Chapter 1

DIAGNOSTIC TESTS

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CONTENTS

INTRODUCTION

In this chapter we provide an overview of the laboratory diagnosis of syphilis. In the first section we briefly describe the natural history of syphilis. In the second section of this chapter, we review syphilis tests grouped according to testing method, and grouped within testing method according to the test's status in the four stages of test evaluation. Also, in the second section, we describe general performance characteristics of the tests by testing method. In the third section of this chapter, we briefly review how the tests are used in the diagnosis of the different clinical forms of syphilis. Details of test assay methods and of performance of individual tests are provided in the chapters devoted to individual testing methods.

Before focusing on the diagnosis of infection by T. pallidum, subspecies pallidum, the cause of syphilis, we need to point out that the pathogenic treponemes, T. pallidum, subspecies pallidum; subspecies pertenue, causative

agent of yaws; subspecies endemicum, causative agent of nonvenereal or endemic syphilis; and T. carateum, causative agent of pinta; are morphologically, serologically, and chemically indistinguishable. The standard serologic tests for syphilis are uniformly reactive in yaws, pinta, and nonvenereal endemic syphilis. Western blotting assays do not differentiate the antibodies formed in response to syphilis from those formed in response to yaws or pinta.1,2 Most molecular approaches, such as DNA sequencing, DNA probes, and polymerase chain reaction (PCR) techniques have failed to distinguish the pathogenic treponemes, also. 2,3 However, animal studies suggest differences in susceptibility of the hamster and the rabbit to infection with T. pallidum subspecies pallidum, pertenue, or endemicum. 4,5 In humans the diseases are diagnosed based on distinctive lesions, the age of populations infected, mode of disease transmission and progression of disease. Thus, the diagnostic tests described below for syphilis are also applicable to the diagnosis of the other treponemal diseases; however, none of the current tests distinguish between the organisms. Fortunately, in the United States, the treponematoses other than syphilis are quite uncommon, and it is seldom necessary to distinguish between infection with T. pallidum, subspecies pallidum, and infection with the other T. pallidum subspecies.

NATURAL HISTORY OF TREPONEMA PALLIDUM INFECTION

Individuals usually acquire Treponema pallidum infection upon contact with the infectious lesions of syphilis during sexual intercourse with an infected sex partner. A pregnant woman with T. pallidum infection may also transmit the infection to her fetus transplacentally. Much less commonly, individuals may acquire T. pallidum infection through nonsexual contact with the infectious lesions of syphilis or through exposure to contaminated blood or body fluids.

Early Syphilis

T. pallidum persists as a chronic infection, and progresses through discrete stages. The initial stages of infection acquired through sexual or nonsexual exposure to a lesion, termed primary, secondary, and early latent syphilis, are collectively referred to as early syphilis. Primary syphilis is defined by the development and spontaneous resolution of one or more ulcers (chancres) at the site of infection. It is thought that treponemes disseminate throughout the body within hours after initial contact with the organism; however, preferential multiplication of treponemes occurs at the site of entry. The primary-stage ulcer usually appears within 3 weeks after infection, although the incubation period between the time of infection and formation of a lesion is 10 to 90 days.6,7 Ulcers spontaneously resolve in 1 to 5 weeks. Regional lymph nodes may be enlarged but are rarely tender. Humoral antibodies, as detected by the standard nontreponemal and treponemal serologic tests for syphilis, usually do not appear until 1 - 4 weeks after the chancre has formed.

By the secondary stage of syphilis, the organism has invaded every organ of the body and virtually all body fluids. Usually, nonspecific symptoms develop 1 - 5 weeks after the primary lesion has healed and may include fever, headache, sore throat, arthalgias, and anorexia. Occasionally the primary stage ulcer is still evident as the onset of secondary stage symptoms and signs begin. The most characteristic signs of secondary syphilis are a generalized rash, mucous patches, and, in moist intertriginous areas, wart-like growths called condylomata lata. These manifestations also spontaneously resolve, usually within 2 to 6 weeks, but relapses may recur during the first four years of infection if the patient is not treated. Circulating immune complexes composed

of IgG and C3, detected in approximately 80% of the secondary cases, may lead to immune complex deposition in the kidneys and subsequent renal damage.8 Infrequently, symptoms and signs of meningeal infection occur. As the disease reaches the secondary stage, with few if any exceptions, all serologic tests for syphilis are reactive, and treponemes may be found in lesions by direct microscopic examination.

During the early latent stage, a relapse to the secondary stage may occur. Serologic tests are reactive in the early latent stage, but the reactivity in the nontreponemal tests decreases with increasing latency. Infection of < 1 year's duration, which is asymptomatic, is arbitrarily defined for epidemiologic purposes as "early latent stage." Because lesions are not usually present after the first year, the disease is not considered infectious and the term late latent is assigned to this stage. However, in pregnancy, T. pallidum can still be transmitted to the fetus up to four years after the initial infection though with decreasing frequency as the infection persists.

Late syphilis

Disease manifestations of late stage syphilis occur in approximately one-third of untreated syphilis cases. The pathogenesis of the various forms that the late or tertiary stage of syphilis may take is not completely understood. In the majority of cases, symptoms of late stage syphilis occur 10 - 20 years after the initial infection.6 Although rare, earlier onset has been reported for individuals coinfected with HIV.9

There are three forms of late syphilis: gummatous syphilis (also called benign), cardiovascular syphilis and neurosyphilis. Gummatous syphilis was reported in the preantibiotic era to occur in 16% of the cases of untreated syphilis from 2 to more than 40 years after the initial infection.8 The gummas resemble the granuloma of tuberculosis and may occur in the skin, bones, mucosae, viscera, muscles, and eyes. With osseous gummas, reactive new bone formation and osteolysis are characteristic.8 Because organisms are rare and the lesions are characteristic of an inflammatory response, some have suggested that the gummas are a result of hypersensitivity to the few treponemes or T. pallidum antigens remaining in foci of longstanding infections.

Literature from the pre-antibiotic era states that cardiovascular syphilis occurred in 10% of the patients with untreated syphilis.6 The lesions in the aorta in cardiovascular syphilis appear to be related to the local multiplication of treponemes.10 Apparently, treponemes spread via the lymphatics and lodge preferentially in the proximal aorta producing endarteritis, which may also involve the coronary arteries near the ostia with stenosis that may lead to syphilitic angina. This inflammatory process may last for years and eventually affect all three layers of the aortic wall. Degeneration of the intima with atherosclerotic plaque formation results in "tree barking.” 11 Ultimately, aortic insufficiency or thoracic aortic aneurysm develop.

