



Laboratory Tests for Sexually Transmitted Infections --- A National Perspective.

One mandate of the National Microbiology Laboratory is to participate in surveillance of communicable diseases.

Effective communicable disease control depends on effective disease surveillance.

Surveillance is a tool that can facilitate the prevention of infection and the amelioration of its immediate and long term effects by providing the necessary information for action.

Source: WHO Recommended Surveillance Standards, 2nd edition
(WHO/CDS/CSR/ISR/99.2)



National Surveillance of STI: (National Notifiable Diseases)

Acquired Immunodeficiency Syndrome (AIDS)

Human Immunodeficiency Virus (HIV)

Gonorrhea

Syphilis

Chlamydia genital infection

Source: PHAC website on Notifiable Diseases
(<http://www.phac-aspc.gc.ca/id-mi/index.html>)





Goals of the National Surveillance System:

A) To facilitate the control of diseases under surveillance by identifying

1. Prevailing incidence levels, impacts and trends to assist the development of objectives for prevention and control as well as evaluation of control programs;
2. Epidemiologic patterns and risk factors associated with diseases to assist development of intervention programs;
3. Outbreaks for timely investigation and control.

B) To provide information for government, health care professionals, voluntary agencies, and the public on risk patterns and trends in communicable disease.



Goals of the National Surveillance System:

C) To monitor progress towards national and international control / eradication targets.

Sources:

- 1) Canada Communicable Disease Report, Volume 16, Supplement S3 (May 2000).
- 2) WHO Recommended Surveillance Standards, 2nd edition (WHO/CDS/CSR/ISR/99.2)





Surveillance of infectious diseases requires clinical data and **laboratory data**.

Quality of the surveillance data will depend on many factors, and one of these is **accurate diagnosis of the disease**.

Laboratory data allows confirmation of the clinical diagnosis and also provides the opportunity for more detailed characterization of the causative micro-organism. Such characterization, through speciation, grouping, typing (including molecular typing) assists in the more precise analysis of clusters of disease.



Laboratory data

Information on antimicrobial susceptibility is of value in determining the most appropriate treatment for both individuals and groups who have a particular clinical symptoms.

Source: Surveillance standards for antimicrobial resistance.
(WHO/CDS/CSR/DRS/2001.5)





References:

- 1) The Canadian STI Best Practice Laboratory Guidelines were published in the Jan/Feb and Mar/Apr 2005 issues of the Canadian Journal of Infectious Diseases and Medical Microbiology, volume 16 (issues 1 and 2).
- 2) A Recommendation Report on “Screening Tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae*” published by the US CDC in Morbidity and Mortality Weekly Report, Oct. 18, 2002, volume 51 (RR-15).
- 3) A review on “Nucleic Acid Amplification Testing for *Neisseria gonorrhoeae*: an ongoing challenge” in Feb. 2006 Journal of Molecular Diagnostics, volume 8: pp. 3-15.



Laboratory issues and challenges for Syphilis

- a) **EIA** using recombinant *T. pallidum* proteins as antigens;
- b) **Molecular diagnostics:**

EIA

- 1) The most reliable method for lab diagnosis of syphilis, regardless of the stage of infection, is still serology.
- 2) With the introduction of EIA and recombinant DNA technology, the conventional 2-step approach of screening with non-treponemal test followed by a treponemal-specific confirmation test is being challenged ?





One school advocates the use of EIA with treponemal antigens as a screening test to be confirmed by another test (preferably using a Different technology or method but with equal or higher specificity).

The other school recommends the traditional approach of using non-treponemal tests such as VDRL and RPR for screening followed by a treponemal antigen confirmation test such as TP-PA or FTA-ABS.

Some issues concerning the use of EIA with recombinant Ags:

- 1) Evaluations of specificity of some of the EIA tests were done using serum from normal blood donors. When applied to a patient population, the specificity rate may change.



