Collect fluid into a capillary tube or directly onto a slide.
- Store and ship to the laboratory at room (ambient) temperature within 24 hours.
- For information on syphilis serology refer to the *Diagnosis of specific infections* section under [Treponema Pallidum](#) in this chapter, and to the [Syphilis](#) chapter.

Chancroid

- Specimens of choice for the detection of *Haemophilus ducreyi* are taken from the base of the ulcer using a calcium alginate or cotton swab, or an aspirate if [buboes](#) are present.
- Where available, culture is the current method of choice, using two media in a biplate.¹³
 - Consult with your local laboratory for more information.
- NAAT detection of *H. ducreyi* is not commercially available but is available at the National Microbiology Laboratory (NML). Estimated turn-around time for results is 14 days. Refer to the NML [Guide to Services](#) for more information.

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- Cleanse the area by flushing with sterile saline and then collect material from the base of the ulcer using a Dacron or cotton swab.
- Swabs can be shipped dry or in a Universal Transport tube containing 1 mL of fluid.
- For detailed information related to the epidemiology and management of chancroid, refer to the [full chapter](#).

Diagnosis of specific infections

Patients presenting with cervicitis, vulvovaginitis, urethritis, pharyngitis or proctitis

Chlamydia trachomatis (non-LGV and LGV serovars)

- NAATs and, to a lesser extent, culture and serology are used for diagnostic purposes.
- Culture is the preferred method for medico-legal purposes.
 - NAATs may provide valuable adjunctive testing. For such purposes, positive NAAT findings should be confirmed.^{2,14}
 - Consult with your local laboratory regarding the availability of such testing and refer to the [Medico-legal considerations](#) section for information on confirming positive NAAT test results.
- Serology is not recommended for the diagnosis of non-LGV and LGV genital chlamydia infections, given cross-reactions with other chlamydia species, and difficulties interpreting variations in titres.
- Routine tests for *C. trachomatis* may be positive in patients with LGV, but generally do not include typing to distinguish LGV serovars from non-LGV serovars.
- Definitive diagnosis of LGV requires serovar-specific testing (i.e., genotyping).
 - Refer to the [Lymphogranuloma Venereum Supplementary Statement](#) for more information on laboratory diagnostics and genotyping for positive chlamydia results.

NAAT

- NAATs are the most sensitive and specific tests.
- The recommended sample for men is a [first-void urine](#) (FVU).²
- [Vaginal swabs](#) or urine can be used for NAATs, making testing more acceptable to women.
 - Data show that NAATs for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* may identify as many or more infected women using vaginal swabs than cervical swabs, urethral swabs or urine.¹⁵
 - [Cervical swabs](#) can also be submitted.
- There are promising data on the performance of some NAATs using pharyngeal and rectal swabs.²
 - Consult your local laboratory and refer to the [Genital and extra-genital specimen collection](#) section above for specific information on the use of validated NAATs for pharyngeal and rectal specimens.
- NAAT may be done at the time of presentation without individuals having to wait 48 hours post-exposure. This is based on expert opinion, which assumes that NAAT is able to detect small amounts of DNA or RNA.

Culture

- [Anogenital and oropharyngeal specimens](#) can be submitted.
- In patients with conjunctivitis, a conjunctival swab can be submitted.
- Nasopharyngeal aspirate can be submitted in infants <6 months of age.
- Consult with your local laboratory for guidance on specimen collection.

Serology

- *C. trachomatis* IgM serology is useful for diagnosing *C. trachomatis* pneumonia in infants less than 3 months of age—that is, infants who may have been exposed to chlamydia during the perinatal period.¹⁶

Neisseria gonorrhoeae

- Due to the higher sensitivity of the most recently approved commercial NAATs, they can increase the number of cases diagnosed.^{2,17} However, culture allows for testing of antimicrobial susceptibility. Therefore, depending on the [clinical situation](#), consideration should be given to using both culture and NAAT, especially in symptomatic patients.²
 - NAATs may be the only available testing method in some laboratories.
- For medico-legal purposes, positive NAAT findings should be confirmed.^{2,14}
 - Consult with your local laboratory regarding the availability of such testing and refer to the [Medico-legal considerations](#) section for information on confirming positive NAAT test results.

