

Update on RTI/STI Diagnostics

Definitions

Antibody - substances the body produces in response to an infectious organisms

Antigen - the organism itself

False negatives - people who test negative but are actually infected (missed infections)

False positives - people who test positive but who are actually not infected

Sensitivity

- how good a test is at identifying people who are infected
- the higher the sensitivity, the lower the rate of false negatives (missed infections)
- Example: if sensitivity of a test is 95% and 100 infected people are tested, 95 will have positive test results and 5 will have negative test results (even though they are infected)

Specificity

- how good a test is at identifying people who are not infected
- the higher the specificity, the lower the rate of false positives
- Example: if specificity of a test is 95% and 100 people who are not infected are tested, 95 will have negative test results and 5 will have positive test results (even though they are not infected)

Sensitivity & Specificity are used to give an indication of how good a diagnostic test is. Ideally one would like a test that has 100% sensitivity (i.e. everyone who is infected tests positive) and 100% specificity (i.e. everyone who is not infected tests negative).

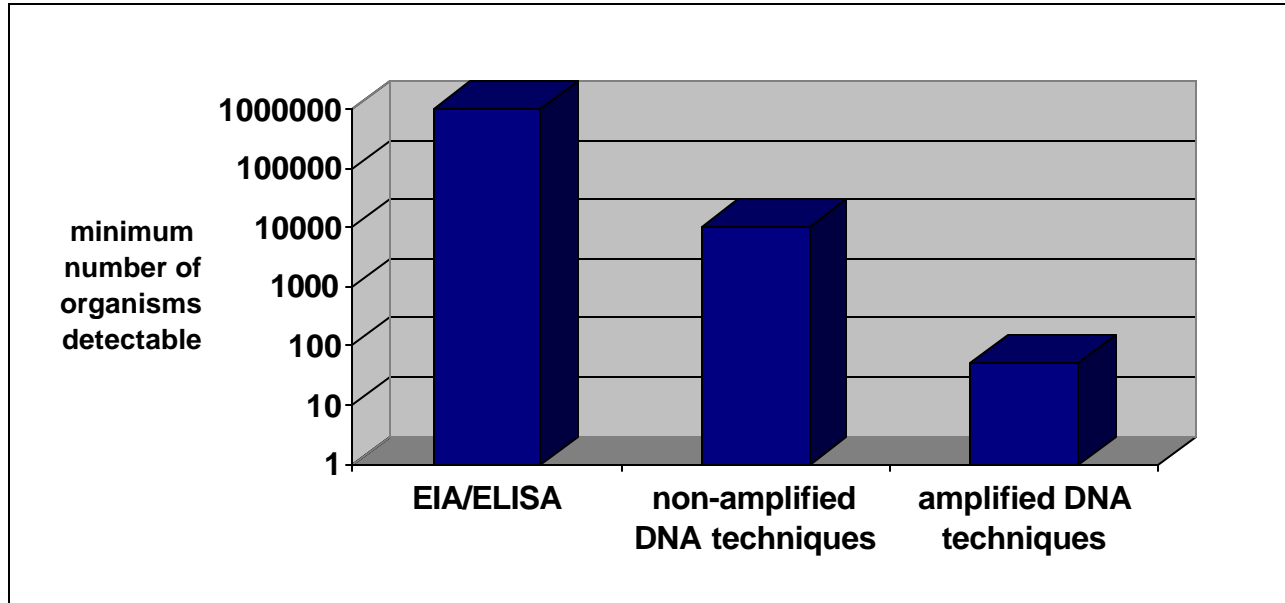
Type of Diagnostic Tests for RTIs and STIs

- A. Directly visualizing the organism under the microscope. Examples: gram staining for gonorrhea, wet mount for yeast, and dark field microscopy for syphilis
- B. Using antibody tests that measure the body's response to the organism. Examples: testing for syphilis or HIV antibodies.
- C. Culturing the organism (i.e., growing it in the laboratory). Examples: culturing chlamydia and gonorrhea.
- D. Methods for detecting antigen (the organism itself). Example: enzyme immunoassay (EIA) for chlamydia.
- E. Non-amplified techniques for detecting DNA from the organism. Example: nucleic acid hybridization.
- F. Amplified techniques for detecting DNA from the organism. Examples: PCR (polymerase chain reaction), LCR (ligase chain reaction) and TMA (transcription mediated amplification).