2. Although it has been reported that using multiple *T. pallidum* recombinant proteins as antigens in EIA contributes to increase sensitivity, it is equally possible the increase in sensitivity may come at the expense of specificity if one or more of the antigens used is not specific.

3. One researcher cautioned that further tests to gather more data on specificity should be done before implementing EIA for syphilis routinely in all setting.

4. Nevertheless, there are definite merits of using EIA with *T. pallidum* recombinant antigens in the following settings:





- a) Psychiatric patients because of greater ability to detect late stage syphilis with EIA.
- b) Patients with HIV since HIV infection may reduce or delay the antibody response detected in conventional tests in primary syphilis.
- c) Screening in blood banking because of sensitivity and specificity and the ability for automation and objective results reading.



Syphilis Molecular Diagnostics offers unique opportunities for

- a) Early and rapid diagnosis

In incubating syphilis, all antibody tests are negative.

Diagnosis of syphilis at this stage as well as at the very early acute phase is best accomplished by direct detection of either the organism (by various types of microscopy) or its DNA (by PCR).

Currently there is no licensed PCR test for diagnosis of syphilis. Nevertheless, there have been many experimental or home grown PCR assays described, and some of the common targets include the *bmp*, *tpp47*, and *polA* genes.





- a) Molecular typing and strain identification for outbreak analysis
Based on detection / amplification and characterization of two genes exhibiting intrastain variability or the so-called variable genes: the *arp* gene which encodes for a putative acidic repeat protein and the treponema pallidum repeat gene, *tpr*.
- b) Detection of azithromycin resistance:
Based on detection and characterization of a segment of the 23S rRNA gene important for macrolide antibiotics susceptibility.



Limitations of these Syphilis Molecular Diagnostics:

- 1) Specimen collection: must contain either the *T. pallidum* or its DNA.
- 2) Sensitivity of the method, esp. those related to molecular typing and antibiotics susceptibility testing, depends on the amount of DNA present in the specimens as well as inhibitory substances that can interfere with the amplification reaction.





Laboratory issues and challenges for detection and characterization of *Neisseria gonorrhoeae*.

Lab methods:

- a) Culture, identification, and subsequent strain characterization
- b) Nucleic Acid Amplification Tests (NAATs)
- c) Nucleic Acid hybridization (Nucleic acid probe) tests
- d) Point-of-Care tests: Gram smear



Culture / Identification

- a) Isolates sensitive to antibiotics in the selective culture medium.
- b) Although not as sensitive as NAATs, culture is the method of choice for pharyngeal and rectal specimens as well as for diagnosis of gonococcal conjunctivitis in neonates.
- c) Bacterial culture should be used for specimens obtained from individuals suspected of child / sexual abuse.
- d) The most current recommendation for definitive identification of Gram-negative oxidase-positive cocci (including *N. gonorrhoeae*) is a combination of carbohydrate utilization test, preformed enzyme detection, and serological or nucleic acid based methods.





- e. Global transmission of prolyliminopeptidase (PIP)-negative *Neisseria gonorrhoeae* strains – implications for changes in diagnostic strategies ?

Reference: August 2006 issue of Sexually Transmitted Infection.

As a result of the prevalence of Pip-negative *N. gonorrhoeae* in the UK, a medical device alert was issued in Sept. 2005 by the regulatory agency in the UK to warn the possibility of false negative or ambiguous results on using biochemical test kits for the identification of *N. gonorrhoeae*.



Nucleic Acid Amplification Tests (NAATs)

Advantages:

a) sensitive; b) rapid; c) non-invasive and self taken specimens;

Disadvantages:

a) expensive; b) complex; c) inhibitory substance in specimens can lead to false-negative results; d) contamination can lead to false positive results; e) sequence variation of target gene in test organism can lead to false-negative results; f) cross-reactions with other *Neisseria* species or other bacteria can lead to false positive results; g) not recommend for testing of pharyngeal and rectal specimens; h) not recommend for tests involved in child / sexual abuse and medicolegal cases





