

THE STD LABORATORY

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Role of the laboratory in STD control

The primary role of the STD laboratory is to support decision making in clinical practice and for public health. Strengthening of laboratory infrastructure should always be subordinate to requirements for STD control interventions.

Clinical practice

Laboratory tests improve the diagnostic specificity of symptomatic STDs as well as the diagnostic sensitivity of symptomatic STDs. A laboratory can become involved in three types of STD control activities:

1. Diagnosis of symptomatic patients: the role of the laboratory is to assist in management of patients seeking health care for symptoms of STD. Given that symptoms and signs of lower genital tract infections are not specific, particularly in women, laboratory tests are helpful to differentiate serious infections, i.e., cervicitis, from milder but more common infections, i.e., vaginitis. Simple laboratory tests incorporated in syndromic management of urethral discharge also help distinguish mixed and single infections, reducing the administration of unnecessary antibiotics.
2. Case finding in asymptomatic individuals: the laboratory can play an important role for the detection of STDs in patients seeking health care for other reasons. Laboratory tests increase substantially the sensitivity of STD diagnosis. Case finding of asymptomatic STDs is most important in female patients who carry the burden of STD complications and sequelae. STD case finding in pregnant women is extremely important for prevention of adverse consequences of syphilis, HIV, gonococcal and chlamydial infection in newborns.
3. Screening of populations: laboratory testing is essential for assessment of STD among (target) populations not seeking health care.

Public health

Laboratory tests play a key role in public health decision making. They help document the epidemiology of STDs in target populations, regardless of the symptoms. Results are used to advocate STD control interventions and assess their impact. Laboratory tests are essential for operational research such as validation of guidelines for syndromic management of symptomatic STDs or definition of appropriate STD treatment guidelines. Reference laboratories are also used to monitor the quality of results produced by clinical laboratories and to train their staff.

Criteria for laboratory test selection

The criteria for determining which laboratory test to use are similar to those for choosing any evaluation indicators and include validity, reliability, feasibility, and acceptability (affordability).

Validity: refers to test sensitivity (percent of true positives) and specificity (percent of true negatives) of a test compared to those of the gold standard. The gold standard is the best available diagnostic test for a disease. In some cases, no single diagnostic test is available, and a combination of tests is needed, such as the "expanded gold standard", e.g. positive culture or confirmed DNA

amplification technique, presently in use for the diagnosis of *Chlamydia trachomatis* infection. Laboratory test sensitivity partly depends on the prevalence of the disease. As the prevalence increases, so does the probability of encountering the disease in its acute stage with higher concentrations of detectable particles. Usually, cost increases with validity.

Reliability: is the ability to produce similar results for the same biological sample. Reliability increases with the validity of the technique, but also depends on the ease of use. Techniques involving automated measurement of substances, such as enzyme immunoassays (EIAs), are intrinsically more reliable than those involving the technician's interpretation, such as microscopy. Direct fluorescent antibody testing (DFA) for *C. trachomatis* can be extremely sensitive and reliable in expert hands, but even a simple test as Gram stain examination of urethral discharge can have a low validity when it is used by a sloppy technician.

Increasingly reliable techniques ("idiot-proof") are constantly being developed by test manufacturers, but they can be extremely expensive. In addition, even the most reliable technique yields poor results when it is improperly standardised and not monitored through quality control procedures.

Feasibility: beyond the ease of use, the technical feasibility of a test for a laboratory depends on the operational requirements for the test, such as space, clean water, stable power supply, sophisticated equipment, and refrigerated storage of reagents. As health facilities become more distant from urban centres, these requirements become more difficult to meet. Even in major central level and reference laboratories, provision of spare parts and reagents is frequently overlooked.

Acceptability: of a test for patients usually depends on the type of biological sample requested. Saliva and urine production is painless and easier than having blood drawn. In some cultures blood samples can be extremely difficult to obtain. Not surprisingly, urethral swabbing in men is probably the least acceptable. Painful or invasive specimen collection techniques may be more acceptable to symptomatic patients, but painless and non invasive sampling is critical for case finding or screening of asymptomatic individuals. Acceptability also increases when results are obtained quickly, which in turn depends on the organisation of laboratory activities. Acceptability also includes affordability when patients must pay for laboratory services. As a general rule, laboratory expenditures should not exceed the treatment costs saved by testing. Acceptance of laboratory expenditures is the lowest for case finding or screening of asymptomatic subjects, including asymptomatic contacts of STD patients.

Laboratory organisation for clinical practice

Clinical infrastructure in the public sector is organised at three successive levels. Most patients are seen in peripheral (primary health care or first-encounter level) health units. Patients who need to be referred are sent to intermediate (regional) health facilities and eventually from there to central (national) facilities. Clinical laboratories are generally organised at the same three levels (see **Table 1** on next page).

Peripheral laboratories are attached to health centres and first referral (health district) hospitals. Laboratory procedures at this level usually include microscopic examination of fresh and stained specimens. In syndromic management of urethral discharge, microscopy help single out nongonococcal infection. For vaginal discharge, microscopy helps differentiate trichomoniasis from candidiasis and bacterial vaginosis.

Table 1: Recommended diagnostic tests by level of laboratory capability

Disease	Laboratory test	Laboratory level		
		Peripheral	Intermediate	Central/STD Centre
Gonorrhoea	Smear (Gram, methylene blue, safranin)	+	+	+
	Culture	-	+	+
	β-lactamase	-	(+)	+
	Antimicrobial susceptibility	-	-	+
Chlamydia trachomatis infection	Antigen detection			
	DFA	-	(+)	+
	ELISA	-	-	+
	DNA hybridization	-	-	(+)
	Culture	-	-	+
	DNA amplification	-	-	(+)
Syphilis	Serology	-	-	+
	Darkfield microscopy	-	(+)	+
	RPR	(+)	+	+
	TPHA	-	(+)	+
	FTA	-	-	+
Genital herpes	IgM	-	-	+
	Antigen detection	-	-	+
	Culture	-	-	+
Chancroid	Serology	-	-	+
	Culture	-	-	+
Antimicrobial susceptibility	Culture	-	-	+
	Antimicrobial susceptibility	-	-	+
Donovanosis	Smear (Leishman, Wright)	-	(+)	+
	Histopathology	-	-	+
Trichomoniasis	Wet mount microscopy	+	+	+
	Culture	-	-	+
Candidiasis	Wet mount (ev.+10%KOH)	+	+	+
	Culture	-	-	+
Bacterial vaginosis	Wet mount, stained smear	+	+	+
	pH, KOH sniff test			
HIV	rapid/simple AB test	(+)	+	+
	classic ELISA	-	(+)	+
	Western blot/line immunoassay	-	-	+

+ yes
 (+) yes, if possible
 - no

Laboratory procedures may also include rapid HIV tests and simple nontreponemal syphilis tests (RPR or VDRL).

Intermediate-level laboratories are commonly situated in regional or provincial hospitals. Their diagnostic requirements are greater, and better infrastructure and skilled manpower are available. Laboratory tests might include culture of *Neisseria gonorrhoeae* and identification of penicillinase-producing strains. They might also include antigen detection of *C. trachomatis*, depending on staff

workload and available resources. Serology might include enzyme-linked immunosorbent assays (ELISA) or particle agglutination for HIV as well as confirmatory microhaemagglutination test for syphilis.

Central laboratories are usually located in the capital city or in an university hospital. The range of diagnostic tests performed varies according to the available human, material, and financial resources and the workload. There is no sharp distinction between what should and could be performed at the intermediate versus the central level. These decisions depend on the organisation of health services in a country. One central reference laboratory may be quite satisfactory in a small country. In big countries centralisation of reference activities in the capital city is unrealistic; therefore some decentralisation of laboratory services at the regional level is necessary.

Constraints to providing laboratory services in developing countries include:

- Shortages of supplies and reagents
- Failure of water and power supplies
- Inadequate maintenance of equipment
- Weak technical ability of personnel
- Lack of supervision and quality control
- Absence of continuing education and training
- Lack of primary and continuing education for health care providers on the usefulness of laboratory diagnostics.

All these factors contribute to low motivation among laboratory technicians and may result in poorly maintained and dirty workplaces, delays in specimen processing, use of out-of-date reagents, mislabelling of specimens, poorly maintained registers, and frequent personnel absences.

Introduction of STD diagnostic activities tests is difficult and expensive. In planning STD laboratory development and diagnostic activities, it is essential to consider the following factors:

- The prevalence of STDs should be of sufficient magnitude to justify the effort.
- Sufficient logistic support and financial and human resources should be available to maintain the laboratory.
- The technology and methodologies used should be appropriate for the technical capacity and educational levels of laboratory staff.
- Clinicians should choose treatment or preventive measures based on laboratory test results.
- Additional tests should not be introduced at the expense of higher priority needs; the most cost-effective STD case management approach should be employed.
- Follow-up, evaluation, coordination, and quality control should be assured.

Public health laboratory services

Among the major public health needs for STD control are prevalence studies for guideline validation or HIV/STD surveillance and antimicrobial susceptibility surveillance of *N. gonorrhoeae*. The most important requirement for public health data is reliability, which depends on skilled manpower and quality control procedures. It is critical that centralised laboratories are reliable because samples taken for public health purposes should be forwarded from different sites in the country to one reference laboratory, usually located in the capital city. When this is not technically feasible, however, some decentralisation should be considered.

The cost of public health testing should be supported by the public sector, since patients cannot be expected to pay for tests they have not requested. Also, because people do not request these tests, the least invasive methods of specimen taking should be used to increase acceptability.

Total separation of public health and clinical testing is not a cost-effective use of equipment and trained personnel. In fact, some laboratories combine both activities because fees for clinical tests help raise money for public health testing. However, the two should not be combined at the expense of public health priorities.