The minimum number of organisms needed in a sample for a test to be positive varies from one type of test to another. The lower the number of organisms that can be detected, the greater the sensitivity of the test. The new amplified DNA techniques (e.g.,

PCR, LCR) are extremely sensitive and can detect between 1 and 50 organisms in the sample tested. (See Figure 1)

Fig. 1. Sensitivity of Different Types of Diagnostic Tests



Bacterial Vaginosis

caused by anaerobic bacteria, *Gardnerella vaginalis*, and *Mycoplasma hominis*

Diagnosis is based on a combination of findings from clinical exam and laboratory tests. Three of these four criteria indicate bacterial vaginosis:

- vaginal pH > 4.5
- clue cells - squamous epithelial cells which are coated with bacteria (presence of clue cells is the overall best indicator of bacterial vaginosis)
- amine odor after addition of KOH (most specific, but insensitive)
- presence of milky discharge

Vaginal cultures are not recommended for the diagnosis of bacterial vaginosis.

Chancroid

caused by *Hemophilus ducreyi*

Diagnosis is usually made based on clinical signs and ruling out other causes of genital ulcers, in particular syphilis.

Culture

- Sample from genital lesion or lymph node can be used
- Culture for *H ducreyi* is not always a standard test available at diagnostic labs
- The organism can be difficult to isolate and requires selective media.

- It is best to inoculate the culture media immediately after sample collection because no transport system has been developed and the organism does not survive for more than a few hours without refrigeration

Gram stained smear of lesions are not recommended, although gram stained smears of lymph node aspirates may be helpful in diagnosing chancroid if *H ducreyi* is seen (but do not necessarily rule out chancroid if the organism is not seen). Other diagnostic tests have not been well described.

Chlamydia

Caused by *Chlamydia trachomatis*

A. Serology: rarely of value; high background prevalence and infrequent rises and falls in antibodies (both IgG & IgM); may be useful in tissue invasive infections such as perihepatitis, lymphogranuloma venereum (LGV) and PID.

B. Cytology:

- Giemsa staining, detects characteristic reticular bodies (small inclusions in the cytoplasm) within epithelial cells. Little value for urethral or cervical specimens because of low sensitivity and specificity; low numbers of infected cells and the presence of mucus, cell debris and other microorganisms make identification difficult. Provides adequate diagnosis for chlamydial conjunctivitis.
- Papanicolaou staining, identifies chlamydia-induced cervical cell changes. Not recommended for diagnosis of chlamydia due to low specificity. Also, smears are often inadequate.

C. Cell culture: labor intensive; slow turnaround; expensive and difficult; rigorous transport requirement; recently improved with microtiter method and monoclonal antibody staining; still considered gold standard, though varies widely in sensitivity depending on the lab.

D. Antigen detection methods – detect *C trachomatis* organisms themselves; more rapid, less expensive, less technically demanding than tissue culture; less effective with asymptomatic infections than PCR & LCR. Two generally used methods:

- Enzyme immunoassay (EIA) - Microtrak® EIA (produced by Syva) or Chlamydiazyme® (produced by Abbott)
- Direct fluorescent antibody slide test (DFA) - Microtrak® DFA (produced by Syva). Requires a fluorescent microscope.