In the pre-antibiotic era, neurosyphilis was reported to occur in 6.5% of the cases. The lesions in the central nervous system in neurosyphilis are also related to the multiplication of treponemes.10 Although neurologic manifestations of syphilis, (e.g., syphilitic meningitis) may develop during the secondary stage, neurosyphilis is usually a complication of late syphilis but may occur as early as 2 years after initial infection. Neurosyphilis may take many forms. All forms have in common chronic meningitis, producing vascular or parenchymatous lesions in the cerebrum and spinal cord.12 In acute syphilitic

meningitis, granular ependymitis and endarteritis may develop, which may lead to thrombosis, vascular occlusion, and cerebral infarction. As a result of obstruction of the cerebrospinal fluid (CSF) flow, several forms of hydrocephalus can occur. In generalized paresis, gross findings include cerebral atrophy, demyelinization of the cortical white matter, and varying degrees of thickening of the meninges consistent with chronic meningitis. Cerebral atrophy, particularly of the frontal poles and tips of the temporal lobes, is prominent. Whorls of sub-ependymal astrocytes forming granular ependymitis are a characteristic finding on microscopic examination. Treponemes can be demonstrated in brain tissue or CSF by direct immunofluorescence, PCR 13 or silver stain. The symptoms and signs of neurosyphilis usually follow years of asymptomatic subclinical infection of the central nervous system.14 Asymptomatic neurosyphilis is usually recognized when a lumbar puncture is performed on a person with a reactive serologic test for syphilis and no history of treatment.15

Congenital Syphilis

In congenital syphilis, a primary stage does not occur because the organisms directly enter the fetal circulation. Necrotizing funisitis may or may not be present .16 Treponemes, or the effects thereof (such as damage from circulating immune complexes), are detectable in almost every tissue of the infant.17 Clinical signs of early congenital syphilis, analogous to adult secondary stage syphilis, include hepatosplenomegaly, cutaneous lesions, osteochondritis, and snuffles.18

The lesions of early congenital syphilis may contain high concentrations of organisms and are infectious. Stillbirth is also a common outcome of pregnancy in mothers with early syphilis, presumably due to overwhelming infection of the fetus.16 Death may also ensue following live birth. However, most infants enter an extended period of latent infection analogous to the late latent stage of the adult. Upon entering latency children with congenital syphilis are no longer considered to be infectious. Late congenital syphilis, defined as congenital syphilis which has persisted for >2 years, may be distinguished from adult disease by stigmata consisting of malformations due to infection of growing tissues. Characteristic stigmata include interstitial keratitis, Moon’s molars, Hutchinson’s teeth, eighth nerve deafness, Clutton’s joints, sabre shins, and perforation of the hard palate

LABORATORY PROCEDURES

Standardization

Since 1939 the United States Public Health Service has been charged with the challenge of standardizing the tests for syphilis. In 1977, guidelines from the Centers for Disease Control (CDC) for the evaluation and acceptance of new tests for syphilis were made available to reagent manufacturers and test developers. In 1989, a supplement to the guidelines included the evaluation of treponemal tests as the initial tests for syphilis. According to the guidelines each new or improved test should progress through four stages of evaluation. These stages can briefly be defined as follows: 1) Research stage - A test in this status must have a written technique; however, the technique may be modified depending on the outcome of studies during development of the test. A limited number of stored samples should be evaluated and the results should indicate that the test has a sensitivity and specificity equal to that of the standard status test to which it was compared. In addition, a sufficient quantity of reagents should be produced to enable initiation of preliminary clinical

studies. During the research stage, reagent stability studies should begin, the range of specimens (e.g. serum, plasma, CSF) for testing determined, and variations between lots of reagents eliminated. 2) Investigational stage - A test in the investigational stage should have a written technique that is based on substantial research. The test should be in a form suitable for studies conducted in parallel with a standard status test to define the test's performance when applied to the population for which its use is intended. Laboratories using an investigational technique may report results to physicians along with the results of the standard status tests currently in use in the laboratory; however, the results must be identified as from testing with an investigational test and the following disclaimer should accompany the results: " A result obtained using an investigational status test cannot be used as the sole criterion for medical management decision making." 3) Provisional stage - A test in the provisional status should have been evaluated in multiple clinical trials and the results published. The test should have been found to be as specific and sensitive as the standard tests to which it was compared, have FDA approval (510K) and be included as a technique in a CLIA- recognized proficiency testing program. Results of a test in the provisional status may be reported to physicians for medical management, if the laboratory performing the provisional status test has run it in parallel with the standard status test currently in use in the laboratory and has found the test results comparable. The test should have a written technique incorporating revisions Standard stage - A test that has achieved standard status should have undergone extensive large scale evaluations conducted independently of the test developer with results published in peer reviewed journals that document that the test is as useful for medical management decision-making as the other standard tests. Usually a test has been commercially available for several years before achieving standard status. A test in this stage should have passed through the preceding stages of research, investigational and provisional status and met the requirements of each stage.

Categories of Tests

Because of the lack of a culture system 19,20 for T. pallidum, alternative methods for detection of the treponemes or antibody against T. pallidum subspecies have been developed. The laboratory tests for syphilis fall into two broad categories, tests that detect T. pallidum organisms or components of organisms (antigen detection tests), and tests that detect antibodies produced in response to T. pallidum infection (antibody detection tests). 21 In general, antigen detection tests in combination with serologic tests for syphilis are the basis for the diagnosis of primary and secondary syphilis, and for the diagnosis of congenital syphilis when infectious lesions are present. Diagnosis of syphilis during early and late latent syphilis is based on antibody detection tests alone. Clinical and other laboratory findings, plus reactive serologic tests for syphilis, must be considered in the diagnosis of late syphilis and congenital syphilis.

T. pallidum detection tests

Detection methods for T. pallidum have been most useful with specimens collected from the epidermal and mucosal lesions of primary, secondary, and early congenital syphilis, because these specimens may be obtained without using invasive procedures and the lesions tend to contain large concentrations of treponemes.22,23 Tests involving staining of T. pallidum in histologic preparations from various tissues may be useful, although organism concentrations tend to be low during late adult and congenital syphilis, and in resolving lesions of early syphilis.16,24 Currently, nucleic acid amplification

methods are being investigated as potentially more sensitive methods of detecting T. pallidum. Meanwhile, inoculation of rabbits with T. pallidum remains the only standardized method adequate for isolation and growth of the treponemes.

Microscopic Examination of Fluid or Smears from Lesions

Darkfield microscopy 21,22 is the standard test of choice when the patient has moist lesions and when the specimens can be examined immediately in wet mount for motile organisms. The demonstration of motile organisms with morphology characteristic of T. pallidum by darkfield microscopy strongly supports a diagnosis of syphilis in primary, secondary, early neonatal congenital, and secondary relapse stages. A test, such as darkfield microscopy, that can be performed during a patient's visit is especially useful during early syphilis to facilitate the timely diagnosis and treatment of patient and sex partners. Because nonpathogenic treponemes in the mouth are indistinguishable from T. pallidum by darkfield examination, samples from this source cannot be used in the test.

The direct fluorescent antibody test for T. pallidum (DFA-TP)24,25 test is another standard microscopic test for syphilis; this test is a practical alternative to the direct darkfield examination when smears cannot be examined immediately because motile organisms are not required. In addition, oral lesions can be examined by DFA-TP because conjugate specific for pathogenic treponemes is used.24,25 DFA-TP is the most specific means of diagnosing syphilis in lesion exudates or body fluids. 25-28

A failure to find the organism by either of the direct microscopic tests does not exclude a diagnosis of syphilis. Failure to demonstrate T. pallidum from typical lesions may be due to a low concentration of organisms or large amounts of debris in the specimen, prior treatment of the patient, spontaneous healing of the lesion, and, most commonly, poor technique.27

