Control of Sexually Transmitted Diseases

A HANDBOOK FOR THE DESIGN AND MANAGEMENT OF PROGRAMS

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The STD Laboratory

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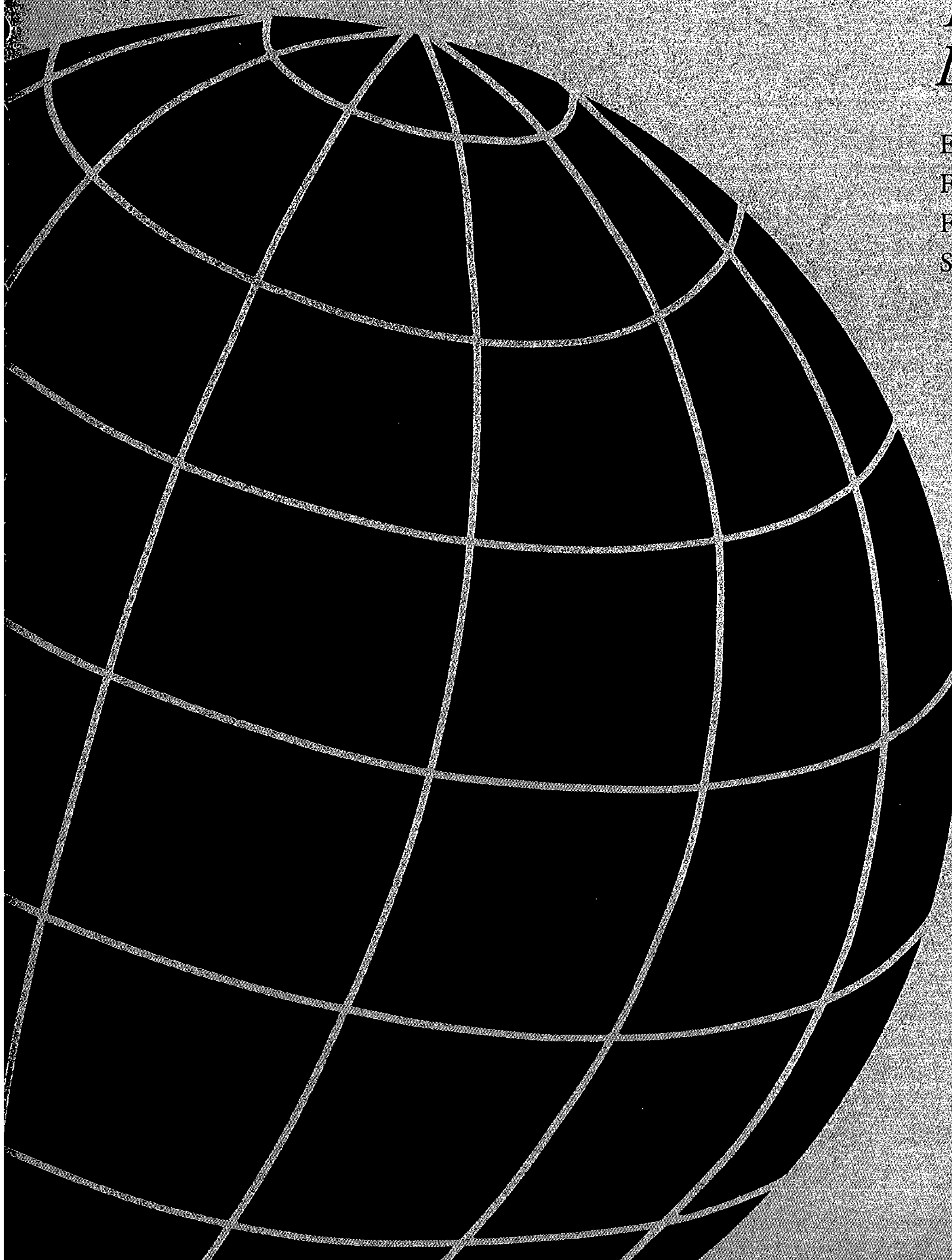

preview CHAPTER 12

The STD Laboratory

THE STD LABORATORY

This chapter helps STD program managers determine the appropriate level of laboratory support for their programs by providing criteria for selecting lab tests and describing how testing can complement the syndromic approach to STD case management.