Rapid antigen detection tests (both use swab as sample & require no instrumentation):

- Optical immunoassay: BioStar® Chlamydia OIA® (Murex Diagnostics Inc.) - ~\$15/test; results in 25 minutes; [sp=100%, sn=83.6%, ppv=100%]
- Direct antigen immunoassay: QuickVue® (Quidel Corporation) - ~\$13.10/test; results in 12-15 minutes

E. DNA probes

- Non-amplified DNA techniques (nucleic acid hybridization): Pace 2® (GenProbe)
- Amplified DNA techniques (LCR, PCR): marked improvement in sensitivity, with high specificity
 1. PCR – Amplicor® (Roche)
 2. LCR – LCX® (Abbott); URlprobe™ combined GC & CT LCR (Abbott)
(SKCDPH Lab LCR \$15.00; U Wash LCR \$35.95)

F. Rapid tests

There are some rapid tests available for diagnosis of chlamydia infection. In general sensitivity is lower than for other types of testing, ranging from 65-87%. e.g. Surecell® (produced by Kodak) and Clearview® (produced by Unipath).

Comparative Performances of Tests for detection of *C. trachomatis*

Test	Sensitivity Relative to Expanded Gold Standard*	Specificity
Cell culture	50-90%	99.9%
Enzyme immunoassay (EIA)	40-60%	99.5%
Direct fluorescent antibody (DFA)	50-80%	99.8%
Non-amplified genetic probe (GenProbe)	40-65%	99.0%
Polymerase chain reaction (PCR)	Cervix 60-92% urethra Female urine 82-93% Male urine 87-100%	99.6%
Ligase chain reaction (LCR)	Cervix 81-100% Female Urine 69-96% Male urine 90-96%	99.7%

*defined using a combination of different test methods, including culture, DFA, and PCR or LCR directed against a target sequence distinct from that used in routine PCR or LCR assays.

Genital Herpes

caused by Herpes Simplex Virus

A. Tzanck prep (Giemsa stain) and Papacicolau stain

- Demonstration of multinucleated giant cells and eosinophilic inclusion bodies
- Least sensitive method, but is rapid, easy to perform and inexpensive
- Consistent with, but not diagnostic of HSV infection

B. Viral culture (recommended)

- Yield depends on the stage of the lesion and the proper collection technique
- Viral typing possible with monoclonal antibodies
- Vesicular or ulcerative lesions 50-80% culture positive

Culture Sensitivity	
Stage of Lesion	Sensitivity
Vesicle	90% +
Pustule	80-90%
Ulcer <5days	65-75%
Ulcer > 5 days	50%
Crust	20-30%

- C. Antigen detection by enzyme immunoassay (EIA) or immunofluorescence
- 70% sensitivity for samples from active lesions
 - May be better than culture for healing lesions
- D. Serologic methods (EIA, western blot, complement fixation tests)
- Commercially available assays do not distinguish HSV-1 and HSV-2 accurately
 - Accurate type-specific tests: western blot (not commercially available), IgG2 immunoassay (good specificity, but modest sensitivity. May be adequate to confirm presumptive diagnosis of herpes in a person with a history of recurrent genital lesions)
- E. PCR
- Useful in central nervous system herpes syndromes
 - Utility in diagnosis of genital disease not defined

Genital Warts caused by Human Papilloma Virus

In most cases, genital warts can be diagnosed based on visual appearance of the lesions and ruling out other potential causes of such lesions, including syphilis (condyloma lata) or cancerous conditions. Diagnostic tests are not commonly done.

Diagnostic tests which may be available include:

- Histological exam of biopsies or excised warts may sometimes be helpful.
- Nucleic acid hybridization detects viral DNA. Multiple techniques are available, but sensitivity and specificity can be quite variable.
- Amplified DNA Tests (PCR) – good specificity, but detection of HPV in absence of visible disease is of uncertain significance.