NAAT

- NAATs are the most sensitive tests.
- If there is a clinical reason to question a positive result repeat testing should be considered.²
- Some of the NAATs may generate false positive results due to possible cross-reaction with other *Neisseria* species.
 - If false positivity is suspected, the original specimen could be confirmed with a second (i.e., different) NAAT.
 - Consult with your laboratory for guidance.
- The recommended sample for asymptomatic men is a [first-void urine](#) (FVU).²
- [Vaginal swabs](#) or urine can be used for NAATs, making testing more acceptable to women.
 - Data show that NAATs for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* using vaginal swabs may identify as many or more infected women than cervical swabs, urethral swabs or urine.¹⁵
 - [Cervical swabs](#) can also be submitted.
- NAAT may be done at the time of presentation without individuals having to wait 48 hours post-exposure. This is based on expert opinion, which assumes that NAAT is able to detect small amounts of DNA or RNA.
- There are promising data on the performance of some NAATs using pharyngeal and rectal swabs.²

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- Consult your local laboratory and refer to the [Genital and extra-genital specimen collection](#) section above for specific information on the use of validated NAATs for pharyngeal and rectal specimens.

Culture

- Successful culture of specimens requires proper collection and transportation of appropriate specimens, or immediate inoculation of medium.¹⁸ Consult with your local laboratory.
- Cultures obtained less than 48 hours after exposure, may give negative results.
- Refer to the *Gonococcal Infections* chapter for a list of situations in which [test of cure](#) is recommended.
 - NAATs may be the only available testing method in some laboratories.

Smears

- The presence of Gram-negative diplococci inside polymorphonuclear leukocytes (PMNs) seen on direct microscopic examination of smears is highly predictive of *N. gonorrhoeae* in symptomatic males.¹⁸
- The sensitivity and specificity of the Gram stain depends on the type of specimen.¹⁸
 - Urethral specimens from symptomatic young adult males have a sensitivity and specificity of 95%.
 - Endocervical specimens from adult females have a sensitivity of 45–65% and a specificity of 90%; and as such are not routinely recommended.
 - Gram stain smears are not suitable for oropharyngeal and rectal specimens, and should not be used.

Trichomonas vaginalis

- *T. vaginalis* infections are often asymptomatic, but may cause urethritis in men and vulvo-vaginitis in women. In women, the vaginal pH is elevated (>4.5).¹⁹
- Commercial NAATs for *T. vaginalis* performed on vaginal swabs, cervical swabs or urine are the most sensitive and specific assays²⁰⁻²² (consult your local laboratory regarding their availability).
 - Wet mount microscopy, antigen detection, and nucleic acid hybridization assays are also available, but are less sensitive.²³⁻²⁵

Mycoplasma genitalium

- *M. genitalium* is emerging as a cause of persistent non-gonococcal/non-chlamydial urethritis in men and may be associated with cervicitis, urethritis, endometritis, pelvic inflammatory disease and infertility in women.^{26,27}
- Although the ideal genital specimen type for the detection of *M. genitalium* has not been thoroughly assessed, appropriate specimens are:
 - FVU (first 10-20 mL), urethral or penile meatal swabs from men;
 - FVU (first 10-20 mL), cervical or vaginal swabs from women.
- Laboratory derived and commercially available research-use-only (RUO) or analyte-specific reagent (ASR) NAATs are effective in detecting *M. genitalium* in vaginal, cervical and urethral swabs, and urine.

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- Laboratories need to perform validation studies to determine performance specifications if they are to be used for diagnosis.¹
- Consult with your local laboratory concerning the availability of *M. genitalium* testing.
- In the absence of validated tests, testing is available through the National Microbiology Laboratory (NML). Refer to the NML [Guide to Services](#) for more information on specimen collection and transportation requirements.
- Culture is impractical and of little diagnostic value.

Candida albicans

- The vaginal pH is normal (<4.5), and the Whiff test is negative (an amine [fishy] odour is *not* emitted when a drop of potassium hydroxide is mixed with a vaginal discharge on a slide).²⁸
 - Note: vaginal pH is not a reliable indicator in post-menopausal women.
- Gram stain and wet-mount preparations can be used to identify budding yeast and branching pseudohyphae.¹¹
 - For symptomatic women vaginal cultures are a consideration.
 - Cultures are not useful for asymptomatic women because *Candida* species may be part of the normal vaginal microbiota.
 - A culture for identification and susceptibilities should be done in women with a positive culture or wet mount who still have symptoms after therapy.