Cost of laboratory testing

Careful attention should be given to the cost-effectiveness of laboratory testing. Such testing is expensive and adds to the complexity of providing care. In many low-income countries, per capita health care expenditures are less than \$ 10 per year; therefore, widespread testing for most STDs, including HIV, using currently available tests is unlikely. Detailed cost-effectiveness studies of health care based on laboratory testing and diagnosis are very complex and require assessment of indirect costs. Direct costs, which include equipment, supplies, reagents, drugs, and labour, can be calculated more easily. An illustration of how to calculate the direct costs for screening and treatment of syphilis is given in **Table 2**.

Table 2: Cost of rapid plasma reagin (RPR) screening in an asymptomatic population of 10,000 people for three different prevalence rates of disease (RPR sensitivity 85%, specificity 98%)			
Prevalence	1%	5%	25%
Positive Predictive Value (PPV)	30%	69.1%	93.4%
N of true positives detected	85	425	2,125
Total N of reactivities detected	283	615	2,275
Screening cost ^a (U.S. dollars)	\$ 9,000	\$ 9,000	\$ 9,000
Cost for management of reactivities detected ^b :			
• laboratory (RPR titration)	\$170	\$369	\$1,365
• medical care	\$849	\$1,845	\$6,825
Total cost	\$10,019	\$11,214	\$17,190
Cost to detect one true positive	\$107.90	\$22.00	\$4.90
Total cost per true positive	\$117.90	\$26.40	\$8.10
a blood collection: \$0.5; Laboratory testing: \$0.4 b Laboratory testing: \$0.6; medical care: \$3.0 * For a prevalence of syphilis of 1%, a test with a sensitivity of 85% and a specificity of 98% will detect in a population of 10,000 people 85 cases of syphilis (true positives) and 198 reactive cases who do not have the disease (false positives); 15 true cases of syphilis will not be detected (false negatives) and 9,702 people will correctly be identified as not having the disease (true negatives)			

Syndromatic STD management approach

Developing-country health providers at all levels, in both the public and private sector, are likely to be confronted with many STD patients. Primary health care facilities in resource-poor settings face several constraints to the optimal management of patients with STDs. These constraints include:

- Lack of access to the laboratory technology, necessary for making an aetiological diagnosis
- Shortage of well-trained staff
- High workloads with limited time available per patient.

- The syndromic approach to STD case management involves the detection of a syndrome (symptoms and signs) associated with a number of well defined aetiological agents. This allows health care workers to make a correct diagnosis in many patients:
- without sophisticated laboratory tests
- without specialized skills
- within a short time, and preferably without the need for a repeat visit by the patient.

Once a syndrome has been identified, adequate treatment can be provided for the majority of the organisms responsible for that syndrome.

Although of adequate sensitivity for urethritis in males and genital ulcers in both sexes, the syndromic approach shows relative low sensitivity and specificity when used for management of female genital discharge. Recently, attempts have been made to supplement identification of signs and symptoms with risk assessment questions and with non-specific simple diagnostics like leukocyte esterase in vaginal fluid or in urine with varying success. On the other hand, management of certain STD conditions, such as pelvic inflammatory disease, requires a syndromic approach, regardless of available resources.

Diagnostic laboratory approach

Urethral discharge

Cases of urethral discharge can be treated on clinical examination according to syndromic management guidelines, but laboratory tests are required to distinguish between nongonococcal and gonococcal urethritis. The presence of leukocytes and typical intracellular diplococci (gonococcal urethritis) or the presence of leukocytes without intracellular diplococci (nongonococcal urethritis) can be detected through microscopic examination of a smear of urethral exudate stained with methylene blue, safranin or Gram's method. Concomitant gonococcal and nongonococcal infections will not be identified with this method.

Since microscopy of stained smears has a sensitivity of greater than 95 percent for symptomatic gonococcal urethritis, culture for the isolation of *N. gonorrhoeae* is not essential for diagnosis and clinical management¹. However, isolation of gonococci, along with clinical monitoring of treatment response, may be important in monitoring antimicrobial susceptibility trends.

For screening or case finding of urethritis among asymptomatic men, the collection of urethral specimens is an invasive method, not much appreciated by the participants. A non-invasive alternative is the collection of first-catch urine for the detection of leukocyte esterase. This method has a reported sensitivity for detecting infectious urethritis ranging from 41 to 100 percent, but it does not allow differentiation between gonococcal and nongonococcal urethritis. The specificity of the leukocyte esterase test varies between 35 and 100 percent; consequently, the predictive value of a positive test may be very low (2-7).

For screening of urethritis in male populations, the newer DNA amplification techniques for the detection of *N. gonorrhoeae* and *C. trachomatis* can be performed on first-catch urine samples.

Vaginal discharge / Lower abdominal pain

Wet mount microscopy is very useful for the diagnosis of trichomoniasis, candidiasis, and bacterial vaginosis (BV). A pH test and a KOH sniff test may help diagnose BV, but the most reliable diagnosis is based on a Gram stained smear to detect the typical bacterial flora and the presence of clue cells. Diagnosis of gonococcal and chlamydial cervicitis, infections with potentially serious

sequelae, is even more important, but unfortunately, simple methods for diagnosing these infections are not available. Gram stain microscopy has a low sensitivity for detecting gonorrhoea among women; culture remains the method of choice⁸. Diagnostic assays for *C. trachomatis* include DFA, EIA, DNA hybridisation, cell culture and DNA amplification techniques (9).

Genital ulcer disease

Genital ulcers may result from syphilis, chancroid, herpes, donovanosis, or (rarely) Lymphogranuloma venereum (LGV). Management of patients can be based on clinical examination and application of treatment algorithms. Essential procedures for definite diagnosis of the different genital ulcer diseases are:

- Darkfield or DFA microscopy for treponemes
- Wright or Leishman stain for *Calymatobacterium granulomatis*
- Culture for *Haemophilus ducreyi*
- Culture or direct immunoassay for herpes simplex virus
- Serology for syphilis and LGV
- Multiplex PCR for syphilis, chancroid, and herpes.

Aetiological laboratory diagnostic procedures

Gonorrhoea

Gonorrhoea produces a purulent exudate, but signs and symptoms of disease may either be absent or indistinguishable from those of chlamydial infection; therefore, laboratory tests are needed for diagnosis and case finding as well as for test-of-cure. Accurate methods for the diagnosis of gonorrhoea are direct microscopy of a stained urethral discharge smear in men and culture or DNA amplification of all other types of specimens.

Direct microscopy (for males)

Simple staining of a urethral specimen with methylene blue or safranin may offer a quick and reliable diagnosis of gonorrhoea in men, but the Gram stain, which give more specific results for specimens containing mixed bacterial flora, remains the standard method.

Culture and identification

Specimens are cultured on selective enriched media such as (modified) Thayer Martin or New York City. The observation of oxidase-positive Gram-negative diplococci with a gonococcus like colony morphology on 24-48h cultures offers a sufficient and reliable identification of *N. gonorrhoeae* for routine diagnosis in genital specimens. For extragenital isolates as well as for research, further characterization is recommended (10). Carbohydrate degradation tests (glucose, maltose, lactose, and sucrose) are commonly used, sometimes in combination with enzymatic substrate tests (11-14). An immunologic confirmation assay using monoclonal antibodies is a very reliable but more expensive alternative (13-15).

Non-culture gonorrhoea detection techniques

Different culture-independent tests for gonorrhoea detecting oxidase, endotoxin, antigen, or DNA have been compared with a standard culture technique. All these procedures are less efficient and most are more expensive than a culture technique for extragenital specimens and for specimens

containing small numbers of organisms. The only technique that can compete with culture is DNA amplification (10, 16-19).

Several antibody techniques have been used to detect gonococcal antibodies in serum. None of the currently available methods are useful for diagnostic purposes because they cannot differentiate recent from past infection.

Chlamydia trachomatis infection

Chlamydia trachomatis is an important cause of urethritis and cervicitis. Symptoms and signs of chlamydial infection may be extremely mild or totally absent, making early diagnosis and treatment less likely than with other STDs. Untreated chlamydial urethritis in men can evolve to epididymitis. In women, the cervix is most commonly infected and infection frequently spreads to the urethra; *Chlamydia* can also invade the endometrium and fallopian tubes, resulting in endometritis or salpingitis. Coinfection with gonorrhoea is common.

With the advent of new technologies, the current reference test (expanded gold standard) for diagnosing *C. trachomatis* infection is either positive tissue culture or positive DNA amplification test confirmed by another test using a different technique, such as DFA, or the same DNA amplification technique directed toward a different target such as a major outer membrane protein (9). The culture procedure, however, is expensive, slow, labour-intensive, technically difficult and beyond the capacity of most laboratories. In competent hands, the specificity of culture is 100 percent, its sensitivity is estimated to be no more than 70 to 85 percent compared to DNA amplification. Cervical culture for *C. trachomatis* has a 65-90 percent sensitivity compared to DNA amplification of first-catch urine (9).

Other non-culture techniques (DFA, EIA, DNA hybridisation) developed during the 1980s, which are easier to perform and less expensive than culture, are still widely used. Unfortunately, all those tests show lower sensitivity than culture and extremely low sensitivity (45-60 percent) when compared to the expanded gold standard (9,20).

Direct microscopy (for males)

In a patient who has a history of acute onset of urethral discharge and has not received antibiotic therapy, a Gram stain of a urethral specimen demonstrating polymorpholeukocytes without the presence of Gram-negative diplococci has a high predictive value for chlamydial infection.

Cell culture

Many cell lines are suitable for the growth of *Chlamydia*, but the method of choice in most laboratories is to add centrifuged specimens to cycloheximide-treated McCoy cell monolayers, incubate them at 36°C for two or three days, then stain them with fluorescein-labelled monoclonal antibody (21,22). The addition of a blind passage enhances the sensitivity of the culture method, but may create a clinically unacceptable delay in diagnosis (23,24).