ROLE OF THE LABORATORY

The primary role of the laboratory is to support decision making:

- In clinical practice
- For public health

CLINICAL PRACTICE

A laboratory can become involved in the following three types of STD activities:

- Diagnosis
- Case finding
- Screening

PUBLIC HEALTH

For public health purposes, laboratory tests are used to do the following:

- Help document the epidemiology of STDs
- Provide operational research
- Provide quality control

CRITERIA FOR LABORATORY TEST SELECTION

- Validity
- Reliability
- Feasibility
- Acceptability

LABORATORY ORGANIZATION

Laboratory procedures usually available at the three successive levels of clinical infrastructure are the following:

- **Peripheral:** microscopic examination of fresh and stained specimens, possibly KOH sniff test and nontreponemal syphilis screening test.

- **Intermediate:** microscopy, culture of *Neisseria gonorrhoeae,* antigen detection of *Chlamydia trachomatis,* and a number of serological tests, including HIV testing.
- **Central:** may be the same as at the intermediate level or more extensive, depending on the level of decentralization.

ANTIMICROBIAL SUSCEPTIBILITY SURVEILLANCE OF *N. GONORRHOEAE*

The recommended antibiotics for treating gonococcal infections are not available or affordable in many developing countries, so it is particularly important to monitor the susceptibility of less expensive antibiotics in these countries. Surveillance data can be used to develop and update appropriate treatment guidelines for managing gonococcal infections at the primary health-care level.

LABORATORY COSTS

Detailed cost-effectiveness studies of health care based on laboratory testing and diagnosis require assessment of indirect as well as direct costs (see section on cost of laboratory testing).

SYNDROMIC MANAGEMENT APPROACH

Use of the syndromic management approach *(see Chapter 8)* eliminates the need for diagnostic tests or simplifies the testing required. This approach is of adequate sensitivity and specificity for urethritis in males and genital ulcers in both sexes, but tends to have lower sensitivity and specificity when used for management of female genital discharge.

DIAGNOSTIC LABORATORY APPROACH

A laboratory approach is described for four major syndromes: (1) urethral discharge, (2) vaginal discharge, (3) lower abdominal pain, and (4) genital ulcer disease (GUD).

LABORATORY PROCEDURES

Laboratory procedures are described for the major STDs: gonorrhea, *Chlamydia trachomatis* infection, syphilis, genital herpes, chancroid, donovanosis, candidiasis, trichomoniasis and bacterial vaginosis. Table 1 shows the recommended diagnostic tests for each disease at each level of laboratory capability.

CONCLUSION

Many infections are asymptomatic, especially in women; even when symptomatic, they may have different causes. Simple, inexpensive diagnostic laboratory tests are urgently needed, particularly to detect *N. gonorrhoeae* and *C. trachomatis* in women.

INTRODUCTION

The syndrome approach to STD case management *(see Chapter 8)* is designed to improve STD diagnosis and treatment at health care facilities where laboratory tests are not available, but it can also be adapted for use when testing is available. Laboratory support is needed to detect infections in asymptomatic individuals, to identify serious infections that do not have specific signs and symptoms, to monitor resistance to antibiotics and to validate treatment algorithms.

This chapter is designed to help managers determine the appropriate level of laboratory support for their STD programs. It describes the role of the laboratory in STD control, criteria for selecting laboratory tests, and the organization of STD laboratories. It also explains when laboratory tests are needed and summarizes the laboratory procedures for detecting the major STDs.

ROLE OF THE LABORATORY IN STD CONTROL

The laboratory is meant to support decision making in STD control at both clinical and public health levels. Strengthening of laboratory infrastructure should always be subordinate to requirements for STD control interventions.

> **The primary role of the laboratory is to support decision making**
> - In clinical practice
> - For public health

CLINICAL PRACTICE

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

- **Diagnosis:** to assist in management of patients with symptoms of STD
- **Case finding:** detection of STD in patients seeking health care for other reasons
- **Screening:** voluntary assessment of STD among populations or selected individuals not seeking health care

Diagnosis of symptomatic patients

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 between mixed and single infections, reducing the administration of unnecessary antibiotics.