Bacterial vaginosis (BV)

- The vaginal pH is elevated (>4.5), and the whiff test is positive (an amine [fishy] odour is present).^{28,29}
- The Gram stain demonstrates a shift in vaginal flora,^{30,31} with a decrease in large Gram-positive rods (lactobacilli) and an increase in small Gram-variable coccobacilli and clue cells (vaginal epithelial cells covered with numerous coccobacilli).

Patients presenting with ulcers or lesions

Herpes simplex virus (HSV)

- NAATs approach sensitivities and specificities of 100%, with rapid turn-around of results.
- Cultures from primary or recurrent genital herpes lesions are easy to perform and can yield positive results within 24 hours when transported in appropriate media.
- Screening for HSV-1 and HSV-2 in asymptomatic patients is not indicated.
- The use of HSV type-specific serology in Canada is currently limited. Laboratory diagnostics vary across the country.
 - Consult with your local laboratory regarding test availability.
- Where available, and when it is not possible to make a diagnosis of HSV using NAATs or culture, antibody assays may be particularly useful in two clinical situations.³²⁻³⁴
 1. When patients have typical or atypical symptoms of genital herpes but negative cultures or NAATs.
 - Detection of HSV-2 antibodies is useful to support a diagnosis of genital HSV infection.

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- It is important to note that detection of HSV-1 antibodies cannot differentiate between an oral and/or genital infection.
- 2. When a first episode of genital HSV is identified during pregnancy.
 - The presence or absence of type-specific antibodies can help to determine whether the infection is new or recurrent.
 - Type-specific serology should be repeated in patients who test antibody negative to monitor for the development of antibodies prior to delivery. This is important, given the higher risk of mother-to-child transmission in a seronegative mother.
- As care must be taken in the interpretation of results,³⁵ consult with your local laboratory.
- For neonates, obtain a specimen for culture by gently rubbing the conjunctiva with a swab. Use separate swab for the mouth and lips, external ear canal, umbilicus, axillae and groin.

Human Papillomavirus (HPV)

- There are at least 14 high-risk (HR) HPV genotypes associated to cancer, and numerous other low-risk (LR) genotypes causing anogenital warts (AGWs).
 - Visual inspection is the usual means of diagnosing AGWs and laboratory testing is not recommended.
- Evidence suggests that HR HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, are carcinogenic and genotypes 66 and 68 are probably carcinogenic.
 - Persistent infection with high-risk HPV types can cause cell abnormalities, which if left undetected and untreated, may lead to precancerous or cancerous lesions.
 - For more information on HPV genotypes and related cancer risk, refer to [Appendix A](#) of the *Human papillomavirus (HPV) Infections* chapter.
- Several DNA and RNA tests are approved in Canada to detect groups of HR HPV genotypes or specific genotypes such as 16, 18 and/or 45, using liquid-based Pap or specimen transport media.
 - Consult with your laboratory concerning the test used and approved samples.
- The sensitivity and specificity of HPV assays are determined by their ability to detect (predict) precancerous lesions, not the presence of infection.
- The presence of HR HPV in patients with abnormal Pap smears may enable recommendations for follow-up and colposcopy.³⁶
- Sequencing and serology are used for epidemiological purposes and are not helpful in guiding patient management.
- Consult with your local laboratory concerning HPV testing, as few laboratories are currently providing this service in Canada.

Treponema pallidum (syphilis)

- Two types of serologic screening algorithms are currently available in Canada.³⁷
 - The first is a traditional algorithm using a non-treponemal test (such as RPR [rapid plasma reagin] or VDRL [venereal disease research laboratory]) to screen sera, followed by one or two treponemal tests on the positive samples.
 - The other approach is to use a reverse sequence algorithm, which uses a treponemal test to screen and a quantitative non-treponemal test to confirm the positives. A second confirmatory treponemal test may be used in some laboratories.