Issues of NAATs for *N. gonorrhoeae*

- i. Inhibitory substances for amplification Rx in clinical samples
Most commercial NAATs have internal control reaction to identify inhibition of amplification reaction.
- ii. Assay complexity and quality control / proficiency testing
Errors (human errors, reagent failure, carryover contamination) may be introduced into the assay at any one of 3 steps: nucleic acid extraction, amplification, and detection.
- iii. Resource requirement for set up and performing the test.
Separate rooms, equipments, and lab wares for the 3 step.
- iv. Sequence variation between *N. gonorrhoeae* subtypes.
Specimens that are culture positive and negative by NAATs are commonly reported.



- v. Cross-reactions with other *Neisseria* species.
Sequence homology between *N. gonorrhoeae* and *N. meningitidis*.
Frequent interspecies horizontal genetic exchange within *Neisseria* genus and possibly even with other bacteria.
This affects bacterial identification scheme.
- vi. Test of cure: culture test should be performed at least 72 hours after completion of antimicrobial therapy, and NAATs at least 2 or 3 weeks after therapy.
(UK National Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults 2005.)





The National Microbiology Laboratory's Reference / Diagnostic Service for Chlamydia infections.

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Chlamydia trachomatis serovars A, B, Ba, and C are associated with endemic trachoma (rarely encountered in Canada).

Serovars L1, L2, and L3 are associated with lymphogranuloma venereum (LGV). Extra LGV surveillance has been put in place in response to an increase in cases in Canada in 2005.

Serovars D through K are responsible for nongonococcal urethritis and a variety of other syndromes in men and women.





Diagnostic Methods

- 1) Direct cytological examination
- 2) Isolation in cell culture
- 3) Antigen detection
- 4) Nucleic acid hybridization test
- 5) Nucleic acid amplification test (NAAT)
- 6) Serology

For details of these methods, please consult the reference:
The laboratory diagnosis of *Chlamydia trachomatis* infections.
Max Chernesky. 2005 Can J Infect Dis Med Microbiol
Volume 16: pp. 39-44.



Issues with detection and identification of *C. trachomatis*

- 1) NAATs are sensitive, specific, commercially available method, can use non-invasive and self-collected specimens like urine or vaginal swabs.
- 2) Although commercial NAATs are not approved for use on rectal samples, they will detect all *C. trachomatis* serovars, including L1 to L3 strains that cause LGV. Use of 2 or more different NAAT approaches targeting to different sequences is recommended to corroborate a positive test result.
- 3) A NML in-house PCR-based detection system is used to detect LGV serovars.





- 4) Serology not recommended for diagnosis of chlamydial infections except for infection in neonates (high IgM), patients with tubal factor infertility (high IgG), and occasionally for LGV infection when culture is not possible or for retrospective diagnosis.
- 5). In sexual assault or abuse cases, culture is the recommended method for detecting *C. trachomatis* in urogenital, pharyngeal, and rectal specimens. Only *C. trachomatis*-specific antibody (e.g. monoclonal antibody to MOMP) should be used to identify the intracytoplasmic inclusions. If NAAT has to be used, 2 or more NAATs targeting to different sequences should be used.
- 6). Test of cure is not routinely done if recommended treatment is followed, with the exception in pregnant women and prepubertal children. And if required, should be done no less than 3 weeks after completion of antimicrobial therapy.



Laboratory Diagnostic Services available at the NML

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- 1) Three NAAT approaches are used at NML:
 - Roche Amplikor PCR test
 - An in-house PCR assay to detect *C. trachomatis*-specific 16S rRNA sequence
 - An in-house PCR assay that detects the *omp1* gene





- 2) Serology by microimmunofluorescent test that detects antibodies to non-LGV serovars of *C. trachomatis* as well as an in-house MIF test that detects antibodies to LGV serovars. Tests that measure IgM- and IgG-specific antibodies are available.
(consult NML for specimen rejection criteria !).

- 3) NAATs for *C. trachomatis* serovars A to C associated with trachoma.