Case finding in asymptomatic individuals

Laboratory tests increase the sensitivity of STD diagnosis, allowing detection of infections in asymptomatic individuals. Case finding of asymptomatic STDs is most important in female patients, who carry the burden of STD complications and sequelae. Case finding is even more important in pregnant women because laboratory testing helps prevent the adverse consequences of syphilis, gonococcal and chlamydial infection in newborns *(see Chapter 9).*

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. Lab 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 LAB TEST SELECTION

The criteria for determining which lab test to use are similar to those for choosing any evaluation indicators.

> **Selection criteria of laboratory test include:**
> - Validity
> - Reliability
> - Feasibility *(for the laboratory)*
> - Acceptability *(including affordability)* for the patient

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" (positive culture or confirmed DNA amplification technique) presently in use for the diagnosis of *Chlamydia trachomatis* infection.

Lab 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 *Chlamydia* can be extremely sensitive and reliable in expert hands, but even a simple test such as Gram stain examination of urethral discharge can have a low validity and reliability when it is used by a sloppy technician. Increasingly reliable techniques ("idiot-proof") are constantly being developed by lab test manufacturers, but they can be expensive. In addition, even the most reliable technique yields poor results when it is improperly standardized or not monitored through quality control procedures.

Beyond 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 and refrigerated storage of reagents. As health facilities become more distant from urban centers, these requirements become more difficult to meet. Sophisticated techniques also tend to require more spare parts and specific reagents. Even in major public sector laboratories, provision of spare parts and reagents is frequently overlooked. A survey conducted in a Latin American country reached the conclusion that 70 percent of all lab equipment in the public sector nationwide was out of order because of missing spare parts or reagents.

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, especially with a cytobrush, is probably the least acceptable. Painful or invasive techniques may be more acceptable to symptomatic patients, but painless and easy 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 organization of laboratory activities.

Finally, acceptability includes affordability when patients must pay for lab services. As a general rule, lab expenditures should not exceed the treatment costs saved by testing. Acceptance of lab expenditures is the lowest for case finding or screening of asymptomatic subjects, including asymptomatic contacts of STD patients.

LABORATORY ORGANIZATION

CLINICAL PRACTICE

Clinical infrastructure in the public sector is organized at three successive levels. Patients are first 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 from there to central (national) facilities. Clinical laboratories are generally organized at the same three levels *(see Table 1).*

Peripheral laboratories are attached to health centers and first referral (health district) hospitals. Lab procedures at this level usually include microscopic examination of fresh and stained specimens. In syndromic

Table 1

Recommended Diagnostic Tests by Level of Laboratory Capability

Disease	Laboratory test	Laboratory level: Peripheral	Intermediate	Central/STD center
Gonorrhea	Smear (Gram, methylene blue, safranin)	+	+	+
	Culture	-	+	+
	ß-lactamase	-	(+)	+
	Antimicrobial susceptibility	-	-	+
Chlamydia trachomatis infection	Antigen detection			
	DFA	-	(+)	+
	ELISA	-	-	+
	DNA hybridization	-	-	(+)
	Culture	-	-	+
	Serology	-	-	+
Syphilis	Darkfield microscopy	-	(+)	+
	RPR	(+)	+	+
	TPHA	-	(+)	+
	FTA	-	-	+
	IgM	-	-	+
Genital herpes	Antigen detection	-	-	+
	Culture	-	-	+
	Serology	-	-	+
Chancroid	Culture	-	-	+
	Antimicrobial susceptibility	-	-	+
Donovanosis	Smear (Leishman, Wright)	-	(+)	+
	Histopathology	-	-	+
Trichomoniasis	Wet mount microscopy	+	+	+
	Culture	-	-	+
Candidiasis	Wet mount (cv. + 10% KOH)	+	+	+
	Culture	-	-	+
Bacterial vaginosis	Wet mount, stained smear pH, KOH snifftest	+	+	+

+ yes
(+) yes, if possible
- no

management of urethral discharge, microscopy helps single out nongonococcal infection. For vaginal discharge, microscopy helps differentiate trichomoniasis from candidiasis and bacterial vaginosis. Simple additional tests to identify bacterial vaginosis are the KOH sniff test and measurement of pH of vaginal fluid. Lab procedures may also include simple nontreponemal syphilis screening tests: rapid plasma reagin (RPR) or Venereal Disease Research Laboratory (VDRL).