Microscopic examination of tissue specimens

The direct fluorescent antibody tissue for T. pallidum (DFAT-TP)24 test is a modification of the DFA-TP for the specific identification of T. pallidum in paraffin-embedded tissue specimens. Usually, the DFAT-TP test is combined with histologic stains to examine biopsy and autopsy material for the presence of pathogenic treponemes. First, the tissue is screened using a silver stain such as Steiner,29 then if spiral organisms are observed, the tissue is stained for treponemes. Any tissue can be used, but most often DFAT-TP is used to detect T. pallidum in skin lesions of secondary or late syphilis and in diseased tissues of the brain, gastrointestinal tract, placenta, umbilical cord, or skin in congenital syphilis.28 The demonstration of T. pallidum in tissue provides a definitive diagnosis of syphilis, or other pathogenic treponemal infection, because the monoclonal conjugate used is highly specific for T. pallidum subspecies.28 However, the condition of the initial biopsy specimen or necropsy sample and the thickness of the tissue sections affect the sensitivity of the test. 24,28

Isolation and propagation

Despite numerous attempts to grow T. pallidum in vitro,19,20 animal inoculation remains the sole method suitable for isolating and propagating T. pallidum. Even though rabbit infectivity testing (RIT) has not been classified according to test evaluation status, standardized instructions for RIT are available from the

Bacterial STD Branch, Division of AIDS, STD and TB Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333. RIT is technically demanding, expensive, and requires up to 90 days to obtain a result. Therefore, RIT is suitable only for research and as a gold standard for test evaluation purposes. The rabbit infectivity test is highly specific and may be the most sensitive test available for the diagnosis of syphilis.30

Nucleic Acid Amplification

Nucleic acid amplification methods, such as the polymerase chain reaction test (PCR), have the potential for extending the uses of T. pallidum detection methods beyond the applications described above for microscopic methods. Even though T. pallidum is distributed systemically soon after infection, the concentration of treponemes is too low, in most cases, for detection by microscopy in blood and cerebrospinal fluid, and, in some cases, in specimens from lesions.

A number of PCR-based tests have been developed in research laboratories either as potential diagnostic tests 31 or to identify T. pallidum-infected animals in experimental systems.2, 32-34 All of these tests are considered to be in the research stage. In the future PCR may prove to be valuable in diagnosing congenitally acquired infection in infants (passively transferred maternal antibodies now confound interpretation of reactive serologic tests), neurosyphilis (the only test for CSF antibody is only 50% sensitive), and early primary syphilis (the only tests available are microscopic), and, finally, in distinguishing new infections from old infections (now only a rise in antibody concentration can be used).

Two of the PCR-based techniques were described almost simultaneously.3,32 One of these techniques was based on the amplification of a 658-base pair segment of the gene for the 47-kDa surface antigen; 35 this is a lipoprotein that is antigenically dominant in the human immune response to T. pallidum. 36 The test was performed on clinical specimens (amniotic fluid, neonatal sera, and neonatal CSF) for use in the diagnosis of congenital syphilis in neonates.32-34, 36 The test results were verified with the RIT. In the various tissue fluids examined,32,33 the overall sensitivity was 78% among the RIT-positive specimens. Only in the amniotic fluid samples was the PCR sufficiently sensitive to be considered as a diagnostic test; CSF and serum results correlated approximately 60% to 67% with RIT. A lack of sensitivity with PCR has generally been found to be related to nonspecific inhibitors of the polymerase found in the specimens tested. False-positive results did not seem to be a problem37 because none of the samples that were negative by RIT were positive by PCR.

The other PCR technique,3 based on amplification of base sequences in the gene coding for a 39-kDa basic membrane protein, was used to diagnose neurosyphilis. This PCR test, as with the standard tests for syphilis,38-40 did not differentiate between patients with active infections and patients that no longer appeared to harbor living treponemes in their CSF.3 All patients that had been previously infected with T. pallidum were positive by PCR regardless of previous treatment status. However, T. pallidum has also been detected by RIT in the CSF of patients who appeared to have been adequately treated.9,41,42 Positive PCR results could indicate either the persistence of small numbers of viable treponemes, or of DNA from dead organisms.

Two other published clinical studies compare PCR using a primer encoding for the 47-kDa protein with direct microscopic examination or rabbit infectivity.31,43

Another clinical study, using a primer which encodes for a portion of the 16S rRNA of T. pallidum, also compared their results to rabbit infectivity.44 Results from a study comparing PCR with DFA-TP found a concordance of 95.5% when touch preparations of genital lesions were examined.43 Results from a Roche Molecular Systems, Alameda CA study comparing the Roche Multiplex PCR (M-PCR)TM with darkfield found the sensitivity and specificity of the M-PCR for T. pallidum to be 91% and 99%, respectively, and the sensitivity and specificity of darkfield to be 81% and 100%, respectively.31 The M-PCR assay includes DNA primers as targets for Haemophillus ducreyi, herpes simplex virus types 1 and 2, as well as for T. pallidum, for the diagnosis of genital ulcer disease. As of this printing Roche Molecular Systems has terminated further plans for marketing of the M-PCR. A similar test is available at CDC.

Antibody detection tests for adult syphilis

Humoral antibodies produced in response to T. pallidum infection become detectable in the primary stage and increase in concentration during the secondary stage. Antibody detection tests supplement the antigen detection methods used for the diagnosis of primary and secondary syphilis and are the only practical methods of diagnosis during early latent and late syphilis. Syphilis serologic tests are divided into two groups termed nontreponemal and treponemal based on the antigen used.

Nontreponemal tests

The fundamental antigen used in each of these tests is that of the Venereal Disease Research Laboratory (VDRL) test, which contains standardized amounts of cardiolipin, cholesterol, and lecithin.45 All nontreponemal tests measure immunoglobulin G (IgG) and IgM antibodies formed by the host in response to lipoidal material released from damaged host cells as well as to lipoprotein-like material -- and possibly cardiolipin -- released from the treponemes.46, 47

The nontreponemal tests in use today include four standard status tests and a single provisional status test.

Standard status nontreponemal tests

The standard status tests include the Venereal Disease Research Laboratory (VDRL) slide test,46 the unheated serum reagin (USR) test, 49 the rapid plasma reagin (RPR) 18-mm circle card test,50 and the toluidine red unheated serum test (TRUST).51 Each of these is a flocculation test (Table 1:1). The VDRL slide test is the only test that can be used to test CSF. CSF cannot be used in the other flocculation tests, because of a lack of both sensitivity and specificity. 38 With the exception of the VDRL slide test, each of the flocculation tests can be performed using heated or unheated serum or unheated plasma samples. Heated serum samples are required for the VDRL slide test. In the other flocculation tests, the use of plasma or cord serum should be limited, because poorly collected and stored specimens from uninfected individuals can appear as minimally reactive in these tests (see Chapter 3).

All nontreponemal tests are performed in a similar manner. The test antigen is mixed with the patient’s serum on a solid matrix and rotated for a specified number of minutes before reading. Because of the lipid nature of the antigen, the liposomes formed are barely visible. Likewise, the antigen-antibody complex that occurs with serum from individuals with syphilis remains suspended and flocculation rather than agglutination or precipitation occurs. Each of the

standard status nontreponemal tests can be performed as a quantitative test, by testing serial dilutions of the patients serum to reach an endpoint titer.

Microscopic tests

Two of the standard status nontreponemal tests, the VDRL slide 45 and the unheated serum reagin (USR) tests,48 are microscopic flocculation tests. Disadvantages of the VDRL test are that the antigen suspension must be prepared fresh daily and the serum specimen must be heated before testing. In contrast, the antigen in the USR test is stabilized, thereby omitting the need to prepare an antigen suspension daily and to dispose of unused antigen at the end of the day's testing, in addition, as the name implies heating of the specimen is not required. Both the VDRL and the USR are performed on glass slides with paraffin rings, which must be prepared, or on reusable ceramic ring slides, which must be cleaned.