Gonorrhea caused by *Neisseria gonorrhoeae*

A. Microbiologic

- Direct smear (Grams' stain) – sensitivity & specificity highly dependent on skill of person examining the slide
- Culture: 72 hour turn-around time; strict handling/transport requirements for samples; labor intensive

B. DNA Tests

- Non-amplified tests: DNA hybridization
- Amplified tests: PCR and LCR

Comparative Performances of Diagnostic Tests for *N. gonorrhoeae*

Test	Sensitivity	Specificity
Gram stain*	Male urethra >95% Cervix ~50%	Male urethra >99% Cervix > 98%
Culture	Male urethra >99.9% Cervix 80-90% Rectum 80-90% Pharynx ~80%	99.9%
Non-amplified test (GenProbe)	Male urethra 99.9% Cervix 80-90%	99%
Polymerase chain reaction (PCR) & Ligase chain reaction (LCR)	Male urethra 99.9% Cervix 90-99% Male Urine 99% Female Urine 90-99%	> 99.9%

*Highly dependent upon skill of the person examining the slide

C. Other: Leukocyte esterase urine dipstick

- Detect enzyme from WBC and gives an indication of urethritis
- First-void urine in men – sensitivity: high 70s - 100%, specificity: 50s - ~100%
- Can play a role in screening asymptomatic men

Human Immunodeficiency Virus (HIV)

There are two broad categories of HIV tests: screening tests and confirmatory tests. Using these two types of test together allows for highly accurate and reliable diagnosis of HIV infection.

Screening Tests:

Screening tests are used for initial testing because they are easier to perform, well suited to testing large numbers of samples, and are less costly. They are highly sensitive and thus there are few false negatives (i.e. most infected people test positive). However they are not as specific as confirmatory tests so in a small percentage of cases the test result will be positive, even though the person is not infected.

The enzyme-linked immunosorbent assay (ELISA) tests measure antibodies to HIV. Traditional screening tests use a blood sample. There are about two dozen types of ELISA tests in use around the world; 10 are licensed for use in the United States. An ELISA test for detecting HIV in urine samples has been approved for use in the United States. The biggest drawback is however that there is no approved confirmatory test for urine samples, so that if the urine ELISA results are positive, a blood sample must then be drawn for confirmatory testing.

OraSure[®] (produced by SmithKline Beecham) is an HIV test which uses mucosal transudate as the sample. Although some call this a saliva tests, the sample is not technically saliva, but rather a mucosal transudate. The sample is collected by placing the special collection device between the cheek and gum. The sample is then sent to a lab for ELISA testing. Positive ELISA results can be confirmed using the Western blot test. These tests are more expensive than blood tests. OraSure[®] is licensed for use in the United States. OraScreen[®], a similar test marketed for home use, is available in some countries, but is not approved for use in the US.

Rapid serologic tests, those which provide results in less than 30 minutes are available. These tests also measure antibodies to HIV, but by different mechanisms than ELISA tests, including: agglutination tests, immunocomb tests, immunodot tests, and immunochromatographic membrane tests. Most rapid tests are kits that include all of the necessary supplies. These tests are relatively simple and involve a limited number of steps. Most rapid tests require refrigeration. When performed correctly, they are quite accurate. While inherent sensitivity and specificity of ELISA tests may be greater than some of the rapid tests, the field performance of rapid tests is often as good as or better than the ELISA because the former is simpler and easier to do in a low resource setting.

One rapid HIV test is approved for use in the United States (Single Use Diagnostic System for HIV-1; SUDS, manufactured by Murex Diagnostics, Inc. Norcross, Georgia, USA).

HIV Dipstick Test Kit: developed by PATH. This is a rapid (results in approximately 20 minutes), inexpensive (< \$0.50/test) test, that requires no specialized equipment. Sensitivity is >99% and specificity is >98%. The dipsticks are licensed in many countries around the world and are currently being produced in Thailand, India, Argentina and Indonesia. They are not licensed for use in the US.

Confirmatory Test:

A confirmatory test is done when the results of a screening test are positive. The confirmatory test is expensive, labor intensive, and requires subjective interpretation, but it is very specific; in other words false positive results are extremely rare.

The Western blot test is considered by most to be the 'gold standard' for confirmation of positive screening test results. This test also measures antibodies to HIV, but is more specific than screening tests so that false positives are minimal. Another, less commonly used confirmatory test is the immunofluorescence assay (IFA). Positive results from ELISA or rapid tests are commonly confirmed using a Western blot.