It is strongly recommended that lab procedures included in STD diagnosis and case-finding guidelines at first-encounter level be available on site in order to optimize acceptability and coverage. RPR testing in pregnant women is a good illustration of a service that should be available at peripheral health centers. Systematic referral elsewhere of asymptomatic subjects for such a simple test is both expensive and time-consuming and significantly affects coverage.

Intermediate-level laboratories are commonly situated in regional or provincial hospitals. Their diagnostic requirements are greater, and better infrastructure and skilled manpower are available. Lab tests might include culture of *Neisseria gonorrhoeae* and identification of penicillinase-producing strains. They might also include antigen detection of *Chlamydia trachomatis,* depending on staff workload and available resources. Serology might include HIV testing (enzyme-linked immunosorbent assay [ELISA] or particle agglutination) as well as confirmatory tests for syphilis (fluorescent treponemal antibody [FTA] or microhemagglutination assay for antibodies to *T. pallidum* [MHA-TP]).

Central laboratories are usually located in the capital city or in a 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 organization of health services in a country. One central reference laboratory may be quite satisfactory in a small country. In big countries centralization of reference cases in the capital city is unrealistic; therefore, some decentralization of lab testing at the regional level is necessary.

Constraints to providing laboratory services in developing countries include:

- Shortages of supplies and reagents
- Failure of power and water supplies
- Inadequate maintenance of equipment
- Weak technical ability of personnel
- Lack of supervision and quality control
- Absence of continuing education
- 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, mislabeling of specimens, poorly maintained registers, and frequent personnel absences.

A few examples from the field illustrate the kind of problems resulting from these constraints:

- In a clinic with an average of at least 100 STD patients each day, women had to wait several hours for treatment until clinicians received the results of Gram stains performed on three cervical smears per women. When asked, the clinicians reported that they treated for gonococcal infection based upon their clinical impressions, even when the lab results returned negative, demonstrating a lack of confidence in the laboratory support services.
- In another clinic, pregnant women were routinely sent to a laboratory where technicians asked them to self-collect a vaginal specimen for wet mount and Gram stain using a swab. Vaginal swabs often arrived at the laboratory dried out, and Gram stain results mentioning the presence of any Gram-negative diplococci were interpreted by the clinicians as confirmatory of gonococcal infection.
- At a metropolitan clinic with a high volume of patients, lab tests such as wet mounts, Gram stains and gonococcal cultures were performed with

nonstandardized disc diffusion sensitivity testing on all men presenting with urethritis and all women presenting with vaginal discharge. As a result of such high-volume testing, the laboratory could not assure the necessary quality. A critical review of the indications for laboratory tests and laboratory techniques improved the quality of work and morale of the laboratory workers and the clinicians who depended on their results.

Introduction of STD diagnostic 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 logistical 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

Among the major public health needs for STD control are prevalence studies for guideline validation *(see Chapter 8)* or STD surveillance and antimicrobial susceptibility surveillance of *N. gonorrhoeae* *(see Chapter 15)*. The most important requirement for public health data is **reliability,** which depends on skilled manpower and quality control procedures. It is critical that centralized labs 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 decentralization 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.

Antimicrobial Susceptibility Surveillance of *N. gonorrhoeae*

Rationale

Surveillance of the antibiotic susceptibility of *N. gonorrhoeae* is a major public health issue. In many countries of the developing world, antibiotics currently recommended by WHO or CDC for gonococcal infections are neither available to nor affordable for STD patients. Given their variable resistance profile, it may be possible to recommend less expensive antibiotics such as cotrimoxazole or kanamycin, provided their efficacy is regularly monitored. Thus, 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.**

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 *N. gonorrhoeae,* 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.

The **agar-plate dilution technique** is the reference method for quantitative testing of chromosome-mediated resistance to antibiotics. Bacterial growth is examined on culture media with various concentrations of antibiotics incorporated. Results are expressed in MICs (minimal inhibitory concentrations). 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 economical and simple to use. Unfortunately, with *N. gonorrhoeae* this method has been standardized only for a limited number of antibiotics.