Direct fluorescent antibody (DFA) test

Fluorescein-labelled monoclonal antibodies to the species-specific epitope of major outer membrane proteins can detect elementary bodies (EBs) of *C. trachomatis* in clinical specimens²⁵. The procedure is rapid and simple to process, but labourious and tedious to read and not recommended for processing large numbers of specimens. Microscopic reading of results is

subjective, and the reliability of the test depends on the expertise of the observer (26,27). Investigators do not always use the same cutoff number, or number of EBs necessary to consider a specimen positive, which influences the sensitivity of the method (28,30). Overall, DFA shows acceptable accuracy for diagnosis in symptomatic patients and for case finding in high prevalence populations, but lacks the sensitivity to detect the small numbers of organisms often found in asymptomatic subjects, particularly in low prevalence populations (31,32).

Enzyme immunoassay (EIA)

EIA methods are more suitable than DFA for batch processing of large numbers of specimens. This method is also more objective than DFA because the results are read with a photometer. The overall sensitivity and specificity of EIA and DFA are similar (32). Most of the currently available EIAs include a confirmation (neutralisation or blocking) assay to be performed on positive specimens, during retesting by EIA, the presence or absence of chlamydial antigen in a sample previously reactive in EIA can be confirmed by selective inhibition of the antigen by Chlamydia-specific immunoglobulin (33,34).

Newer easy-to-use membrane immunoassays enable rapid diagnosis of chlamydial infection under field conditions. These methods have a lower sensitivity and are therefore less accurate than the more classic immunoassays (9,35,36).

DNA assays

DNA hybridization methods have been applied to chlamydial diagnosis since the 1980s (37). Commercially available tests are easy to perform and have been shown to be highly specific, but their sensitivity appears to be similar to that of DFA and most EIAs (9). Amplification of DNA sequences with polymerase chain reaction (PCR), ligase chain reaction (LCR), and transcription mediated amplification (TMA) offer very high sensitivity (38-40). However, the high cost of these assays, the specialised laboratory equipment necessary, and the ease with which contamination with DNA can occur in the laboratory currently limit the use of these methods in routine diagnosis.

Urine specimens

Obtaining urethral specimens by swab from men is an invasive method that causes some discomfort. The collection of cervical swabs from women requires a clinical setting, skilled personnel and a speculum examination. Urine samples are easier to obtain in both sexes and this makes diagnosis and screening of chlamydial infection more feasible. Several studies have shown significant lower sensitivities of EIA, DFA, and cell culture from urine specimens than those from urethral and cervical swab specimens (41-44). Results obtained by DNA amplification techniques on urine specimens from both men and women have shown that this approach is at least as good as cell culture on classic genital swab specimens (45-47). Another promising approach to noninvasive testing is the use for DNA amplification of vaginal introitus specimens self-collected by the patients (48).

Antibody detection tests

Various serological methods (complement fixation (CF), micro-immunofluorescence (MIF), EIA) have been used to study chlamydial infections in special situations, but their use for diagnostic purposes remains limited. Similar to serologic confirmation of other infections, serologic evidence of chlamydial infection can be obtained by demonstrating seroconversion or by a fourfold or greater rise in antibody titer in paired sera two weeks apart. The use of a single high titer for diagnosis of

chlamydial urethritis and cervicitis is unreliable, it is diagnostically suggestive for LGV, reactive arthritis, epididymitis and pelvic inflammatory disease (27,49).

Active LGV infections in general have CF titres of 1:64 or greater. However, high CF titres can be found in asymptomatic individuals and those with chlamydial infections with the non-LGV serovars. MIF is more sensitive than CF because it is possible to determine the antigenic type of the infecting chlamydial strain. MIF, however, is not routinely available and is used in few specialised laboratories (50).

Syphilis

Venereal syphilis is acquired by sexual contact with an infected person with an open ulcer or with mucocutaneous secondary lesions. Transmission of *Treponema pallidum* requires exposure of non-infected mucous membranes or skin abrasions to infectious lesions.

No structural or metabolic difference has been found that distinguishes between spirochetes that cause venereal syphilis (*T. pallidum*), endemic syphilis (*T. endemicum*), yaws (*T. pertenue*) and pinta (*T. carateum*). Therefore, these organisms cannot be differentiated by laboratory tests, but only by clinical manifestations and through epidemiological studies, including inquiries to determine the mode of transmission.

Syphilis is a chronic infection with diverse clinical manifestations occurring in distinct stages, and each stage requires a different diagnostic approach. Treponemes can be identified in the primary and secondary stages. In the primary stage, treponemes are microscopically detectable in skin or mucosal lesions at the site of entry (primary chancre). During the secondary stage, they can also be detected in papular rash lesions or in condylomata lata.

During the early primary stage serological tests are negative; antibodies usually appear one to four weeks after a lesion has formed. During the secondary stage, all serological tests are positive, and nearly all patients will show high antibody titres (>1:8) in nontreponemal tests. Serology remains positive during latency and the tertiary stage, however, approximately 20 percent of patients during late latency and 30 percent of patients with tertiary-stage syphilis may have nonreactive nontreponemal tests.

Congenital syphilis is acquired by transplacental transmission of *T. pallidum* from a pregnant woman to a foetus. Diagnosis of congenital syphilis is based on (1) microscopic demonstration of *T. pallidum* in nasal discharge or skin lesions, when present; (2) detection of specific IgM antibody in serum; (3) demonstration of rising nontreponemal test antibody titres in serial serum samples during the first eight months of life.

Direct microscopy

Darkfield microscopy is the standard method to provide an instant diagnosis of syphilis in the primary and secondary stages. For reliable results, however, appropriate technical conditions usually found only in specialised laboratories are essential, including well trained staff and adequate equipment and time.

The direct fluorescent antibody (DFA) test may be a more practical alternative to darkfield microscopy because the clinical specimens are fixed on a slide with methanol or acetone and laboratory examination can be done after transport. DFA also eliminates confusion with other spiral organisms and does not require motile organisms for syphilis diagnosis, so its specificity and

sensitivity are higher than that of darkfield (51). Failure to visualise the organism, however, does not exclude a diagnosis of syphilis. A negative result may mean that:

- An insufficient number of treponemes were present in the lesion
- The patient was treated or partially treated recently
- The lesion was approaching natural resolution
- The lesion was not syphilitic

Nontreponemal tests

All the current nontreponemal tests for syphilis are flocculation tests using cardiolipin, lecithin, and cholesterol as antigen. The Venereal Disease Research Laboratory (VDRL) test was the first in the series of slide flocculation methods, and the basic antigen composition in all newer tests is that of the VDRL (52). The antigen used in the VDRL test is not stabilised, so a working suspension must be prepared fresh daily. The VDRL should be performed on serum heated at 56°C before testing, and results must be read with a microscope at 100x magnification. This microscopic test, VDRL, is the only appropriate test for spinal fluid. Another microscopic method, the unheated serum reagin (USR) test, can be performed with a stabilised antigen on unheated serum (53).

In other flocculation tests, the reaction is visible to the naked eye. The most popular of these macro-vue methods is the rapid plasma reagin (RPR), which uses plastic-coated cards in place of slides and a stabilised antigen to which charcoal particles are added. The antigen is not coated on these particles. Instead, it is trapped in the lattice performed by the antigen-antibody complex in positive samples, making the reaction visible to the naked eye. The test may be performed on unheated serum or plasma⁵⁴. Modifications of the RPR include the reagin screen test (RST), which uses a lipid-soluble black dye in place of charcoal, the VDRL carbon antigen test, which is similar to the RPR, and the toluidin red unheated serum test (TRUST), which uses toluidin red in place of charcoal to make the reaction visible (55-56).

Treponemal tests

Specific treponemal tests detect antibodies against treponemal cellular components. The three different test procedures are: indirect immunofluorescence, haemagglutination and enzymelinked immunosorbent assay. The fluorescent treponemal antibody-absorption (FTA-Abs) test is the most sensitive of all syphilis tests, but is technically the most difficult. Standard reading, high-quality and appropriate dilution of the conjugate, and the use of good antigen slides are essential for the reliability of the test (57). Microhaemagglutination assay for antibodies to *T. pallidum* (MHATP), *T. pallidum* haemagglutination assay (TPHA) or haemagglutination treponemal test for syphilis (HATTS) are easier to perform than FTA-Abs, have fewer variables, and are more practical for batch processing of large numbers of specimens (58-59). Enzyme-linked immunosorbent assays (EIAs) are designed for batch processing and are suitable for automation of serology (60-61). The different treponemal tests have comparable sensitivity and specificity, except for primary-stage syphilis, where FTA-Abs is more sensitive than the other methods.

Appropriate use of serological tests

The sensitivity and specificity of nontreponemal and treponemal syphilis tests for the different phases of the disease are shown in **Table 3**. A reactive nontreponemal test may indicate a primary infection, a recent infection treated or not treated, or a false positive result. False positive results occur in populations at a rate of 1 to 3 percent. The vast majority of false positive sera show antibody titres of m 1:4. However, low titres do not exclude syphilis and are often found in early primary, late latent and tertiary syphilis. Determination of nontreponemal serum titres through a quantitative procedure may be helpful for more accurate interpretation of results and for evaluation

of patients after treatment. To exclude false positive results, it is necessary to perform a specific treponemal test.

Table 3: Sensitivity and specificity of serological tests for Syphilis

Test	Sensitivity (%) by phase of Syphilis infection				Specificity %
	Primary	Secondary	Latent*	Late*	
VDRL	80 (74-87)	100	80 (71-100)	71 (37-94)	98
RPR card	86 (81-100)	100	80 (53-100)	73 (36-96)	98
FTA-Abs	98 (93-100)	100	100	96	99
MHA-TP	82 (69-90)	100	100	94	99

A reactive treponemal test may indicate a primary or early infection, recent infection treated or not treated, or past infection. Once infected with pathogenic treponemes, the majority of subjects remain treponemal antibody-positive in tests for years - even for a lifetime - whereas nontreponemal tests usually revert to negative over time after successful treatment.