Macroscopic tests

The rapid plasma reagin (RPR) 18-mm circle test 49 and the toluidine red unheated serum test (TRUST)50 are macroscopic flocculation tests. The antigen used in the RPR 18-mm circle card test is a stabilized suspension to which sized charcoal particles have been added. The antigen is not attached to these particles; the charcoal is trapped in the lattice formed by the antigen-antibody combination when reactive serum is tested. Thus, the reaction can be seen without the aid of a microscope. In the TRUST, uniform particles of the azo pigment, toluidine red, are added to a stabilized USR antigen to aid in the visualization of reactive sera, much as charcoal does in the RPR card test. The tests are performed on disposable plastic-coated cards. 49

Table 1:1 Standard Status Diagnostic Tests for Syphilis

Nontreponemal:
- Venereal Disease Research Laboratory (VDRL) Slide
- Unheated serum reagin (USR)
- Rapid plasma reagin (RPR) 18-mm circle card
- Toluidine red unheated serum test (TRUST)

Treponemal:
- Fluorescent treponemal antibody absorption (FTA-ABS)
- Fluorescent treponemal antibody absorption double-staining (FTA-ABS DS)
- Microhemagglutination assay for antibodies to T. pallidum (MHA-TP)
- Darkfield microscopy
- Direct fluorescent antibody test for T. pallidum (DFA-TP)

Interpretation of standard status nontreponemal test results

Qualitative test results for the microscopic tests, VDRL and USR, are reported as reactive, weakly reactive or nonreactive. Qualitative test results for the macroscopic tests, RPR card test and TRUST, are reported as reactive (regardless of the size of the flocculant) or nonreactive.

diseases of an acute and chronic nature in which tissue damage occurs.57 The incidence of false-positive reactions depends on the test used and the population studied. False-positive results occur in the general population at a

rate of 1% to 2% regardless of the nontreponemal test used.58-61 Acute false-positive reactions lasting less than 6 months usually occur after febrile diseases or immunizations (Table 1:3). Rates of false-positive reactions during pregnancy are no greater than seen in the general population.62 Chronic false-positive reactions are more often associated with autoimmune diseases such as arthritis and all forms of lupus, or with chronic infections such as leprosy (Table 1:3). More than 10% of intravenous (IV) drug users may give false-positive results.63 HIV infection has not been associated with an increase in false-positive nontreponemal test results in individuals with a low risk of drug addiction. 61 As a rule, 90% of the false-positive titers are less than 8, but low titers are also seen in latent and late syphilis. Not only do patient factors cause false positive results, false positive results may occur if the temperature of the laboratory, reagents or specimen is >29?C (<85?F).

In addition to the failure to recognize prozone reactions that occur in 1% to 2% of patients with secondary syphilis,56 false-negative nontreponemal test results are also seen in incubating primary and late syphilis or when the temperature of the laboratory, specimen or reagents is < 23?C (<73?F).64

Table 1:3 Causes of acute and chronic false-positive reactions in the nontreponemal tests

Acute	Chronic
hepatitis	connective tissue/autoimmune diseases
viral pneumonia	immunoglobulin abnormalities
measles	narcotic addiction
malaria	aging
pregnancy	leprosy
infectious mononucleosis	malignancy
chicken pox	
other viral infections	
immunizations	
drug use	
lab or technical error	

When laboratory results contradict the physician’s opinion or the patient’s history, a repeat specimen should be submitted. The diagnosis of syphilis must be based on serologic tests as well
as history, a thorough physical examination, and a plausible explanation for the source of infection.

Provisional Status Nontreponemal Test

The VISUWELL Reagin test65 is the only nontreponemal serologic test currently in the provisional stage of evaluation. In 1987, Pedersen, Orum and Mouritsen developed a VDRL/enzyme immunoassay (EIA) to detect IgG.66 The VISUWELL Reagin test is based on the Pedersen method. In the indirect EIA procedure, the wells of a microtiter plate are coated with VDRL antigen. The patient's serum is added and reagin (nontreponemal) antibodies attach to the VDRL antigen. These antibodies are then detected with an antihuman immunoglobulin conjugate labeled with an enzyme. Finally, at a specified time a stop solution is added, and results are read spectrophotometrically. Results for the VISUWELL Reagin are reported as reactive, nonreactive or indeterminant. Studies have shown the test to have a sensitivity in untreated syphilis of 97% and a specificity of 97%.65,66 Using the VISUWELL Reagin test, several hundred tests can be performed in a day. Like the other nontreponemal tests, the VISUWELL Reagin test

usually becomes nonreactive with treatment of the patient. The major disadvantage of the VISUWELL Reagin test is the lack of an evaluated protocol for obtaining quantitative test results. Prozone reactions have been reported to occur infrequently in the VISUWELL Reagin test by those laboratorians routinely using the test (Larsen, personal communication).

Treponemal Tests

All treponemal tests use T. pallidum or its components as the antigen. Treponemal tests are used primarily to verify reactivity in the nontreponemal tests. The treponemal tests also may be used to confirm a clinical impression of syphilis in patients with nonreactive nontreponemal test results, such as might occur in late syphilis.

Standard status treponemal tests in current use consist of tests based on indirect fluorescent antibody and hemagglutination techniques (Table 1:1), including an automated hemagglutination test. A single provisional status treponemal test employs the EIA method. Investigational status treponemal tests include one test that employs the EIA and a recombinant treponemal protein and another test that employs an immunoblotting method.

Standard status treponemal tests

Currently two treponemal tests based on indirect fluorescent antibody techniques are considered as standard tests for syphilis; the fluorescent antibody absorption (FTA-ABS),67 and the FTA-ABS double staining (DS) tests.68 These treponemal tests use T. pallidum subspecies pallidum as the antigen and detect antibodies directed against treponemal cellular components.

In the FTA-ABS test, serum must first be diluted 1:5 in sorbent. Sorbent is an extract of cultures of T. phagedenis, Reiter treponeme, used to remove antibodies to nonpathogenic treponemes, which otherwise may cause nonspecific results from the serum samples of persons presumed to be normal. 67 Next, the serum is placed on the microscope slide to which the antigen, a suspension of T. pallidum subspecies pallidum organisms, has been fixed. Finally the conjugate, fluorescein-labeled antihuman globulin, is added. The FTA-ABS test is extremely sensitive but must be well controlled. The 1+ reading standard control, correct conjugate dilution, and proper adjustment of the fluorescent microscope are critical to the reproducibility and accuracy of the test results.

The FTA-ABS DS test68 is designed specifically for use with fluorescent microscopes that
have incident rather than transmitted illumination. The test is the basic FTA-ABS procedure with the addition of a contrasting fluorochrome-labeled counterstain for T. pallidum as a final staining step. The counterstain eliminates the need to use transmitted light with darkfield microscopy to locate treponemes that are not stained when the antihuman IgG fluorochrome-labeled conjugate is added. The test also eliminates errors in the standard procedure caused by improper alignment of the darkfield condenser. The current FTA-ABS DS includes the use of a rhodamine-labeled primary conjugate and a fluorescein-labeled counterstain.

Reactive results of both FTA-ABS tests cover a range of staining intensities from 1+ (reactive minimal) to 4+. Because beaded fluorescence (atypical staining) has been observed in serum from patients with active systemic lupus erythematosus and from patients with other autoimmune diseases, this observation

should also be reported. Although the FTA-ABS tests are the most sensitive of all the tests, they are also the tests affected most by variation in equipment, reagents, and test interpretation.