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

Frequency

Some Western countries, such as the United States and Canada, 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.

Sample Size

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

Sample Population

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.

Selection of Sample Sites

The number and geographic 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.

Table 2

COSTS OF RAPID PLASMA REAGINS (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 reactives detected	283	615	2,275
Screening cost [a] (U.S. dollars)	$9,000	$9,000	$9,000
Cost for management of reactives 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) *aboratory 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 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).*

		Syphilis +	Syphilis –		
RPR	+	85	198	283	specificity: 9702/9900 =98%
	–	15	9,702	9,717	positive predictive value: 85/283 = 30%
		100	9,900	10,000	negative predictive value: 9702/9717 = 99.8%

sensitivity: 85/100 = 85%

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 person; therefore, widespread testing for most STDs 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 labor, 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.

SYNDROMIC STD MANAGEMENT APPROACH

The use of a cluster of signs and symptoms to determine therapy without making a definitive etiologic diagnosis is referred to as syndromic management *(see Chapter 8)*. Use of these methods either eliminates the need for diagnostic tests or simplifies the testing required.

Although of adequate sensitivity for urethritis in males and genital ulcers in both sexes, the syndromic approach tends to have lower 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 or with non-specific, simple diagnostics like detection of leukocyte esterase, an enzyme in white blood cells. 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 based on clinical examination according to syndromic management guidelines, but laboratory tests are required to distinguish between nongonococcal and gonococcal urethritis. The presence of leucocytes and typical intracellular diplococci (gonococcal urethritis) or the presence of leucocytes 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 gonococcal urethritis, culture for the isolation of *N. gonorrhoeae* is not essential for diagnosis and clinical management.[1] 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 noninvasive alternative is the collection of first-catch urine for detection of leucocyte esterase. Leucocyte esterase 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 leucocyte esterase method varies between 35 and 100 percent; consequently, the predictive value of a positive test may be very low.[2-7]

VAGINAL DISCHARGE/LOWER ABDOMINAL PAIN

Wet mount microscopy is very useful for the diagnosis of trichomoniasis, candidiasis and bacterial vaginosis (BV). A pH test, a KOH sniff test and a Gram stain of vaginal discharge may contribute to a reliable diagnosis of BV. 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 very low sensitivity for detecting gonorrhea among women; culture remains the method of choice.[8] Diagnostic assays for *Chlamydia trachomatis* infection include direct fluorescent antibody test, enzyme immunoassays, DNA hybridization, cell culture and DNA amplification techniques (polymerase chain reaction (PCR) and ligase chain reaction (LCR)).

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 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

LABORATORY PROCEDURES

GONORRHEA

Gonorrhea 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 gonorrhea are direct microscopy of a stained urethral discharge smear in men and cultures 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 gonorrhea in men, but the Gram stain, which gives more specific results for specimens containing mixed bacterial flora, remains the standard method.

Nonculture gonorrhea detection techniques

Different culture-independent tests for gonorrhea detecting oxidase, endotoxin, antigen or DNA have been compared with a standard culture technique. All these procedures are more expensive and less efficient than a culture technique for extragenital specimens and for specimens containing small numbers of organisms, (except for DNA amplification tests).[9-13]

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.

Culture identification

The observation of oxidase-positive Gram-negative diplococci with a gonococcus-like colony morphology in a cultured specimen 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.[9] Carbohydrate degradation tests (glucose, maltose, lactose and sucrose) are commonly used, sometimes in combination with enzymatic substrate tests.[14-17] An immunologic confirmation assay using monoclonal antibodies is a very reliable but more expensive alternative.[16,18]

In vitro *antimicrobial susceptibility testing*

Antimicrobial susceptibility testing of gonococcal isolates is not required for guidance of gonorrhea treatment in an individual patient because currently recommended treatment regimes by WHO are adequate to cure gonococcal infections resistant to penicillin, ampicillin and tetracycline. 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 standardization, *in vitro* antimicrobial susceptibility testing may generate unreliable results.