For borderline reactions, discordant nontreponemal-positive treponemal-negative reactions, and discordance with clinical impression, the tests should be repeated on a new serum sample. If disagreement persists, a different treponemal test may be done for final judgement. In incubating syphilis all antibody tests are negative. In early primary syphilis, different combinations of results may be obtained (nontreponemal-positive microhaemagglutination-negative, nontreponemal-negative microhaemagglutination-positive, or nontreponemal-negative microhaemagglutination-negative). To diagnose or exclude syphilis, the tests must be repeated after two to three weeks on a new serum sample. In such cases, it may be helpful to perform an FTA-Abs, since it is the most sensitive test for primary syphilis. Finally, when adequate treatment is started early in primary syphilis, patients may remain antibody-negative.

Seroreversion of nontreponemal tests to negative in patients adequately treated usually occurs within a period of six months to a few years and is associated with the duration of infection, previous infection, and the antibody serum titer at the moment of treatment. Seroreversion of treponemal tests also occurs within a few years in a minority of patients; this phenomenon is not yet clearly understood.

For diagnosis as well as for case finding of syphilis, serum samples should first be screened with a nontreponemal test. To date, the most popular nontreponemal test is the RPR 18-mm circle card test with mechanical rotation. In poorly equipped laboratories with low numbers of specimens to test, hand rotation of the card is appropriate. The sensitivity of a "hand rotation" RPR is only slightly lower than that of a RPR with mechanical rotation. Thus, some samples with low antibody titer of m1:2 may appear negative with the hand rotation procedure (62). Specific treponemal antibody tests are used to confirm positive nontreponemal samples as well as for epidemiological studies. The most appropriate of these tests for routine work is the TPHA.

With nontreponemal tests, undiluted serum samples with high antibody titer occasionally appear nonreactive because of excess antibody. This phenomenon, known as prozone effect, is sometimes observed in patients with secondary syphilis. Consequently, nonreactive undiluted samples from symptomatic patients should be diluted 1:16 to 1:256 and retested with a quantitative procedure.

Demonstration of treponemal IgM in serum

The synthesis of specific IgM antibodies is the first humoral immune response after infection in syphilis as well as in other bacterial or viral infections. In syphilis, treponemal IgM antibody is

present not only in patients with early primary syphilis, but may also be found during the latent period and in patients with late disease. IgM decreases more slowly after spontaneous resolution of infection than after successful therapy.

Detection of IgM antibody is also very useful for the diagnosis of congenital syphilis. The presence of IgM antibody in the blood of newborns indicates prenatal infection of the child. In most children, however, IgM antibody only appears a few weeks to a few months after birth. The appearance of IgM in the cerebrospinal fluid (CSF) of patients with an intact serum/CSF barrier (i.e. normal serum/CSF albumin ratio) indicates active neurosyphilis because the molecular size of IgM prevents it from passing the intact serum/CSF barrier as well as the placental barrier.

The sensitivity of treponemal IgM detection is not optimal, but its observation may contribute to a more reliable interpretation of congenital syphilis, early primary syphilis, late syphilis, and reinfection of patients with a previous history of syphilis or other treponematoses. Disappearance of IgM can be a helpful test-of-cure for patients with early infection before seroreversion of nontreponemal tests is observed.

Genital herpes

Herpes simplex virus (HSV) belongs to the group of alpha-viruses that become latent and cause persistent infections. Genital herpes is caused by HSV-2 in approximately 85 percent of cases; the remainder are caused by HSV-1. Primary HSV infection may be asymptomatic or characterised by the appearance of extensive vesicular or ulcerative genital lesions associated with inguinal lymphadenopathy, dysuria and fever. Recurrent genital herpes episodes are usually milder (except in an immunocompromised host) and are nearly always caused by HSV-2. Genital herpes is mainly diagnosed on clinical grounds; laboratory diagnosis is usually not essential.

Neonatal herpes is the most serious consequence of genital herpes infection. The virus is transmitted from the infected mother to the child during vaginal delivery. There is a need for rapid and reliable laboratory tests to detect HSV in asymptomatic infected pregnant women shortly before delivery, as well as to monitor neonates exposed to HSV at delivery.

Virus culture

Isolation of HSV in tissue culture remains the diagnostic laboratory method of choice. Culture performed on fresh vesicular lesions has a sensitivity of more than 90 percent. Culture from pustular lesions is positive in 70 to 80 percent of cases, whereas only 25 percent of crusted lesions give a positive culture result. Cultures from primary infection lesions recover a significantly higher amount of virus than culture from recurrent lesions (63-65).

Cytopathic effect (CPE) typical of HSV can be recognised by a rounding of scattered culture cells, visible after one to seven days of incubation, depending on the concentration of virus in the clinical specimen. Other viruses may exhibit CPE similar to HSV; definite identification of HSV is recommended when an unusual type of CPE occurs or when specimens come from asymptomatic people. Identification and typing of HSV may also be useful for epidemiological and research purposes. Virus isolates can be confirmed as HSV and typed as HSV-1 or HSV-2 by neutralisation tests, immunologic assays or nucleic acid hybridization.

Direct detection methods

Nonculture procedures are more practical for routine diagnosis of HSV infections because tissue culture facilities are not widely available. Detection of HSV antigen by immunologic techniques is currently the most common rapid diagnostic method. Immunologic procedures include immunofluorescence, immunoperoxidase, and enzyme-linked immunosorbent assay. The sensitivity of these antigen detection methods seems to vary between 70 and 95 percent (66,67).

A more recent approach to rapid HSV diagnosis is DNA hybridization (68). The amplification of DNA sequences by polymerase chain reaction offers a highly sensitive method useful for detecting HSV in asymptomatic pregnant women at term (69).

Papanicolaou and Tzanck staining are no longer considered appropriate techniques for the diagnosis of genital herpes (63). Although they are simple, inexpensive methods and can be used to demonstrate cytologic changes, such as giant cells or cells with intranuclear inclusions in smears from lesions and cervical specimens, they are not specific for HSV and have a low sensitivity compared to cell culture.

Serology

Serological tests for HSV antibody detection can contribute to diagnosing a primary infection episode if seroconversion or a fourfold or greater rise in antibody titer is observed between an acute phase serum sample and a convalescent serum obtained 10 to 14 days later. In patients with recurrent infection, a significant antibody rise occurs in less than 10 percent of cases.

HSV antibody procedures include complement fixation, indirect immunofluorescence, neutralisation technique, latex agglutination, haemagglutination, and enzyme immunoassay. All these methods are sensitive for detection of IgG antibodies but cannot discriminate between recent and past HSV infection on a single serum sample. Most of the commercially available tests cannot effectively differentiate between HSV-1 and HSV-2 infection because of extensive cross-reactivity. The major targets of serum antibodies are viral surface glycoproteins, and most of the immunogenic epitopes are common to both HSV-1 and HSV-2 types. Recently several new proteins specific for HSV-1 and HSV-2 have been defined, including the glycoprotein gG of HSV-1, which differs significantly from the gG of HSV-2, and solid phase ELISA procedures using purified recombinant gG2 glycoprotein to specifically detect antibody to HSV-2 have become available. These tests can be used to determine specific IgG antibodies in patients exposed to HSV-2, including individuals who were previously infected with HSV-1 or other herpes viridae (70).

Chancroid

Chancroid is caused by *Haemophilus ducreyi* and is transmitted sexually by direct invasion of the organism through healthy or abraded skin and mucosa. The disease starts with a painful papule at the site of infection, resulting in a single or in multiple ulcers. Inguinal lymphadenopathy may be present in up to 50 percent of patients. Extensive and persistent genital ulcers without inguinal bubo development may be observed in patients with immunosuppression caused by HIV infection. Due to the atypical presentation and superinfection of the ulcers, the accuracy of a clinical diagnosis varies between 40 and 80 percent.

Isolation and identification of H. ducreyi

To date, an accurate diagnosis of chancroid depends on the ability to culture *H. ducreyi*. Different media formulations have been used to isolate the organism with varying success. It has been shown

that parallel use of both gonococcal (GC) and Mueller-Hinton (MH) media may increase the isolation rate of *H. ducreyi* from ulcers to above 80 percent.

A presumptive identification of *H. ducreyi* may be based on colony characteristics, Gram stain, production of β -lactamase and oxidase reaction. Colonies are nonmucoid, raised, granular, grayish-yellow in colour, and can be pushed intact across the surface of the agar with an inoculating loop. They can be either translucent or opaque, and this variability in opacity gives the impression of a mixed, nonpure culture. *H. ducreyi* is a fastidious organism with limited biochemical activity. Haemin is required to initiate growth. Nitrate reduction and alkaline phosphatase are also important characteristics. *H. ducreyi* is oxidase-positive when tested with tetramethyl-p-phenylenediamine and almost 100 percent of isolates are β -lactamase positive.

Direct detection methods

Direct examination of ulcer material on Gram stained smears may contribute to the diagnosis of chancroid if typical small, Gram-negative bacilli grouped in chains or as "schools of fish" are observed. These typical features, however, are infrequently seen on smears from patients with culture-proven chancroid, resulting in a sensitivity of much less than 50 percent for the direct Gram stain (71-73). In addition, because most genital ulcers harbour polymicrobial flora due to secondary contamination, the presence of Gram-negative bacilli may be misleading and frequently results in a false-positive diagnosis (73-74). As a result of its low sensitivity and low specificity, a Gram stained smear is not recommended for the diagnosis of chancroid.

Non-culture antigen and nucleic acid methods for *H. ducreyi* have been developed, but are not yet used in routine diagnosis. Fluorescein-labelled and enzyme-labelled monoclonal antibodies have been used to detect *H. ducreyi* in clinical specimens with varying success (75,76). DNA probes have been shown to be 100 percent sensitive and specific for the identification of bacterial isolates, but their usefulness for direct diagnosis is not been established (77,78). DNA amplification of different *H. ducreyi* sequences offers diagnostic technology superior to culture. The most accurate diagnostic procedure for diagnosing genital ulcer disease is a multiplex PCR to detect simultaneously *T. pallidum*, herpes simplex virus, and *H. ducreyi* (79).