A standard status confirmatory test that laboratories without a fluorescent microscope find attractive is the microhemagglutination assay for antibodies to T. pallidum (MHA-TP).69,70 The MHA-TP is less expensive and less complex than the FTA-ABS tests. The MHA-TP is a qualitative hemagglutination test in which tanned, formalinized sheep red cells are used as the carrier for the T. pallidum antigen. The patient's serum is first diluted in absorbing diluent containing sheep and bovine red cell membranes, normal rabbit extract, Reiter treponeme sonicate, normal rabbit serum, and stabilizers. Serum is tested with sheep red cells which have been sensitized with T. pallidum; unsensitized sheep red blood cells are used as a control for heterophile reaction. The degree of hemagglutination is reported, ranging from a smooth mat of agglutinated cells surrounded by a smaller red circle of unagglutinated cells with hemagglutination outside the circle (1+), to a smooth mat of agglutinated cells covering the entire bottom of the well (4+). Inconclusive results are reported when a heterophile reaction that cannot be diluted out is observed.

Because the test is based on agglutination, quantitation of treponemal antibodies is possible, but is not useful. Most studies demonstrate no practical relationship between the titer and either the progression of the disease or the clinical stage of syphilis diagnosed, and unlike the quantitative nontreponemal tests, the quantitative hemagglutination test does not seem useful in posttreatment evaluation.70-73 The most common sources for error with the hemagglutination tests include the use of dusty or improper plates, pipetting errors, and vibrations in the laboratory. Although the MHA-TP test is less complicated than the FTA-ABS test, the MHA-TP is less sensitive in primary syphilis.58,74,75

An automated hemagglutination test is the Olympus PK-TP. This test was developed mainly for use in blood banking as a screening test for syphilis. The instrument used is a PK7100 or -7200, an automated pretransfusion blood testing system, which is the basic equipment for blood grouping and typing used by the American Red Cross. The instrument can run 240 samples per hour. The PK-TP reagent is composed of chicken erythrocytes that have been fixed, then sensitized with components of sonicated T. pallidum. A sequence of testing is performed for screening in blood banks. Plasma is tested first. Since the PK-TP is less specific with plasma than serum, serum is tested if the plasma specimen is reactive. If the serum is reactive, then the serum is tested in the RPR card test, and if reactive in the RPR, it is confirmed by the FTA-ABS test. This sequence of testing is done first to eliminate false-positive results that may occur when plasma is used. The RPR card test is used to identify individuals most likely to have infectious syphilis rather than long-standing noninfectious, or previously treated, infection, or false positive results. The FTA-ABS test is used to identify false positive results in either of the two previous tests, because both the RPR card and PK-TP test are less specific than the FTA-ABS test. At this time, the use of the PK-TP as a screening test in the United States is restricted to blood banking organizations. Early experience using the PK-TP as the initial screening test in blood banks indicates that the PK-TP is at least twice as specific as the RPR card test.76

Interpretation of standard status treponemal test results

The FTA-ABS test results are reported as reactive, reactive minimal, nonreactive or atypical staining. MHA-TP results are reported as reactive,

nonreactive or inconclusive (equivocal). Results reported as reactive minimal or inconclusive imply the need to submit a second specimen drawn approximately two weeks after the first sample.

The treponemal tests are nonreactive during incubating-stage syphilis. Treponemal tests become reactive in early primary syphilis and may remain reactive for years with or without treatment (Table 1:4). For the diagnosis of late untreated syphilis, reactivity in the treponemal test may be the only indication of a previous treponemal infection. For 85% of persons successfully treated, test results can remain reactive for years, and for some persons a lifetime.52 A somewhat smaller proportion of persons treated early in primary syphilis remain seroreactive. However, in general, a nonreactive treponemal test result indicates no past or present infection.

Misinterpretation of treponemal test results most often occurs when these tests are misused as screening procedures.

Table 1:4 Performance of Standard Status Treponemal Tests

	Sensitivity by Stage of Untreated Syphilis				Specificity
Test	Primary	Secondary	Latent	Late	Nonsyphilis
FTA-ABS	84(70-100)a	100	100	96	97(84-100)
FTA-ABS DS	80(70-100)	100	100		98(97-100)
MHA-TP	76(69-90)	100	97(97-100)		99(98-100)

a Range of sensitivity in CDC studies.

Limitations of the standard status treponemal tests

When treponemal tests are used for screening purposes about 1% of the general population will have false-positive results77 (excluding individual with reactive results following successful treatment for syphilis). Although false-positive results in the treponemal tests are often transient and their cause is unknown, associations have been established with specific conditions that differ for the fluorescent antibody and hemagglutination tests. An association between false-positive FTA-ABS test results and the diagnosis of systemic,78,79 discoid, and drug-induced lupus erythematosus80-82 has been established (Table 1:5). Patients with systemic lupus erythematosus can have false-positive FTA-ABS test results that exhibit an "atypical beading" fluorescence pattern. To resolve these types of false-positive reactions, absorption with calf thymus DNA can be used to remove the anti-DNA antibodies in the serum.79 False positive FTA-ABS test results also occur among patients with other collagen diseases, leprosy, intravenous drug abuse, and Lyme disease. Some of these false-positive reactions may be due to the failure of the sorbent used in the tests to remove all cross-reacting group, genus, or family antibodies; e.g., Lyme disease.83,84

Table 1:5 Causes of chronic false-positive reactions in the treponemal tests

Systemic lupus erythematosus
Drug-induced lupus erythematosus
Narcotic addiction
Aging

The treponemal hemagglutination methods may give fewer false-positive test results than the fluorescent antibody methods.58,59,85,86 In general, the occurrence of false-positive hemagglutination tests is rare in "healthy" persons (< 1%). Inconclusive hemagglutination tests have been reported for patients with infectious mononucleosis, especially in the presence of a high heterophile antibody level.73 Presumably, false-positive hemagglutination tests also occur in samples from drug addicts, patients with collagen disease, patients with leprosy, and in patients with other miscellaneous conditions.59,85,86 However, the MHA-TP test is nonreactive in Lyme disease.83 In some cases, the results of the hemagglutination test for T. pallidum are difficult to assess because syphilis may coexist with these other conditions. If a nontreponemal test is reactive, but either the FTA-ABS or hemagglutination test is nonreactive, and the clinical presentation does not suggest syphilis, the reactive result is most likely a false positive result.86

Provisional status treponemal test

The Captia Syphilis-G is the single provisional status treponemal test. The test is an indirect EIA in which the microtitration plates have been coated with a sonicate of T. pallidum. Diluted patient's serum is added to the microtiter well, then after incubation and washing, biotinylated anti-human IgG labeled with strepavidin-peroxidase is added to detect the antibody to T. pallidum in the patient's serum. After rinsing off the excess antibodies, an enzyme substrate is added for detection. Test results are reported as reactive, nonreactive or equivocal. Overall sensitivity in syphilis, regardless of stage, has been reported to range from 98.3% to 100%.87-91 In a study in which syphilis was staged, the test was reported to have a sensitivity of 82% in early primary syphilis.90 The reported specificity of the Captia Syphilis G ranges from 70.2% to greater than 99%.87-91 More extensive test use and evaluation of its performance in proficiency testing programs are necessary before this test will achieve standard status. Limitations of the EIA test are time and costs when small numbers of samples are to be processed. The main advantages are the capacity to process large numbers of samples and to use automated readout. The reading of the test is not subjective, as for the FTA-ABS and hemagglutination tests, but an objective spectrophotometric reading provided as a printout.