Detection of plasmid-mediated antimicrobial resistance in vitro

Penicillinase-producing *N. gonorrhoeae* (PPNG): many isolates of *N. gonorrhoeae* are resistent to penicillin and ampicillin because they produce an enzyme, ß-lactamase, that results in hydrolysis of these drugs. Common rapid ß-lactamase detection techniques include: the acidometric method, which uses a pH indicator to detect increased acidity from cleavage of the ß-lactam ring of penicillin; the idometric method, which detects a color change caused by the reduction of iodine by penicilloic acid; and the chromogenic cephalosporin method, which detects a color change of a chromogenic cephalosporin after hydrolysis of the ß-lactam ring.[19-21]

Tetracycline-resistant *N. gonorrhoeae* (TRNG): high-level tetracycline-resistant isolates of *N. gonorrhoeae* carrying a conjugative plasmid have become endemic in different geographic areas. TRNG can be determined by testing its ability to grow on a medium containing 10 mg of tetracycline per liter. The minimum inhibitory concentration (MIC) of tetracycline is ≥16 mg/liter.[22]

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 gonorrhea is common.

With the advent of new technologies, the current reference test for the diagnosis of *C. trachomatis* infection is either positive tissue culture or positive DNA amplification test confirmed by another test using a different technique, such as direct fluorescent antibody (DFA), or the same DNA amplification technique directed toward a different target, such as the MOMP (major outer memebrane protein) gene. The culture procedure, however, is expensive, slow, labor-intensive, technically difficult and beyond the capacity of most laboratories. In competent hands, the specificity of culture is 100 percent. Because technical difficulties can interfere with the reliability of the culture within a laboratory and unquestionably from one laboratory to the other, its sensitivity is estimated to be no more than 70 to 80 percent.[23] Cervical culture for *C. trachomatis* has a 65 percent sensitivity compared to the LCR assay of urine.[24]

Different nonculture techniques (immunofluorescence, enzyme immunoassay, DNA hybridization) developed during the 1980s, which are easier to perform and less expensive than culture, are now widely used. Many publications compare the evaluation of nonculture methods with that of cell culture, but an estimation of their actual sensitivity and specificity is extremely difficult since the reference culture technique does not meet the criteria of a real "gold standard."[25]

Direct microscopy (for males)

In a patient who has a history of acute onset of urethral discharge and has not received any 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 to three days, then stain them with fluorescein-labeled monoclonal antibody.[26,27] The addition of a blind passage enhances the sensitivity of the culture method, but may create a clinically unacceptable delay in diagnosis.[28,29]

Direct fluorescent antibody (DFA) test

Fluorescein-labeled monoclonal antibodies to the species-specific epitope of major outer membrane proteins can detect elementary bodies (EBs) of *C. trachomatis* in clinical specimens.[30] The procedure is rapid

and simple to process, but laborious 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.[31,32] 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.[33-35] 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.[36,37]

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.[37] Most of the currently available EIAs include a confirmation (neutralization or blocking) assay 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.[38,39]

New easy-to-use membrane immunoassays enable rapid diagnosis of chlamydial infection under field conditions.[40] These methods have a lower sensitivity and are therefore less accurate than the more classic immunoassays.

DNA probes, PCR and LCR

DNA hybridization methods have been recently applied to chlamydial diagnosis.[41] 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. Amplification of DNA sequences with PCR and LCR offer very high sensitivity. However, the high cost of PCR and LCR, the specialized laboratory equipment necessary, and the ease with which contamination with DNA can occur in the laboratory currently limit the use of this method in routine diagnosis.

Urine specimens

Obtaining urethral specimens by swab from men is an invasive method that causes some discomfort. The collection of first-catch urine samples is noninvasive and therefore more acceptable. Several studies have shown comparable sensitivities for nonculture methods (EIA or DFA) on urethral swabs and centrifuged sediments from urine samples.[42,43] Other studies have shown a significantly lower sensitivity of cell culture from urine samples than from urethral swab specimens.[44,45] The utility of urine samples from women for culture detection instead of cervical swabs has been debated.[23,46,47] Results obtained by DNA amplification techniques on urine specimens from both men and women and clinical samples (PCR and LCR) are very promising.[24]

Antibody detection tests

Various serological methods (complement fixation, microimmunofluorescence, enzyme immunoassay) 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 at least two weeks apart. The use of a single high titer for diagnosis of chlamydial urethritis and cervicitis is unreliable. It is diagnostically suggestive for lymphogranuloma venereum (LGV), reactive arthritis, epididmytis and pelvic inflammatory disease.[23,32]

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

SYPHILIS

Venereal syphilis is acquired by sexual contact with an infected person with an open ulcer; the transmission of *T. pallidum* requires exposure of non-infected mucous membranes or skin abrasions to infectious lesions.