Alternate Testing Strategies

Other testing strategies besides a screening test followed by a confirmatory test have been proposed by WHO and UNAIDS for use in low resource settings where the Western blot (the most common confirmatory test used in developing countries) may not be readily available or affordable. These strategies include using a combination of two screening tests (ELISA or rapid tests) without using Western blot. Studies have shown that use of two screening test together can give results similar to, or in some cases

better than, use of a screening test followed by a confirmatory test, at a much lower cost.^{1,2,3,4} It is important to note that results will vary depending on the combination of screening tests used, so it is necessary to evaluate the intended combination before wide-spread implementation.

Other HIV/AIDS-Related Diagnostic Tests:

There are a number of other tests used to monitor disease progression in individuals infected with HIV including those that detect the level of virus itself, viral nucleic acids or those that measure the levels of CD4 lymphocytes (the white blood cells that steadily decline in people with HIV infection) in the blood.

Syphilis – caused by *Treponema pallidum*

I. Identification of the causative organism in lesions and tissue

A. Dark field microscopy

1. Advantages:

- Definitive immediate diagnosis
- Inexpensive, rapid

2. Disadvantages

- Requires specialized microscope & experienced microscopist
- Must be performed immediately – motility of the organism is important for identification
- Not specific for *T. pallidum*, possible confusion with non-pathogenic spirochetes

B. Direct fluorescent antibody (DFA-TP)

- Identification of organism using immunofluorescence in smear made from lesion
- Compares favorably with dark field microscopy but specific for *T. pallidum*

C. Monoclonal antibody test - not commercially available

II. Serological Tests

A. Nontreponemal tests: Measure antibodies directed against a cardiolipin-lecithin-cholesterol antigen (material released from damaged cells in response to infection) and thus is not specific for syphilis.

1. Microscopic include:

- VDRL - venereal disease research lab slide test
- USR - unheated serum reagin

2. Macroscopic card tests include:

- RPR - rapid plasma reagin

¹ Tamashiro H. et al. Reducing the cost of HIV antibody testing. *Lancet* 1993;342:87-90.

² World Health Organization, *Weekly Epidemiological Record*. 1997; 72: 81-88.

³ Andersson S et al. Field evaluation of alternative testing strategies for diagnosis and differentiation of HIV-1 and HIV-2 infections in an HIV-1 and HIV-2-prevalent area. *AIDS* 1997;11:1815-1822.

⁴ Stetler HC et al. Field evaluation of rapid HIV serologic tests for screening and confirming HIV-1 infection in Honduras. *AIDS* 1997;11:369-375.

- TRUST - toluidine red unheated serum test

Advantages of nontreponemal tests

- Highly sensitivity (negative test indicates syphilis is highly unlikely)
- Quantitative
- RPR and TRUST are rapid and inexpensive (~ \$0.25/test, results available in 10 min), easy to perform and can be done in clinic or office

Disadvantages of nontreponemal tests

- Specificity is fair. False positive reactions = 1-2% (positive test may be due to infection other than syphilis); false positives more likely in some circumstances e.g. autoimmune diseases, during pregnancy, after febrile disease, in IV drug users, and with chronic diseases like leprosy

Diagnostic devices for use with RPR and TRUST, developed by PATH. Available from Omega Diagnostics Limited, Alloa Business Centre, Whins Road, Alloa FK10 35A, Scotland, UK, Tel: 44-125-921-7315, fax: 44-125-972-3251

- Battery-powered rotator platform
- Plasma separator card that uses capillary blood obtained with a lancet

B. **Treponemal tests:** Measure antibodies directed against *T. pallidum* antigens and are thus specific for syphilis infection

1. Immunofluorescence tests
 - FTA-ABS: fluorescent treponemal antibody-absorption
2. Hemagglutination tests
 - MHA-TP: micro- hemagglutination *Treponema pallidum*
 - TPHA: *Treponema pallidum* hemagglutination assay

Advantages

- Highly specific and highly sensitive

Disadvantages

- Expensive and time consuming so not used for screening; primarily used for confirmation of positive non-treponemal tests