Serology

The usefulness of serological tests for the diagnosis of active *H. ducreyi* infection is very limited, but serology has proven to be valuable for research and epidemiologic purposes.

The development of reliable serological tests depends on detailed information about the host immune response as well as antigen presentation by the infectious organism. Data on these mechanisms for chancroid and *H. ducreyi* are very limited. Clinical experience and experimental inoculation studies in humans suggest that there is probably no acquired immunity to *H. ducreyi*. More research is needed, however, to determinate its antigenic composition, including specific immunogenic epitopes, and the kinetics of the humoral immune response to *H. ducreyi* in treated and nontreated patients with primary and repeated episodes of chancroid.

Circulating serum IgG and IgM antibodies to *H. ducreyi* have been detected with dot immunobinding and enzyme-linked immunosorbent assays (75,80). With both methods, a qualitative and quantitative variation in antibody response is observed in patients with recent or past history of chancroid but the factors influencing these response variations are not yet clearly understood.

Antimicrobial susceptibility testing

During the past two decades chromosomal resistance and high-level plasmid-mediated resistance have significantly increased among *H. ducreyi* isolates. Resistance patterns of clinical isolates, however, can vary greatly in geographically diverse areas. It has been observed that chancroid treatment failures are much more common in HIV-positive patients, but it is not yet clear whether treatment failure is significantly associated with HIV status or with increased antimicrobial resistance of *H. ducreyi* isolates.

To date, no standardised procedures exist for susceptibility testing of *H. ducreyi*; the only practical and reliable method is the agar dilution technique for determining minimum inhibitory concentrations. Commonly used media are MH agar or GC agar enriched with 1 percent haemoglobin, 5 percent foetal calf serum, and 1 percent IsoVitaleX. Antimicrobial susceptibility testing of *H. ducreyi* is a cumbersome technically delicate procedure and can only be successfully performed in specialised reference laboratories.

Donovanosis

Donovanosis (granuloma inguinale) is a chronic infection involving the skin, mucous membranes and lymphatics of the genitalia and perineal area. The disease starts with a subcutaneous nodule at the site of infection. This nodule erodes through the skin to form a beefy, red, granulomatous ulcer. Inguinal lymph nodes may become involved; the disease may spread haematogenously and may even result in cutaneous lesions at extragenital body sites.

Donovanosis is caused by *Calymatobacterium granulomatis*, which cannot be cultured on artificial media. Laboratory diagnosis depends on the visualisation of Donovan bodies in smears from clinical lesions.

Direct microscopy

The sensitivity of microscopy of tissue samples crushed between two slides is below 40 percent for patients with clinically suspected lesions of donovanosis. Histological aspects in sections of a biopsy may be helpful to differentiate between donovanosis and other conditions. An ulcer with a mixed inflammatory infiltrate of plasma cells, neutrophils and histiocytes and with a conspicuous absence of lymphocytes, suggests granuloma inguinale. Demonstration of characteristic intracellular organisms (Donovan bodies) by Warthin-Stary silver impregnation is diagnostic (81).

Serology

Antibodies to *C. granulomatis* have been observed using a complement fixation method in sera from patients whose lesions persisted for more than three months. More recently, a successful indirect immunofluorescence technique has been described. Sera from patients are applied to biopsy tissue sections containing Donovan bodies and treated with antihuman IgG conjugated with fluorescein isothiocyanate. In the absence of culture methods or reliable and simple nonculture detection tests, this serological assay may prove valuable for the diagnosis of donovanosis (82).

Candidiasis

Vulvovaginal candidiasis is caused by *Candida albicans* in approximately 85 percent of cases, with the remaining cases caused by other species, particularly by *C. glabrata* (83). Classic clinical symptoms and signs of candidiasis include vaginal itching, vulvar burning, external dysuria, curdy

white discharge (that looks like cottage cheese) without malodour, and erythema of the labia and vulva. Symptoms and signs, however, are often less specific, and laboratory diagnosis is essential for accurate differential diagnosis.

Direct microscopy

The yeast form is easily recognised in a wet mount preparation of vaginal fluid as round to oval cells of 4–8 µm diameter with typical budding. Adding a drop of 10 percent KOH to the preparation may facilitate the detection of yeasts, in particular the recognition of mycelia (pseudohyphae). Yeasts are Gram-positive and can easily be observed in a Gram-stained smear. The sensitivity of a wet mount, however, is superior.

Culture

Culture remains the most sensitive method currently available for the detection of *Candida*. However, it should be stressed that a positive culture does not necessarily indicate that *Candida* is responsible for vaginal symptoms, as more than 20 percent of healthy women may harbour *Candida* in the vagina. Microscopy has a much higher diagnostic value. Few patients with symptomatic vaginal candidiasis have negative microscopy. Consequently, culture may only be useful if vaginal candidiasis is clinically suspected in the presence of a negative wet mount preparation (84).

Colonies of yeasts appear after one or two days incubation at 36°C and are white opaque to creamy. The only important identification of isolates consists of microscopic differentiation from bacteria. Additional confirmation is not essential for routine diagnosis of vaginal candidiasis.

Trichomoniasis

Trichomoniasis is considered mainly sexually transmitted. Nonvenereal acquisition through fomites may be possible, but is not well documented. *Trichomonas vaginalis* elicits an acute inflammatory response resulting in vaginal discharge containing large numbers of polymorphonuclear neutrophils. Typical symptoms associated with trichomoniasis include vaginal itching or irritation and frothy gray to green-yellow discharge. Vaginal malodour and dysuria may be present.

The infection is caused by *T. vaginalis*, an ovoid globular, pear-shaped flagellated protozoan. Although certain symptoms and signs are predictive for trichomoniasis, visualisation of the parasite is required to establish the diagnosis (85).

Direct microscopy

Trichomonads are easily recognised in a wet mount preparation of vaginal fluid by their typical jerky motility. An increased number of polymorphonuclear leukocytes is usually observed, but small numbers of leukocytes do not rule out infection.

Other diagnostic methods

Culture of *T. vaginalis* is currently the most sensitive method for diagnosing trichomoniasis and may be recommended when vaginal infection is suspected despite negative wet mount results, for diagnosis of trichomoniasis in men, and for research.

Various direct detection methods for *T. vaginalis*, including immunofluorescence, latex agglutination, and enzyme-linked immunosorbent assay, have been described. A recently developed

antigen immunoassay seems to be comparable in sensitivity and specificity to culture (86). Several methods for antibody detection against *T. vaginalis* in serum and in vaginal washings have been evaluated but did not contribute to a more adequate diagnosis of trichomoniasis.

Bacterial vaginosis

Bacterial vaginosis (BV) is a clinical entity characterised by slightly increased quantities of malodourous vaginal discharge. It is associated with overgrowth of the normal bacterial flora of the vagina with *Gardnerella vaginalis*, *Mycoplasma hominis* and various anaerobic bacteria, such as *Bacteroides* and *Mobiluncus* species.

Diagnostic procedures

The diagnosis of BV is based on the presence of at least three of the four following characteristics (87,88):

- Homogenous white-gray adherent discharge: Interpretation of this clinical sign is subjective. Discharge seen in women with BV is often not markedly increased over that seen in healthy women; the application of vaginal douches may reduce the amount of discharge.
- Increased vaginal pH: The normal mature vagina has an acid pH of w 4.0. In BV, the pH is generally elevated to more than 4-5. The vaginal pH test has the highest sensitivity of the four characteristics, but the lowest specificity. An elevated pH is also observed if vaginal fluid is contaminated with menstrual blood, cervical mucus or semen, and in *T. vaginalis* infection.
- Malodour: Women with BV often complain of vaginal malodour, which is due to the release of amines produced by anaerobic bacteria that decarboxylate lysine to cadaverine and arginine to putrescine. If a drop of 10 percent KOH is added to the vaginal fluid, the amines immediately become volatile, producing a typical fishy amine odour.
- Presence of clue cells: These cells are squamous epithelial cells covered with many small coccobacillary organisms. Microscopy of a wet mount shows stippled granular cells without clearly defined edges because of the large numbers of adherent bacteria present and an apparent disintegration of the cells. The adhering bacteria are predominantly *G. vaginalis*, sometimes mixed with anaerobes.

Confirmatory laboratory testing

A Gram stain of a vaginal smear has a higher specificity for the detection of clue cells than a wet mount preparation. Moreover, a Gram stain allows good evaluation of the vaginal bacterial flora. Normal vaginal fluid contains predominantly *Lactobacillus* species and exceedingly low numbers of streptococci and coryneform bacteria. In BV, lactobacilli are replaced by a mixed flora of anaerobic bacterial morphotypes and *G. vaginalis*. The Nugent scoring system for Gram stain is a weighted combination of lactobacilli, *G. vaginalis* or *Bacteroides* (small Gram-variable or Gram-negative rods) and curved Gram-variable rods (*Mobiluncus*) (89). This standardized 0-10 scoring system is presented in **Table 4**. Each morphotype is quantitated from 1 to 4 + with regard to the number of morphotypes per oil immersion field. The sum of the weighted quantitations of the three morphotypes yield a score of 0 to 10. The criterion for BV is a score of 7 or higher, a score of 4 to 6 is considered intermediate, and a score of 0 to 3 is considered normal.