No structural or metabolic difference have been found that distinguish between spirochetes that cause venereal syphilis *(Treponema pallidum)*, endemic syphilis *(T. endemicum)*, yaws *(T. pertenue)* and pinta *(T. corateum)*. 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 the papular rash lesion 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 titers (>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 fetus *(see Chapter 9)*. Diagnosis of congenital syphilis is based on:

- Microscopic demonstration of *T. pallidum* in nasal discharge or skin lesions, when present
- Detection of specific IgM antibody in serum
- Demonstration of rising nontreponemal test antibody titers in serial serum samples during the first eight months of life

Direct microscopy

Darkfield microscopy is the only method that provides an instant diagnosis of syphilis in the primary and secondary stages. For reliable results, however, appropriate technical conditions usually found only in specialized 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.[48] Failure to visualize 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 methods 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,[49] and the basic antigen composition in all newer tests is that of the VDRL. The antigen used in the VDRL test is not stabilized, 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 stabilized antigen on unheated serum.[50]

Table 3
SENSITIVITY AND SPECIFICITY OF SEROLOGICAL TESTS FOR SYPHILIS

	Sensitivity (%) by *Phase of syphilis infection*				*Specificity*
Test	**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

* *widely variable results reported in the literature*

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) test, which uses plastic-coated cards in place of slides and a stabilized antigen to which charcoal particles are added. The antigen is not coated on these particles. Instead, it is trapped in the lattice formed 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 plasma.[51] Modifications of the RPR include the reagin screen test (RST), which uses a lipid-soluble black dye in place of charcoal;[52] the VDRL carbon antigen, which is similar to the RPR, and the toluidine red unheated serum test (TRUST), which uses toluidine red in place of charcoal to make the reaction visible.[53]

Treponemal tests

Specific treponemal tests detect antibodies against treponemal cellular components. The three different test procedures are: indirect immunofluorescence, haemagglutination and enzyme-linked 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.[54] Microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP), *T. pallidum* hemagglutination assay (TPHA) or hemagglutination 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.[55,56] Enzyme-linked immunosorbent assays (ELISAs) are designed for batch processing of specimens and are suitable for automation of serology.[57,58] 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 general populations at a rate of 1 to 3 percent. The vast majority of false-positive sera show antibody titers of $\leq$ 1:4. However, low titers do not exclude syphilis and are often found in early primary, late latent and tertiary syphilis. Determination of nontreponemal serum titers 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.

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 the nontrepenomal 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, combinations 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 cards 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 $\leq$ 1:2 may appear negative with the hand rotation procedure. 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 a 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 syphilis 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 placental barrier as well as the intact serum/CSF 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 patient with early infection before seroconversion of nontreponemal tests is observed.

Genital Herpes

Herpes simplex virus (HSV) belongs to the group of alpha herpes 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 characterized 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 asymptomatically 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.[59-61]

Cytopathic effect (CPE) typical of HSV can be recognized by a rounding of scattered 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 neutralization 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.[62,63]

The most recent approach to rapid HSV diagnosis is DNA hybridization.[64] The amplification of DNA sequences by polymerase chain reaction may eventually offer a highly sensitive method useful for detecting HSV in asymptomatic pregnant women at term.[65]

Papanicolaou and Tzanck staining are no longer considered appropriate techniques for the diagnosis of genital herpes.[59] 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 assays 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, neutralization technique, latex agglutination, hemagglutination 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. None of the commercially available tests can 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 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.[66]