Investigational status treponemal tests

Although several recombinant proteins have been used to develop EIAs in research laboratories,91 the only EIA using a recombinant antigen currently in the investigational stage is the VISUWELL Syphilis test. This test is available in Canada and the United States. The cloned antigen used in the VISUWELL Syphilis test is the 47-kDa lipoprotein35,93 which is now thought to be a penicillin-binding protein.94 This protein has been shown to be immunodominant, produced in large quantities by the treponemes, and does not cross-react to a significant extent with similar proteins from the commensal treponemes. The VISUWELL Syphilis test has received limited evaluation. Results of two unpublished studies indicate a sensitivity of greater than 92% and a specificity of greater than 94% for the VISUWELL Syphilis test (personal communication Richard C. Alexander, San Bernardino County Public Health Department, CA and CDC study).

The Western blot technique developed by Hanff et al.95 for T. pallidum can be used to detect either IgG or IgM antibodies.36,96-98 Performance of the Western blot techniques for syphilis is similar to the techniques used for confirmation of antibodies to the human immunodeficiency virus (HIV). To date, many investigators agree that acquired syphilis may be diagnosed if antibodies to immunodeterminants with molecular masses of 15.5 kDa, 17 kDa, 44.5 kDa, and 47

kDa are detected.92 Antibodies to at least three of the four immunodeterminants must be present for the results to be reported as reactive. The Western blot technique using IgG conjugate appears to be at least as sensitive and specific as the FTA-ABS tests, and efforts have been made to standardize the procedure.99 Currently, two commercial companies are conducting preliminary evaluations of Western blot test kits (Centocor UK Ltd, Surrey and MarDx, Carlsbad, CA). The Western blot offers the advantage of direct visualization of the immunodeterminant reactions with the patient's antibody. At present, some reference and research laboratories are using their own version of the Western blot to resolve questionable FTA-ABS test results.

IgM Antibody Detection for Congenital Syphilis

A standard status test for treponemal IgM antibody is not available.

Provisional status IgM test

A variation on the Captia Syphilis-G ELISA test is the Captia Syphilis-M test.90,100,101 The test is recognized as a provisional status test for the detection of congenital syphilis in newborns, not for the diagnosis of sexually acquired syphilis. Because IgM rheumatoid factor (IgM produced against the Fc region of IgG) anti-IgG idiotype antibodies can cause false positive results if the antigen-antibody reaction takes place in the presence of IgG antibody, the Captia Syphilis M test uses anti-human IgM antibody to capture IgM in the infant's serum, followed by the addition of a purified biotinylated anti-T. pallidum antibody-T. Pallidum antigen complex to detect those IgM antibodies directed toward T. pallidum.100,107 One study found that the IgM capture EIA was more sensitive than the FTA-ABS 19S IgM test (see below) in detecting probable cases of congenital syphilis; 101 another study found the test to be equal in sensitivity to the IgM Western immunoblot in neonatal congenital syphilis, but less sensitive than the Western blot in detecting delayed onset congenital syphilis.102 Current interpretations of the IgM capture test results are given in Chapter 15. The interpretation of the test result is linked to the treatment status of the mother and her stage of syphilis.

Investigational status IgM tests

Several variations on the FTA-ABS test have been used on an experimental basis for the diagnosis of congenital syphilis by replacing the IgG conjugate with an IgM conjugate. The FTA-IgM and the FTA-ABS IgM were reported to be both nonspecific and lacking in sensitivity.18,103-106 The specificity of the FTA-ABS-IgM test for neonatal congenital syphilis, as well as other indirect immunofluorescent IgM tests for prenatal infections, has been questioned by the observation that newborns may produce IgM antibodies in response to passively transferred maternal IgG antibody (rheumatoid factor and anti-idiotypic antibodies) rather than in response to the infectious agent itself.107 The fractionation of the infant's serum in the FTA-ABS 19S IgM test appears to eliminate most of the problems with the specificity of the test. The FTA-ABS 19S IgM test is not available in kit form, and matching the reagents to be used in the test is extremely difficult. Nevertheless, a standardized method for the FTA-ABS 19S IgM is available from the Bacterial STD Branch, Division of STD, AIDS and TB Laboratory Research, National Center for Infectious Diseases, Center for Disease Control and Prevention, Atlanta, GA 30333. False-positive reactions in the current version of the FTA-ABS-IgM test with the 19S fraction of IgM have been reported in normal infants, but they are infrequent. 101, 106 Still the major drawback of the FTA-ABS-IgM 19S test for neonatal congenital syphilis lies in its insensitivity.100,106 Therefore, while the FTA-ABS 19S IgM test for

congenital syphilis may be useful to confirm a clinical impression of congenital syphilis, at this time it cannot replace careful repeated clinical assessment combined with serial quantitative nontreponemal tests in the evaluation of the newborn with possible congenital syphilis.

The Western blot used to diagnose congenital syphilis is identical to the IgG method,95 except an IgM conjugate replaces the IgG conjugate.36,97,98 Most studies have shown the IgM Western blot superior to either the FTA-ABS IgM test or IgM EIA for the detection of congenital syphilis.96-98 Currently an IgM Western blot test is not available in a kit form. See Chapter 19 for a standardized method for performing and reporting test results.

Methods for Detecting Antibody in CSF

The VDRL slide test is the only standard status test that can be used with cerebrospinal fluid (VDRL-CSF test),38 but its use should be limited to CSF specimens from patients with reactive serum treponemal tests. The VDRL-CSF test is highly specific, but may be insensitive in some types of neurosyphilis.6,38,40 The FTA-ABS test has been adapted for use with CSF (FTA-ABS CSF test).38,39 The FTA-ABS CSF test is considered an investigational status test. In contrast to the VDRL-CSF, the FTA-ABS CSF test is highly sensitive, but detects treponemal antibodies that may be unrelated to a current active infection.38-40 Nevertheless, a nonreactive result in the FTA-ABS CSF test may be used to rule out neurosyphilis.39,40

ROLE OF LABORATORY TESTS IN THE DIAGNOSIS OF SYPHILIS

Nontreponemal tests are the usual initial serologic tests for screening for syphilis because of low cost, ease of performance, and a sensitivity similar to the treponemal tests (Tables 1:2 and 1:4).
The advantage of the higher sensitivity of treponemal tests for the detection of late syphilis and late latent syphilis of long duration, is countered by the higher specificity of nontreponemal tests due to their tendency to become nonreactive following treatment of syphilis. In addition, both the laboratory costs and the costs to follow-up individuals with reactive screening tests are lower with the nontreponemal tests and the advantages of screening with treponemal tests are poorly documented. The introduction of automated treponemal tests may reduce the laboratory costs of screening with a treponemal test, but the increased costs associated with the follow-up of individuals with false positive treponemal screening tests will remain.76

Individuals with false positive results to both nontreponemal and treponemal tests are uncommon, but they may constitute a significant proportion of confirmed positive test results, especially in a low prevalence screening population (e.g. <1%).58-62,85,86,108 Immune system abnormalities, such as occur with lupus erythematosis, account for some of these combined nontreponemal-treponemal false positive test results,82 but many remain unexplained.86,108 Treatment is frequently offered along with counseling concerning the uncertainty of the diagnosis.