Table 4: Nugent's scoring system for Gram-stained vaginal smears^a			
<i>Score^b</i>	<i>Lactobacillus morphotype</i>	<i>Gardnerella & Bacteroides spp morphotypes</i>	<i>Curved Gram variable rods</i>
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

a: Morphotypes are scored as the average number seen per oil immersion field. Note that less weight is given to curved Gram-variable rods. Total score = lactobacilli + *G. vaginalis* and *Bacteroides* spp + Gram-variable rods.

b: 0 = no morphotypes present; 1 = <1 morphotype present; 2 = 1 to 4 morphotypes present; 3 = 5 to 30 morphotypes present; 4 = > 30 morphotypes present.

HIV

Several different types of laboratory tests for detecting HIV antibody in human serum exist today. The selection of the most appropriate test or combination of tests to use depends on three criteria: (1) the objectives of HIV testing, (2) the sensitivity and specificity of the tests being used, (3) the prevalence of HIV infection in the population being tested.

Objectives of HIV antibody testing

The three main objectives for which HIV antibody testing is performed are:

- Transfusion and transplant safety: Screening of blood and blood products, and of organs, tissues, sperm or ova from donors.
- Surveillance: Unlinked and anonymous testing of serum for monitoring the prevalence and trends in HIV infection over time in a given population.
- Diagnosis of HIV infection: Voluntary testing of serum from asymptomatic individuals or from persons with clinical signs and symptoms suggestive of HIV infection or AIDS.

Sensitivity and specificity of HIV tests

Sensitivity and specificity are two major factors that determine a test's accuracy in distinguishing between infected and uninfected persons. Only tests of the highest possible sensitivity should be used when there is a need to minimize the rate of false negative results (e.g., in transfusion). A test with a high specificity will have few false positive results and should be used when there is a need to minimize the rate of false positive results (e.g., in diagnosis).

Prevalence of HIV infection

The probability that a test will accurately determine the true infection status of a person varies with the prevalence of HIV infection in the population from which the person comes. In general, the higher the HIV prevalence, the greater the probability that a person testing positive is truly infected. Thus, with increasing prevalence, the proportion of false positive samples decreases; conversely, the likelihood that a person with a negative test result is truly infected, decreases as prevalence increases.

Strategies for HIV antibody testing

As HIV antibody assays have become extremely sensitive over the years, the probability of a false positive reaction in two assays based on a different principle is not negligible. Therefore, if test combinations are not carefully selected, individuals may be wrongly diagnosed as HIV seropositive. Conversely, the most specific assays are slightly less sensitive as compared to the average HIV antibody test, which may result in a false negative diagnosis. The choice of the most appropriate HIV tests also depends on the HIV variants present in a particular geographical area (e.g. HIV-1 group O).

Studies have shown that combinations of ELISA and/or simple rapid assays such as dot immunoassays and agglutination can provide results as reliable, and in some instances more reliable than the ELISA/Western blot (WB) combination, and at much lower cost. Confirmatory tests such as WB or line immunoassays, should only be used to resolve indeterminate results for diagnostic purposes.

Strategy I (for transfusion/transplant safety)

All serum/plasma is tested with one ELISA or simple/rapid assay. Reactive samples are considered HIV antibody positive and nonreactive samples are considered HIV antibody negative. The test selected for this strategy should preferably be a combined HIV-1/HIV-2 assay which is highly sensitive. The specificity of the chosen test should be at least 95 percent. If a blood or tissue donor is to be notified of a test result, testing strategy III for diagnosis must be applied.

Strategy II (for surveillance)

All serum/plasma is first tested with one ELISA or simple/rapid test. Any sample found reactive on the first assay is retested with a second ELISA or simple/rapid assay based on different antigen preparation and/or different test principle (e.g., indirect versus competitive). Samples that are positive on both tests are considered HIV antibody positive. Samples, nonreactive on the first test, are considered HIV antibody negative. Any sample that is reactive on the first test but nonreactive on the second test, should be retested with the two assays. Concordant results after repeat testing will indicate a positive or negative result. If the results of the two assays remain discordant, the sample is considered indeterminate. For surveillance no further testing is needed; indeterminate results should be reported and analysed separately.

Strategy III (for diagnosis)

All serum is first tested with one ELISA or simple/rapid assay, and any reactive samples are retested using a different assay. Serum that is nonreactive on the first test is considered HIV antibody negative. Serum that is reactive in the first test but nonreactive in the second assay should be repeated with both tests. Concordant negative results after retesting will indicate HIV antibody negative. Sera found positive in the first assay and positive or negative in the second assay, even after retesting, should always be tested with a third assay. Serum reactive on all three tests is considered HIV antibody positive. Samples reactive in the first test only or reactive in two of the three tests are considered indeterminate for individuals who may have been exposed to HIV in the last three months and negative for those who have not been exposed to any risk for HIV infection.

For newly diagnosed HIV seropositives, an additional blood sample should be obtained and tested to help eliminate possible technical or clerical error.

Serum from people with clinical signs of AIDS may have an indeterminate result due to a decrease in antibodies. In this case, serum does not normally need to be retested.

For diagnosis of HIV infection in asymptomatic individuals, with an indeterminate result, a second blood sample should be obtained after a minimum of 2 weeks following the first sample and should be tested with the same strategy. If the second serum sample also produces an indeterminate result, it should be tested with a confirmation test (WB or line immunoassay). However, if this result is also indeterminate longer follow-up may be required. If the results remain indeterminate after one year, the person is considered to be HIV antibody negative (90).

Antimicrobial susceptibility surveillance of *N. gonorrhoeae*

Rationale

Surveillance of the antibiotic susceptibility of *N. gonorrhoeae* is a major public health issue. In many countries antibiotics currently recommended by the World Health Organisation (WHO) or Centers for Disease Control (CDC) for gonococcal infections are neither available to nor affordable for STD patients. It may be possible to recommend less expensive drugs (i.e. trimethoprim/sulfamethoxazole or kanamycin), provided their efficacy is regularly monitored. Susceptibility surveillance data are used primarily to help develop and update appropriate treatment guidelines for managing gonococcal infections at the primary health care level. Susceptibility testing on an individual basis is not justified.

Susceptibility testing may be recommended in the following circumstances:

- In reference laboratories for epidemiological investigations to provide susceptibility information and monitor trends in drug resistance.
- In STD laboratories that conduct a high volume of tests to help monitor the clinical efficacy of recommended treatment regimens.
- In studying new antimicrobial agents.
- In providing information to clinicians in cases of treatment failure.

It is important to remember that treatment failure may be due to gonococcal resistance to currently recommended drugs, but more common reasons include poor compliance with therapy, reinfection or coinfection with *Chlamydia trachomatis*. If performed without rigorous standardisation, *in vitro* antimicrobial susceptibility testing may generate unreliable results.

Antibiotics to be tested

Penicillin and tetracycline are tested for epidemiological rather than practical purposes, since gonococcal resistance to both drugs is usually high in many countries. Less expensive drugs that should be tested include trimethoprim/sulfamethoxazole and the aminoglycosides. Resistance to fluoroquinolones is rapidly increasing when they are widely used, making monitoring essential. Spectinomycin and second- and third-generation cephalosporins are still highly effective, but monitoring could document the emergence of resistant strains.

Laboratory methodology

Antimicrobial resistance in *N. gonorrhoeae* is both chromosome- and plasmid-mediated. Penicillinase-producing *N. gonorrhoeae* (PPNG), also termed beta-lactamase-positive gonococci, refers to plasmid-mediated resistance to penicillin, which is detectable with simple and inexpensive tests. Tetracycline-resistant *N. gonorrhoeae* (TRNG) refers to plasmid-mediated resistance to tetracycline. No simple test can detect TRNG. The corresponding plasmid must be identified with

biomolecular techniques. Alternatively, resistance to tetracycline is assumed to be plasmid-mediated when reaching levels far beyond those observed with chromosomal resistance.

Antimicrobial susceptibility testing

The agar-plate dilution technique is the reference method for quantitative testing of chromosome-mediated resistance to antibiotics (91). Bacterial growth is examined on culture media with various concentrations of antibiotics incorporated. Results are expressed in minimal inhibitory concentrations (MICs). Unfortunately, the technical requirements for this method make it inaccessible in developing countries, except in a few experienced reference laboratories.

The disc-diffusion technique involves examining bacterial growth on culture plates around calibrated paper discs impregnated with antibiotics. It is both economic and simple to use. Unfortunately, with *N. gonorrhoeae*, this method has been standardised only for penicillin, tetracycline, spectinomycin, and cephalosporins (92).

The more recent E-test, produced by AB Biodisk of Sweden, combines the advantages of the agar-dilution technique with the simplicity of disc testing (93). Plastic strips with continuous antibiotic gradients, allowing MIC determination, are applied onto the surface of culture plates. Several studies have shown that the E-test is a reliable alternative to agar-dilution for *N. gonorrhoeae*. However, it is expensive and has not yet been fully recognised as a reference method.

Detection of plasmid-mediated antimicrobial resistance

PPNG: Common rapid β -lactamase detection techniques include (1) the acidometric method, which uses a pH indicator to detect increased acidity from cleavage of the β -lactam ring of penicillin; (2) the iodometric method, which detects a colour change caused by the reduction of iodine by penicilloic acid, (3) the chromogenic cephalosporin method, which detects a colour change of a chromogenic cephalosporin after hydrolysis of the β -lactam ring (94-96).

TRNG: High-level tetracycline-resistant isolates of *N. gonorrhoeae* carrying a conjugative plasmid have become endemic in different geographical areas. TRNG can be determined by testing its ability to grow on a medium containing 10 mg of tetracycline per liter. The MIC of tetracycline is ≤ 16 mg/liter (97).

Sampling for surveillance

Some Western countries conduct ongoing gonococcal susceptibility surveillance in sentinel STD clinics throughout the country. In developing countries, however, surveillance data are used primarily to validate treatment guidelines. Guideline validation is an intermittent process, so susceptibility surveillance may be conducted through specific surveys at regular intervals, particularly when laboratory resources are limited.

It is estimated that 100 to 150 gonococcal isolates need to be collected to measure a significant shift in antibiotic resistance between two surveys.