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- Several commercial immunoassays (IA) have been developed to detect IgG or IgM to specific *T. pallidum* antigens. Tests such as the treponemal-specific enzyme immunoassay (EIA) may provide a more sensitive screening test for syphilis.
 - Although IA is highly sensitive, the test can lack specificity; therefore if the treponemal-specific EIA is positive, a second treponemal-specific test (e.g., TP-PA, MHA-TP, FTA-ABS, INNO-LIA™) can be used as a supplementary test to confirm the diagnosis.
 - Refer to the Syphilis chapter for more information on cerebrospinal fluid examination, comprehensive recommendations on the management of syphilis in pregnant women and for guidance on interpretation of serologic test results.

Bloodborne infections

Hepatitis A virus (HAV)

- The presence of HAV IgM antibodies is diagnostic of acute infection.
 - HAV IgM may persist for 3 to 6 months.³⁸
- HAV IgG antibody testing can demonstrate immunity.
 - Refer to the [Canadian Immunization Guide, Part 4, Active Vaccines, Hepatitis A Vaccine](#) for testing and vaccination recommendations.

Hepatitis B virus (HBV)

- Patients acutely infected with HBV will have positive EIA results for hepatitis B surface antigen (HBsAg) and/or anti-hepatitis B core (anti-HBc) IgM.
- Most patients (90%) develop immunity within 6 months of infection, lose their HBsAg and have it replaced by anti-HBc IgG and anti-hepatitis B surface antibodies (anti-HBs).³⁹
- Persistence of HBsAg for 6 months or more is indicative of a chronic infection.
- The presence of hepatitis B e antigen (HBeAg) or high viral load of HBV DNA in acutely or chronically infected individuals indicates greater infectivity for contacts and for babies born to positive mothers⁴⁰: e antigens may eventually be replaced by antibodies (anti-HBe).
- For additional information, refer to the [Primary Care Management of Hepatitis B-Quick Reference](#).

Hepatitis C virus (HCV)

- For information on screening, follow-up testing, treatment and monitoring of individuals with a positive anti-HCV test result refer to :
 - the Primary Care Management of Chronic Hepatitis C: Professional Desk Reference 2009;
 - An update on the management of chronic hepatitis C: 2015 Consensus guidelines from the Canadian Association for the Study of the Liver.

Human immunodeficiency virus (HIV)

- There are many different types of HIV screening tests that are licensed for use in Canada; type and availability can vary by jurisdiction.
- The detection of HIV antibody is the most widely used means of diagnosing HIV.

Antibody detection tests

- Sera are initially screened for antibodies against HIV1 and HIV2 by enzyme immunoassay (EIA). All positive EIAs should be confirmed using a different EIA, Western blot or an RNA assay.
 - 3rd generation HIV EIA tests are able to detect antibody at 20 to 30 days after exposure.
 - 4th generation combination tests permit the detection of p24 antigen during the acute phase of infection and reduce the window period to 15 to 20 days.⁴¹
- Currently, one rapid HIV test is licensed for use as a point-of-care (POC) device in Canada. It allows for an accurate presumptive diagnosis within 60 seconds.
 - Positive POC test results require laboratory confirmation.
- Note that Health Canada requires that rapid test kits only be used in settings where pre- and post-test HIV counselling is available.⁴²
- Each laboratory develops and validates its own algorithm for confirmatory testing to ensure that it provides the most accurate results possible.
- Consult with your local laboratory for more information.

HIV viral detection tests (NAAT)

- HIV infection can also be diagnosed by detecting the presence of the virus itself.
 - Qualitative NAAT is used to detect small amounts of nucleic acid in babies born to HIV-infected mothers, for individuals who may still be in the window period, and for those with advanced disease or marked compromised immunity.
 - Quantitative NAAT (viral load testing)^{41,43} is used to monitor HIV-positive patients prior to and during antiretroviral therapy.
 - Genotyping, phenotyping and serum levels of antiretrovirals are used to detect drug resistance, enabling appropriate antiretroviral drug combinations and adjustment of dosage if required.⁴⁴

For more information on HIV tests, their sensitivity and specificity, as well as interpretation of results, refer to the [Human Immunodeficiency Virus: HIV Screening and Testing Guide](#)

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