Below, we classify criteria for the diagnosis of syphilis into three categories -- definitive, presumptive, and suggestive -- according to recommendations published by CDC following consultation with syphilis experts.109

Incubating T. pallidum Infection

The T. pallidum infection status of sex partners may be useful information in the diagnosis of syphilis, especially early syphilis, when the patient could have been recently infected.

Approximately 30% of persons who have sex with an infected partner with active skin or mucosal lesions will develop syphilis;7 however, none of the tests currently available reliably detect T. pallidum infection during the incubation period. For these reasons, treatment is recommended for patients with a known exposure to syphilis within the previous 90 days, even without a specific diagnosis of T. pallidum infection.109

Primary Syphilis

Microscopy for the direct detection of T. pallidum, a nontreponemal serologic test for syphilis with quantitative results, and historical information regarding prior treatment for syphilis and the syphilis status of recent sex partners are the key information items needed to diagnose primary syphilis. Use of darkfield microscopy and a rapid nontreponemal test for syphilis permits arriving at the diagnosis and initiating treatment and partner notification at the initial visit.

Observation of T. pallidum in genital or extra-genital ulcers that are potentially due to syphilis infection is definitive evidence of primary syphilis. The DFA-TP is potentially useful, when dark field microscopy is not available, or is too nonspecific as in oral lesions, or used to confirm results from darkfield microscopy; however, at present, the DFA-TP is not widely available. A lymph node draining a chancre may be aspirated or the chancre may be biopsied to obtain a suitable specimen for microscopy, although neither is a common practice for the diagnosis of syphilis. A nonreactive nontreponemal test result is consistent with a positive darkfield or DFA-TP result because of the usual delay in humoral response in primary syphilis.7

T. pallidum may not be detectable in ulcer exudate of an important minority of patients with primary syphilis because of an inadequate specimen, self-treatment by the patient, or declining concentration of organisms during spontaneous resolution of the lesion.

A reactive nontreponemal serologic test result for a patient who has not been treated for a previous episode of syphilis, or a four-fold increase in titer for a patient with a history of previous treatment for syphilis, is considered to be presumptively diagnostic of primary syphilis if T. pallidum is not detected in ulcer fluid by microscopic examination, or if a microscopic examination is not performed.109

Since both direct detection of T. pallidum by microscopy and serologic tests may be somewhat insensitive in early primary syphilis, serologic examination may be repeated over a 2 to 12 week period to rule out syphilis. Also, the presence of a lesion and sexual contact within the preceding 90 days with a person in whom syphilis has been diagnosed is considered to be suggestive of primary syphilis.

Treponemal tests probably add little to nontreponemal tests for the diagnosis of primary syphilis; however, laboratories may routinely confirm nontreponemal test results to rule out false positive results, especially when chancres are atypical. Because of the insensitivity of the MHA-TP in early primary syphilis, the FTA-ABS test is the recommended confirmatory test.58,59,74,75

For the future, amplification tests such as PCR, offer great promise especially as they have the potential to simultaneously detect T. pallidum, herpes simplex virus, and Haemophilus ducreyi, which not uncommonly coexist in lesions.31

The presence of HIV infection does not influence the choice or interpretation of tests in the diagnosis of primary syphilis.110

Secondary Syphilis

During secondary stage syphilis, serologic tests for syphilis are uniformly reactive, and treponemes may be found in lesions by direct microscopic examination. As with primary syphilis, the sensitivity of direct microscopic detection of T. pallidum varies greatly, with low sensitivity associated with poor specimens and inexperienced or inadequately trained microscopists. Also, as with primary syphilis, the use of darkfield microscopy and a rapid nontreponemal test for syphilis permits arriving at the diagnosis and initiating treatment and partner notification at the initial visit. A presumptive diagnosis of secondary syphilis is based on the presence of typical lesions and a reactive nontreponemal test titer of >1:8 and no previous history of syphilis or, for persons with a history of syphilis, a fourfold increase in the most recent titer compared with past test results. 109 For patients with atypical lesions and/or nontreponemal test titers of <1:8, the nontreponemal tests should be repeated and a confirmatory treponemal test should be performed before a presumptive diagnosis is made.109 Nucleic acid amplification tests should be potentially useful for the confirmation of T. pallidum in atypical lesions, although these tests do not provide a result at the initial visit and the results currently are considered presumptive rather than definitive. A suggestive diagnosis is made only when serologic tests are not available and is based on both the presence of clinical manifestations and sexual exposure within the past 6 months to a person with syphilis.109

Although the titer of serologic test results may be higher among syphilis patients with coexistent HIV infection, the choice and interpretation of tests for the diagnosis of secondary syphilis is the same for HIV infected and uninfected patients.110 Even though T. pallidum can be detected in the CSF of a substantial minority of HIV infected and uninfected patients with secondary syphilis, routine CSF examination is not considered necessary in the absence of neurological symptoms and signs, since the relationship between detection of treponemes in the CSF in early syphilis and the development of late neurosyphilis and the potential for reducing any such increased risk through enhanced therapy has not been established.110

Latent Syphilis

Since, by definition, symptoms and signs are absent in latent syphilis, tests to detect latent syphilis are usually performed as screening tests, or because of exposure to T. pallidum infection. In early latent syphilis the nontreponemal tests are reactive. As the length of time between initial infection and serologic testing increases, the likelihood of a reactive nontreponemal test decreases. With specificities of approximately 99% for both the nontreponemal and treponemal tests in low prevalence populations (e.g. 1-2%), at least 50% of positive test results will be false positives. Fortunately, the causes of false positive test results differ for the treponemal and nontreponemal tests so that a test from either group can be used to confirm the positive result of a test from the other group. Even though they are less sensitive than treponemal tests in long-standing infection, nontreponemal tests are usually used as the initial test in screening because they are less likely to be reactive following successfully treated syphilis and they are relatively inexpensive and easy to

preform. If a treponemal test is performed in the absence of a reactive nontreponemal test, and a reactive result is obtained, then the reactive treponemal test result should be confirmed.

A definitive diagnosis of latent syphilis is not usually obtained since specimens suitable for the direct detection of T. pallidum are not available. A presumptive diagnosis can be based on reactive treponemal and nontreponemal tests and no history of previous treatment for syphilis or, if previously treated, on a fourfold rise in titer of the nontreponemal test.109 For purposes of determining appropriate treatment and management of sex partners a diagnosis of early latent syphilis (less than one year) is made by establishing that within the past year the patient had a nonreactive serologic test, or a four-fold rise in nontreponemal test titer, or sexual intercourse with a person who has early syphilis.109

As with the other stages of early syphilis, the titer of reactive nontreponemal tests may be higher among patients with coexistent HIV and early latent T. pallidum infection,110-113 except for patients with advanced immunodeficiency, who may become falsely seronegative.114, 115 Nevertheless, except to consider the possibility of false negative serologic tests for syphilis in advanced AIDS, the approach to the detection of early latent syphilis is the same for HIV infected and uninfected individuals.110 Routine CSF examination to detect neurosyphilis is probably indicated for the patient with coexistent latent syphilis and HIV infection;110 whereas, the need for lumbar puncture in the HIV uninfected patient with latent syphilis is controversial.15

Late Tertiary Syphilis

Late stage syphilis occurs in approximately one-third of the persons who fail to receive treatment.6 Often symptoms of late-stage syphilis occur 10 to 20 years after the initial infection.
Because results for approximately 30% of patients with late syphilis will be reactive in the treponemal tests but nonreactive in the nontreponemal tests, treponemal test results should be obtained if syphilis in these stages is suspected and the nontreponemal tests are nonreactive. The laboratory should be informed that late syphilis is suspected; otherwise, depending on laboratory policy, a treponemal test may not be performed in the absence of a reactive nontreponemal test. Treponemal test results are almost always reactive and may be the only basis for diagnosis.