Sensitivity of Serological Tests for Syphilis

Test	Stage of Syphilis (% positive)*			
	Primary	Secondary	Latent	Tertiary
VDRL	59-87	100	73-91	37-94
FTA-ABS	86-100	99-100	96-99	96-100
MHA-TP	64-87	96-100	96-100	94 –100

**percentage figures provided should not be interpreted as absolute values because there are small numbers in certain categories and test results vary from study to study*

Trichomoniasis

caused by *Trichomonas vaginalis*

Microscopy: visualization of motile trichomonads on saline wet mount smear (50-65% sensitive). Stained smears are not useful as the trichomonads are difficult to differentiate from white blood cells.

Culture: 85-95% of infections can be detected by culture. Sensitivity varies depending on the type of culture media.

InPouch TV™ is a commercially available culture system for use in diagnostic testing for *Trichomonas vaginalis*. It is produced by BioMed Diagnostics, San Jose, California. The sample is inoculated into culture medium contained in a clear, plastic pouch. The pouch is incubated and then placed directly on the microscope for reading the results. The system is more sensitive than other culture media available. Cost is approximately \$2.00 per pouch and InPouch TV™ has a shelf life of one year at room temperature (18-25° C).

Vaginal yeast infections

caused by *Candida albicans*

Microscopy: wet prep (KOH) shows yeast buds and pseudohyphae; ~ 50-60% sensitive

Culture: Routine culture for diagnosis of yeast infections is not recommended. Up to 50% of women may have normal colonization of the vagina with *Candida* species.

***FemExam*® pH and Amines TestCard™ for diagnosis of Vaginitis**

This is a rapid card test for diagnosis of vaginitis (BV, *Candida*, *Trichomonas*).

- Need the testcard and a swab for taking a sample of vaginal fluid
- No special training is required
- Results are available within 2 minutes
- Information from the manufacturer says accuracy is >98%
- Price is \$4.75 to \$6.50 per test depending on the size of the order

On The Horizon

IC Strip Tests

PATH is working on development of IC (immunochromatographic) Strip Tests for syphilis, gonorrhea and chlamydia infection. These tests will be rapid (within an hour, exact time depends on the test), easy-to-read, low cost (goal is \$0.50, although they will probably first start at \$1.00), and require a minimum of training. No specialized equipment is necessary.

Current status

- Syphilis (farthest along in development): field tests are under way in developing countries and there is a private sector partner producing the strips. They should be available within the next few years, perhaps as early as late 1999. Can use blood, plasma or serum.
- Gonorrhea: lab evaluations have shown the test can identify gonorrhea isolates from all around the world. Clinical evaluation of the tests is underway and a private sector partner to make the strips has been identified. These should be available within the next few years.
- Chlamydia (least developed of all three): Lab tests indicate it has equal or greater sensitivity compared to commercially available rapid diagnostics. PATH has hired a consultant to work on this project who will be looking to identify a private sector partner. Unlikely to be available within the next 5 years.

Sources

- Handouts from AVSC Russia STI Counseling Workshop notebook (handouts adapted from Seattle STD/HIV Prevention and Training Center materials)
- The Practitioner's Handbook for The Management of Sexually Transmitted Diseases. Second edition. Celum CL, Wilch E, Fennell C & Stamm WE. Health Sciences Center for Educational Resources, University of Washington. 1994
- Laboratory Methods for the Diagnosis of Sexually Transmitted Diseases. American Public Health Association. Eds Wentworth BB, Judson FN & Gilchrist MJR. 1991.
- Sexually Transmitted Diseases. Second edition. Eds. Holmes KK, Mardh PA, Sparling PF, Wiesner PJ. McGraw-Hill. 1990.
- Assorted company web sites and product literature
- JAMA Sexually Transmitted Disease Information Center
- Communication with Gretchen Shively, PATH; Gina Dallabetta FHI/IMPACT; Jeanne Marrazzo and Connie Celum, University of Washington, Harborview STD Clinic

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