Consecutive male patients with visible urethral discharge attending primary health care facilities are the first choice for the sample population because gonococcal yields are the highest and it is easier to take specimens from men than from women. Enrolled patients should be representative of STD patients at first-encounter level within the formal health sector. Patients entering the survey should not have been referred from other health care services. Self-medication, widely practiced in many

developing countries, should not lead to exclusion from the survey but should be noted in the data collection process.

In some countries, male patients are very hard to reach because the stigma associated with STDs leads to widespread self-medication. Female commercial sex workers may be a second best option for a sample population, but specimen taking is more difficult because they are women, gonococcal prevalence may be lower, and the representativeness of the overall population questionable.

The number and geographical distribution of sites included in the survey should depend on how representative each site is of resistance patterns in the country as well as logistical constraints.

References

1. Handsfield H.H., Lipman T.O., Harnisch J.P., Tronka E., Holmes K.K. Asymptomatic gonorrhoea in men: diagnosis, natural course, prevalence and significance. *N Engl J Med* 290:117-123 (1974).
2. Sadof M.D., Woods E.R., Emans J.S. Dipstick leukocyte esterase activity in first-catch urine specimens. *JAMA* 258:1932-1934 (1987).
3. O'Brien S.F., Bell T.A., Farrow J.A. Use of a leukocyte esterase dipstick to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* urethritis in asymptomatic adolescent male detainees. *Am J Public Health* 78:1583-1584 (1988).
4. Shafer M.-A., Schachter J., Moscicki A.B., et al. Urinary leukocyte esterase screening test for asymptomatic chlamydial and gonococcal infections in males. *JAMA* 262:2562-2566 (1989).
5. Werner M.J., Biro F.M. Urinary leukocyte esterase screening for asymptomatic sexually transmitted diseases in adolescent males. *J Adolesc Health* 12:326-328 (1991).
6. Mayaud P., Chagalucka J., Grosskurth H., et al. The value of urine specimens in screening for male urethritis and its microbial aetiologies in Tanzania. *Genitourin Med* 68:361-365 (1992).
7. McNagny S.E., Parker R.M., Zenilman J.M., Lewis J.S. Urinary leukocyte esterase test: a screening method for the detection of chlamydial and gonococcal infections in men. *J Infect Dis* 165:573-576 (1992).
8. Goodhart M.E., Ogden J., Zaidi A.A., Kraus S.T. Factors affecting the performance of smear and culture tests for the detection of *Neisseria gonorrhoeae*. *Sex Transm Dis* 9:63-69 (1982).
9. Black C.M. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Microbiol Rev* 10:160-184 (1997).
10. Knapp J.S. Historical perspectives and identification of *Neisseria* and related species. *Clin Microbiol Rev* 1:415-431 (1988).
11. Kellog D.S., Turner E.M. Rapid fermentation confirmation of *Neisseria gonorrhoeae*. *Appl Microbiol* 25:550-552 (1973).
12. Philip A, Garton G.C. Comparative evaluation of five commercial systems for the rapid identification of pathogenic *Neisseria* species. *J Clin Microbiol* 22:101-104 (1985).
13. Dillon J.R., Carballo M., Pauzé M. Evaluation of eight methods for the identification of pathogenic *Neisseria* species: *Neisseria*-Kwik, RIM-N, Gonobio-Test, Minitex, Gonocheck II, GonoGen, Phadebact Monoclonal GC OMNI Test, and Syva MicroTrak Test. *J Clin Microbiol* 26:493-497 (1988).
14. D'Amato R.F., Enriquez L.A., Tomfohrde K.M., Singerman E. Rapid identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* by using enzymatic profiles. *J Clin Microbiol* 7:77-81(1978).

15. Lawton W.D., Battaglioli G.J. GonoGen coagglutination test for confirmation of *Neisseria gonorrhoeae*. *J Clin Microbiol* 18:1264-1265 (1974).
16. Hook E.W., Holmes K.K. Gonococcal infections. *Ann Intern Med* 102:229-243 (1985).
17. Granato P.A., Franz M.R. Evaluation of a prototype DNA probe test for the noncultural diagnosis of gonorrhoea. *J Clin Microbiol* 27:632-635 (1989).
18. Zubrzycki L. Non-culture tests for the diagnosis of gonorrhoea. *Adv Exp Med Biol* 263:77-88 (1990).
19. Rein M.F. Gonorrhoea. *Curr Opin Infect Dis* 4:12-21 (1991).
20. Taylor-Robinson D. The value of non-culture techniques for diagnosis of *Chlamydia trachomatis* infections: making the best of a bad job. *Eur J Microbiol Infect Dis* 11:499-503 (1992).
21. Ripa K.T., Mårdh P.-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 6:328-331 (1977).
22. Stephens R.S., Kwo C.C., Tam M.R. Sensitivity of immunofluorescence with monoclonal antibodies for detection of *Chlamydia trachomatis* in cell culture. *J Clin Microbiol* 16:4-7 (1982).
23. Jones R.B., Katz B.P., Van der Pol B., Caine V.A., Batteiger B.E., Newhall W.J. Effect of blind passage and multiple sampling on recovery of *Chlamydia trachomatis* from urogenital specimens. *J Clin Microbiol* 24:1029-1033 (1986).
24. Schachter J., Martin D.H. Failure of multiple passages to increase chlamydial recovery. *J Clin Microbiol* 25:1851-1853 (1987).
25. Tam M.R., Stamm W.E., Handsfield H.H., et al. Culture independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. *N Engl J Med* 310:1146-1150 (1984).
26. Livengood C.H., Schmitt J.W., Addison W.A., Wrenn J.W., Magruder-Habib K. Direct fluorescent antibody testing for endocervical *Chlamydia trachomatis*: factors affecting accuracy. *Obstet Gynecol* 72:803-809 (1988).
27. Barnes R.C. Laboratory diagnosis of human chlamydial infections. *Clin Microbiol Rev* 2:119-136 (1989).
28. Lipkin E.S., Moncada J.V., Shafer M.A., Wilson T.E., Schachter J. Comparison of monoclonal antibody staining and culture in diagnosing cervical chlamydial infection. *J Clin Microbiol* 23:114-117 (1986).
29. Pothier P., Komierczak A. Comparison of cell culture with two different chlamydia tests using immunofluorescence or enzyme-linked immunosorbent assay. *Eur J Clin Microbiol* 5:569-572 (1986).
30. Hipp S.S., Yangsook H., Murphy D. Assessment of enzyme immunoassay and immunofluorescence for detection of *Chlamydia trachomatis*. *J Clin Microbiol* 25:1938-1943 (1987).
31. Ridgway G.L., Taylor-Robinson D. Current problems in microbiology: 1. Chlamydial infections: which laboratory test? *J Clin Pathol* 44:1-5 (1991).
32. Plummer D., Garland S., Denham I. Testing for *Chlamydia trachomatis*: a double blind exercise? *Venereol* 4:63-65 (1991).
33. Moncada J., Schachter J., Bolan G., et al. Confirmatory assay increases specificity of the Chlamydiazyme test for *Chlamydia trachomatis* infection of the cervix. *J Clin Microbiol* 28:1770-1773 (1990).
34. Van Dyck E., Samb N., Dieng Sarr A., et al. Accuracy of two enzyme immunoassays and cell culture in the detection of *Chlamydia trachomatis* in low and high risk populations in Senegal. *Eur J Microbiol Infect Dis* 11:527-534 (1992).
35. Grossman J.H., Rivlin M.E., Morrison J.C. Diagnosis of chlamydial infection in pregnant women using the Testpack *Chlamydia* diagnostic kit. *Obstet Gynecol* 77:801-803 (1991).

36. Thomas B.J., MacLeod E.J., Taylor-Robinson D. Evaluation of sensitivity of 10 diagnostic assays for *Chlamydia trachomatis* by use of a simple laboratory procedure. *J Clin Pathol* 46:912-914 (1993).
37. Palva A., Jansimies-Somer H., Saikku P., Väänänen P., Söderland H., Ranki M. Detection of *Chlamydia trachomatis* by nucleic acid sandwich hybridization. *FEMS Microbiol Lett* 23: 83-89 (1984).
38. Loeffelholz M.J., Lewinski C.A., Silver S.R., et al. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. *J Clin Microbiol* 30:2847-2851 (1992).
39. Schachter J., Stamm W.E., Quin T.C., Andrews W.W., Burczak S.D., Lee H.H. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. *J Clin Microbiol* 32:2540-2543 (1994).
40. Pasternack R., Vuorinen P., Niittinen A. Evaluation of the Gen-Probe *Chlamydia trachomatis* Transcription-Mediated Amplification assay with urine specimens from women. *J Clin Microbiol* 35:676-678 (1997).
41. Paul I.D., Caul E.O. Evaluation of three *Chlamydia trachomatis* immunoassays with an unbiased, noninvasive clinical sample. *J Clin Microbiol* 28:220-222 (1990).
42. Schwebke J.R., Clark A.M., Pettinger M.B., Nsubuga P., Stamm W.E. Use of an urine enzyme immunoassay as a diagnostic tool for *Chlamydia trachomatis* urethritis in men. *J Clin Microbiol* 29:2246-2249 (1991).
43. Kok T.-W., Payne L.E., Bailey S.E., Waddell R.G. Urine and the laboratory diagnosis of *Chlamydia trachomatis* in men. *Genitourin Med* 69:51-53 (1993).
44. Sellors J.W., Mahony J.B., Jang D., et al. Comparison of cervical, urethral and urine specimens for the detection of *Chlamydia trachomatis* in women. *J Infect Dis* 164: 205-208 (1991).
45. Lee H.H., Chernesky M.A., Schachter J., et al. Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain assay of urine. *Lancet* 345:213-216 (1995).
46. Pankku M., Puolakkainen M., Apter D., Hirvonen S., Paavonen J. First-void urine testing for *Chlamydia trachomatis* by polymerase chain reaction in asymptomatic women. *Sex Transm Dis* 24:343-346 (1997).
47. Mouton J.W., Verkooyen R., van der Meijden W., et al. Detection of *Chlamydia trachomatis* in male and female urine specimens by using the amplified *Chlamydia trachomatis* test. *J Clin Microbiol* 35: 1369-1372 (1997).
48. Hook E.W., Smith K., Mullen C., et al. Diagnosis of genitourinary *Chlamydia trachomatis* infections by using the ligase chain reaction on patient-obtained vaginal swabs. *J Clin Microbiol* 35:2133-2135 (1997).
49. Taylor-Robinson D., Thomas B.J. Laboratory techniques for the diagnosis of chlamydial infections. *Genitourin Med* 67: 256-266 (1991).
50. Wang S.-P., Grayston J.T. Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organisms in a new microtiter indirect immunofluorescence test. *Am J Ophthalmol* 70:367-374 (1970).
51. Yobs A.R., Brown L., Hunter E.F. Fluorescent antibody technique in early syphilis. *Arch Pathol* 77:220-225 (1964).
52. U.S. Public Health Service. Manual of tests for syphilis. 1955. Washington DC: U.S. Government Printing Office, 1955: PHS publication n° 411.
53. U.S. Public Health Service. Serology evaluation and research assembly 1956-1957. Washington, DC: U.S. Government Printing Office, 1959: PHS publication n° 650:92-93.
54. Portnoy J. Modification of the rapid plasma reagin (RPR) card test for syphilis, for use in large scale testing. *Am J Clin Pathol* 40: 473-479 (1963).