A definitive diagnosis of a syphilis gumma (late benign syphilis) can be based on the observation of T. pallidum in biopsy samples by DFAT-TP.116 However, few treponemes are found in gummas by direct microscopic examination. Presumptive diagnosis is based on a reactive treponemal test and no known history of treatment for syphilis. PCR has recently been described for the diagnosis of late benign syphilis in a case report.116

Diagnosis of cardiovascular syphilis is made on the bases of aortic insufficiency or aneurysm, reactive treponemal test results, and no known history of treatment for syphilis.

CSF examinations, such as the VDRL-CSF slide test, total protein, and white-cell counts, should be performed on the spinal fluid of patients with apparent late latent syphilis, and those with clinical symptoms and signs consistent with neurosyphilis. Diagnosis of neurosyphilis requires a reactive treponemal test result with a serum sample, and a CSF cell count of > 5 mononuclear cells per cubic centimeter, and CSF total protein in excess of 45 mg/dl. The VDRL-CSF

test should be performed only if the patient's serum treponemal test is reactive. The VDRL is the only nontreponemal test that should be used for testing CSF.38 The results of the VDRL-CSF test are reported as either reactive or nonreactive. A quantitative test is performed to determine the titer of the antibodies in the CSF by preparing twofold serial dilutions in saline. False-positive VDRL-CSF test results have been reported infrequently,116 but false-negative results may occur in symptomatic or asymptomatic neurosyphilis.38 Although the FTA-ABS CSF test is not considered as a standard test for neurosyphilis, this modification of the FTA-ABS test is more sensitive but less specific that the VDRL-CSF test, and is occasionally used to rule out neurosyphilis if a nonreactive result is obtained39, 40 However, a reactive result by itself may not indicate neurosyphilis, but may, for example, be detecting antibodies from an earlier adequately treated case of syphilis.39, 40

Evaluation after treatment of adult syphilis

To follow the efficacy of therapy, patients should be monitored to ensure that signs and symptoms have resolved and that the antibody titer has declined. Because nontreponemal tests are the only serologic tests for syphilis that can be quantitated they are used to follow the success or failure of treatment. A fall in titer of antibody following treatment confirms cure,51,117-119 whereas a rise in titer following treatment establishes treatment failure or reinfection. To monitor the efficacy of treatment, quantitative nontreponemal tests should be performed on the patient's serum samples. Whenever possible, post-treatment changes in titer should be followed using the same test and laboratory. However, no definitive criteria for cure or failure exist. Patients with signs or symptoms that persist or recur or who have a sustained four-fold increase in titer can be considered to have failed treatment. CDC treatment guidelines also suggest that failure of titers to decline by four-fold by six months after therapy for primary or secondary syphilis identifies patients at risk for treatment failure, as does lack of a four-fold decline within 12 - 24 months for patients with latent syphilis and initially high titers (>1:32). The time after therapy until the titer declines four-fold appears to be longer with the RPR53 than with the VDRL test.53 For most patients treated in early syphilis, the titers decline until little or no reaction is detected after three years.53,54,117,118 Patients treated in the latent or late stages, or who have had multiple episodes of syphilis, may show a more gradual decline in titer.110,118,119 Low titer will persist in approximately 50% of these patients after 2 years.119 As far as can be determined, this persistent seropositivity does not signify treatment failure or reinfection, and these patients are likely to remain serofast even if they are retreated.119

Recent studies indicate that HIV infection may delay the decline in titer of antibody detected by nontreponemal tests for syphilis in patients with primary or secondary syphilis.110 The prognostic significance of this finding is unknown, since these patients did not experience clinical treatment failure during the one-year post-treatment observation period, delayed decline in antibody titer was not associated with detection of T. pallidum in CSF, and enhanced therapy did not appear to prevent the delay.110

At present, the value of performing lumbar puncture and CSF examination in patients without clinically apparent neurological disease, following therapy of patients for uncomplicated early syphilis is uncertain.110

Congenital Syphilis

The control of congenital syphilis can be accomplished by routine serologic screening and treatment of infected pregnant women. False-positive nontreponemal test results have been reported in samples from pregnant women.62, 108 Therefore, nontreponemal test results should be confirmed with a treponemal test and, if it is reactive, the patient should be treated. Also, in pregnancy, nontreponemal titers remaining from previous treatment of syphilis tend to increase nonspecifically.108 This increase in titer may be confused with the diagnosis of reinfection or relapse. An increase in titer may be considered nonspecific if previous treatment can be documented and if darkfield or DFA positive lesions, a fourfold increase in titer, and a history of recent sexual exposure to a person with infectious syphilis are absent.109

Currently, the diagnosis of neonatal congenital syphilis depends on a combination of results from physical, radiographic, serologic, and direct microscopic examinations.17 Although some clinical manifestations may be present at birth, they are more often seen at 3 weeks - 6 months. At birth, up to 50% of the infants with congenital syphilis are asymptomatic;18 other stigmata that may develop later include teeth and bone malformation, deafness, blindness, and learning disabilities.

When screening for congenital syphilis at delivery, CDC recommends the testing of the mother's serum rather than cord blood.6 Recent studies101, 120 compared the reactivity of the mother's serum, cord blood, and infant's serum and found that the maternal sample is the best indicator of infection, followed by neonatal serum, with cord blood being the least reactive. Infant's serum is the specimen of choice for the IgM-specific tests. The standard serologic tests for syphilis, based on the measurement of IgG, may reflect passively transferred antibodies from the mother to the infant, rather than IgM antibodies produced during gestation. Previously, the difference in the mother's nontreponemal test titer and the infant's titer at delivery was thought to be a means of distinguishing infected from uninfected infants. If the infant's titer was higher than that of the mother's, then the infant had congenital syphilis.121 However, the converse has not been found to be true. A lower titer in the infant's serum than in the mother's does not rule out congenital syphilis.101 Examination of serum sample pairs from mothers and infants in cases of congenital syphilis indicated that only in 22% of the cases did the infant have a titer higher than that of the mother. Passively transferred maternal antibodies should be catabolized and undetectable among noninfected infants between the ages of 12 - 18 months.122,123 Recent interest in tests that detect treponemal IgM antibody has been stimulated by the need for a test to diagnose congenital T. pallidum infection in the neonate whose mother has syphilis, but who is born without signs of congenital syphilis. The majority of such neonates are not infected, but, nevertheless, should be treated, since congenital syphilis cannot be ruled out. Demonstration of T. pallidum, by direct microscopic examination, in the umbilical cord, placenta, nasal discharge, skin lesion material, or any tissue is the definitive means for diagnosing neonatal congenital syphilis.16 Since infected infants can produce IgM in utero after 3 months gestation and the fetus can be infected with T. pallidum anytime during gestation, a positive treponemal IgM test in the neonate can, theoretically, be interpreted as presumptive for the diagnosis of congenital syphilis. However, the current IgM tests for congenital syphilis appear to be relatively insensitive; therefore a nonreactive test should not be interpreted as the absence of congenital infection.

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