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 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 ß-lactamase and oxidase reaction. Colonies are non-mucoid, raised, granular, grayish-yellow in color, 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. Hemin is required to initiate growth. Nitrate reduction and alkaline phosphatase are also important characteristics. *H. ducreyi* is oxidase-positive when tested with tetramethyl-p-phenylene-diamine, and almost 100 percent of isolates are ß-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.[67-69] In addition, because most genital ulcers harbor polymicrobial flora due to secondary contamination, the presence of Gram-negative bacilli may be misleading and frequently results in a false-positive diagnosis.[69,70] 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 still experimental. Fluorescein-labeled monoclonal antibodies have been used to detect *H. ducreyi* in clinical specimens. Antibody against a 62 kDa protein and against a 29kDa protein can be detected, respectively, from 50 percent and 74 percent of people with clinically sus-

pected chancroid.[71,72] These results are promising, but further evaluation and extensive screening of clinical specimens is required to determine the reliability of the procedures. DNA probes have been shown to be 100 percent sensitive and 100 percent specific for the identification of bacterial isolates, but their usefulness for direct diagnosis of chancroid remains to be established.[73,74]

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 determine 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 or 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] 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 standardized 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 hemoglobin, 5 percent fetal 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 specialized 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 hematogenously and may even result in cutaneous lesions at extragenital body sites.

Donovanosis is caused by *Calymatobacterium granulomatis,* which cannot yet be cultured on artificial media. Laboratory diagnosis depends on the visualization 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 observed 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 histyocytes and with a conspicuous absence of lymphocytes, suggests granuloma inguinale. Demonstration of characteristic intracellular organisms (Donovan bodies) by Warthin Starry silver impregnation is diagnostic.[76]

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.[77]

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*.[78] Classic clinical symptoms and signs of candidiasis include vaginal itching, vulvar burning, external dysuria, curdy white discharge (that looks like cottage cheese) without malodor, 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 recognized in a wet mount preparation of vaginal fluid as round to ovoid cells of 4 μm diameter with typical budding. Adding a drop of 10 percent KOH to the preparation may facilitate the detection of yeast, in particular the recognition of mycelia (pseudohyphae). Yeast is 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 harbor *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.[79]

Colonies of yeast appear after one to 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 malodor and dysuria may be present.

The infection is caused by *Trichomonas vaginalis*, an ovoid, globular, pear-shaped flagellated protozoan. Although certain symptoms and signs are predictive for trichomoniasis, visualization of the parasite is required to establish the diagnosis.[80]

Direct microscopy

Trichomonads are easily recognized in a wet mount preparation of vaginal fluid by their typical jerky motility. An increased number of polymorphonuclear leucocytes is usually observed, but small numbers of leucocytes 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.[81] 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 characterized by slightly increased quantities of malodorous vaginal discharge. It is associated with an 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:[82,83]

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 ±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.

Malodor: Women with BV often complain of vaginal malodor, which is due to the release of amines produced by anaerobic bacteria that decarboxilate lysine to caverdine 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 odor.

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.

Additional laboratory tests

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.* Culture of bacteria associated with BV, however, is not essential for routine diagnostic purpose.

DIAGNOSTIC NEEDS

STD control programs are hampered by difficulties in diagnosing STDs. Many infections are asymptomatic, particularly in women, and even when symptomatic, may have different etiologies. Although combined therapy can be used effectively, simple, inexpensive tests are urgently needed, particularly for *N. gonorrhoeae* and *C. trachomatis* infections in women. Simple, inexpensive diagnostics are also necessary to identify the different causes of genital ulcers. High-quality, sturdy microscopes, centrifuges, rotators and other essential equipment that will provide years of services should be available at low cost.

The ideal diagnostic test would be:

- **Accurate**—highly sensitive and specific
- **Inexpensive**—affordable in developing countries
- **Simple**—requiring minimal training and uncomplicated or no equipment
- **Rapid**—with results available before a patient leaves the clinic (maximum 20 minutes)
- **Convenient**—so that specimens are simple to collect and socioculturally acceptable (do not require venipuncture or vaginal speculum examination, such as saliva, urine, vaginal swab or capillary blood)
- **Stable**—using reagents with long shelf life that require no refrigeration (35°C and 80 percent humidity)
- **Functional**—packaged simply with easy instructions.

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