55. March R.W., Stiles G.E. The reagin screen test: a new reagin card test for syphilis. *Sex Transm Dis* 7:66-70 (1980).
56. Pettit D.E., Larsen S.A., Harbec P.S., et al. Toluidin red unheated serum test (TRUST). A non treponemal test for syphilis. *J Clin Microbiol* 18:1141-1145 (1983).
57. Hunter E.F., Deacon W.E., Meyer P.E. An improved test for syphilis-the absorption procedure (FTA-Abs). *Public Health Reports* 17:410-412 (1964).
58. Cox P.M., Logan L.C., Norins L.C. Automated, quantitative microhemagglutination assay for *Treponema pallidum* antibodies. *Appl Microbiol* 18:485-489 (1969).
59. Wentworth B.B., Thompson M.A., Peter C.R., Bowdon R.E., Wilson D.L. Comparison of a hemagglutination treponemal test for syphilis (HATTS) with other serologic methods for the diagnosis of syphilis. *Sex Transm Dis* 5:103-111 (1978).
60. Stevens R.W., Schmitt M.E. Evaluation of an enzyme-linked immunosorbent assay for treponemal antibody. *J Clin Microbiol* 21:399-402 (1985).
61. Young H., Moyes A., McMillan A., Robertson D.H. Screening for treponemal infection by a new enzyme immunoassay. *Genitourin Med* 65:72-78 (1989).
62. Van Dyck E., Bogaerts J., Piot P. Rapid Plasma Reagin card test: Evaluation of a hand rotation assay and stability of the RPR antigen. *WHO Bull* 72:741-743 (1994).
63. Corey L., Spear P.G. Infection with herpes simplex viruses. *N Engl J Med* 314:686-691; 749-757 (1986).
64. Corey L. Laboratory diagnosis of herpes simplex virus infections, principles guiding the development of rapid diagnostic tests. *Diagn Microbiol Infect Dis* 4:111 S-119S (1986).
65. Lafferty W.E., Kroft S., Remington M., et al. Diagnosis of herpes simplex virus by direct immunofluorescence and viral isolation from samples of external genital lesions in a high-prevalence population. *J Clin Microbiol* 25:323-326 (1987).
66. Schmidt M.J., Denis J., Devlin V., Gallo D., Mills J. Comparison of direct immunofluorescence and direct immunoperoxidase procedures for detection of herpes simplex virus antigen in lesion specimens. *J Clin Microbiol* 18:445-448 (1983).
67. Nerurkar L.S., Namba M., Braskears G., Jacob A.J., Lee Y.S., Sever J.L. Rapid detection of herpes simplex virus in clinical specimens by use of a capture biotin-streptavidin enzyme-linked immunosorbent assay. *J Clin Microbiol* 20:109-114 (1984).
68. Redfield D.C., Richman D.D., Albanil S., Oxmanil M.N., Wahl G.M. Detection of herpes simplex virus in clinical specimens by DNA hybridization. *Diagn Microbiol Infect Dis* 1:117-128 (1983).
69. Hardy D.A., Arvin A.M., Yasukawa L.L., et al. Use of polymerase chain reaction for successful identification of asymptomatic genital infection with herpes simplex virus in pregnant women at delivery. *J Infect Dis* 162:1031-1035 (1990).
70. Lee F.K., Coleman R.M., Pererira L., Bailey P.D., Tatsuno M., Nahmias A.J. Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. *J Clin Microbiol* 22: 641-644 (1985).
71. Choudhary B.P., Kumari S., Bhati R., Agarwal D.S. Bacteriological study of chancroid. *Indian J Med Res* 76:379-385 (1982).
72. Coovadia Y.M., Kharsany A., Hoosen A. The microbial aetiology of genital ulcers in black men in Durban, South Africa. *Genitourin Med* 61: 266-269 (1985).
73. Sturm A.W., Stolting G.J., Gormane R.H., Zanen H.C. Clinical and microbiological evaluation of 46 episodes of genital ulceration. *Genitourin Med* 63:98-101 (1987).
74. Chapel T.A., Brown W.J., Jeffris C., Stewart J.A. The microbial flora of penile ulcerations. *J Infect Dis* 137:50-56 (1979).
75. Schalla W.O., Sanders L.L., Schmid G.P., Tam M.R., Morse S.A. Use of dot immunobinding and immunofluorescence assays to investigate clinically suspected cases of chancroid. *J Infect Dis* 153:879-887 (1986).

76. Karim H., Finn G., Easmon C., et al. Rapid detection of *Haemophilus ducreyi* in clinical and experimental infections using monoclonal antibody: a preliminary evaluation. *Genitourin Med* 65:361-365 (1989).
77. Parsons L., Shayegani M., Waring A.L., Bopp L.H. DNA probes for the identification of *Haemophilus ducreyi*. *J Clin Microbiol* 27:1431-1435 (1989).
78. Rossau R., Duhamel M., Jannes G., Decourt J.L., Van Heuverswyn H. The development of specific, rRNA-derived oligonucleotide probes for *Haemophilus ducreyi*, the causative agent of chancroid. *J Gen Microbiol* 137:277-285 (1991).
79. Orle K.A., Gates C.A., Martin D.H., Body B.A., Weiss J.B. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum* and herpes simplex virus types 1 and 2 from genital ulcers. *J Clin Microbiol* 34:49-54 (1996).
80. Museyi K., Van Dyck E., Vervoort T., Taylor D., Hoge C., Piot P. Use of an enzyme immunoassay to detect IgG serum antibodies to *Haemophilus ducreyi*. *J Infect Dis* 157:1039-1043 (1988).
81. Freinkel A.L. Histological aspects of sexually transmitted genital lesions. *Histopathol* 11:819-831 (1987).
82. Freinkel A.L., Dangor Y., Koornhof H.J., Ballard R.C. A serological test for granuloma inguinale. *Genitourin Med* 68:269-272 (1992).
83. Oriel J.D., Patridge B.M., Denny M.J. Coleman J.C. Genital yeast infections. *Br Med J* 4:761-764 (1972).
84. Sobel J.D. Vulvovaginal candidiasis. In: Holmes K.K., Mårdh P.-A., Sparling P.F., et al., eds, *Sexually Transmitted Diseases* 2nd ed. New York: McGraw-Hill, Inc: 515-523 (1990).
85. Rein M.F., Müller M. *Trichomonas vaginalis* and trichomoniasis. In: Holmes K.K., Mårdh P.-A., Sparling P.F., et al., eds. *Sexually Transmitted Diseases*. 2nd ed. New York: McGraw-Hill, Inc: 481-492 (1990).
86. Watt R.M., Philip A., Wos S.M., Sam G.J. Rapid assay for immunological detection of *Trichomonas vaginalis*. *J Clin Microbiol* 24:551-555 (1986).
87. Amsel R., Totten P.A., Spiegel C.A., Chen K.C., Eschenbach D., Holmes K.K. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 74:14-22 (1983).
88. Spiegel C.A. Bacterial vaginosis. *Clin Microbiol Rev* 4:485-502 (1991).
89. Nugent R.P., Krohn M.A., Hillier S.L. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J Clin Microbiol* 29:297-301 (1991).
90. World Health Organization and UNAIDS. Revised recommendations for the selection and use of HIV antibody tests (1997).
91. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. NCCLS document M7-A2 vol 10, n° 8 (1991).
92. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard. NCCLS document M2-A4 (1990).
93. Van Dyck E., Smet H., Piot P. Comparison of E test with agar dilution for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. *J Clin Microbiol* 32:1586-1588 (1994).
94. Thornsberry C., Kirven L.A. Ampicillin resistance in *Haemophilus influenzae* as determined by a rapid test for beta-lactamase production. *Antimicrob Agents Chemother* 6:653-654 (1974).
95. Rosenblatt J.E., Neumann A.M. A rapid slide test for penicillinase. *Am J Clin Pathol* 69:351-354 (1978).

96. O'Callaghan C.H., Morris A., Kirby S.M., Shinger A.H. Novel method for detection of β -lactarnase by using a chromogenic cephalosporin substrate. *Antimicrob Agents Chemother* 1:283-288 (1972).
97. Morse S.A., Johnson S.R., Biddle J.W., Roberts M.C. High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal tetM determinant. *Antimicrob Agents Chemother* 30: 664-670 (1986).