Review

Laboratory methods of diagnosis of syphilis for the beginning of the third millennium

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ABSTRACT – Despite that the whole genome of *T. pallidum*, the causative agent of syphilis, has been sequenced, syphilis is, and will remain for some time, diagnosed by direct clinical observation and by laboratory methods. This review presents comprehensively most of the practical techniques used for direct detection of *T. pallidum* and lists all practical methods for phospholipid and treponemal antibodies detection. It describes most novel tests for syphilis, discusses problems with serocrossreactivity in Lyme disease, immune responses in HIV-syphilis coinfected patients, and reviews serologic responses to antibiotic treatment. © 1999 Éditions scientifiques et médicales Elsevier SAS

**1. Introduction**

Syphilis, caused by infection with *Treponema pallidum* subsp. *pallidum*, shares clinical manifestations with other treponemal and nontreponemal diseases, and in some stages, may be asymptomatic. Therefore its clinical diagnosis must be supported by laboratory tests. The causative organism of syphilis was first demonstrated on 17 May 1905 [1], and the first serological test, the complement fixation test, was reported one year later (10 May 1906) [2]. The complement fixation test and a precipitation test were the standard laboratory tests for syphilis for several decades until the introduction of the Veneral Disease Research Laboratory (VDRL) test using a mixture of cardiolipin-lecithin-cholesterol as antigen [3] and the development of a number of tests for detection of treponemal antibodies [4–6]. The scope of these tests has not changed significantly in the last few decades. Although molecular technologies such as polymerase chain reaction (PCR) have been introduced in recent years, they have yet to make a significant mark on the diagnosis of syphilis.

PCR has increased the sensitivity of *T. pallidum* detection to \( <10 \) organisms [7–9], but it still remains a technique performed mostly in research laboratories. The typing of *T. pallidum* [10], based on analysis of repeated genes (*arp* and *tpr*), was just introduced in 1998. It remains to be explored whether typing proves to have practical value in studying outbreaks and in attempts to control syphilis. Recently, the whole genome of *T. pallidum* has been sequenced [11]. This opens the door to various new approaches towards diagnosis, epidemiology, and management of syphilis in the third millennium. However, at best, in the early part of the third millennium laboratories may still use methods described in this review. As frequently happens with breakthroughs in science, overoptimistic predictions compete with highly skeptical ones. Taking the middle ground, we believe that in the near future new laboratory tests for detecting the causative organism, and perhaps even culturing of *T. pallidum*, will be accomplished. However, the newer tests may not have greater sensitivity or specificity than PCR. The highest priorities for improving diagnosis of syphilis should be aimed at developing sensitive and specific tests for diagnosis within the first two weeks of infection, in congenital syphilis and in neurosyphilis, situations when traditional testing has been limited value.

Vaccine development and global elimination of syphilis are noteworthy goals [12]. However, these are distant objectives at the current time. Transmission of *T. pallidum* has been sharply reduced in some countries, but the global occurrence of syphilis varies greatly (table I) [13]. Furthermore, the patterns of occurrence are unpredictable and unexplainable. Examples of these fluctuations include post-World War II France and the United States of America. In France, in the early 1950s the incidence of primary and secondary syphilis was over 20 cases per 100 000 inhabitants, decreasing in the late 1950s to three cases, only to rise to 10 cases per 100 000 population in the middle 1960s [14]. In the USA, by the 1950s syphilis became a clinical rarity, only to resurface as a disease of interest and concern with the appearance of human im-
There are a number of methods available for direct detection of intact organisms or *T. pallidum* DNA. 

2.1.1. Darkfield (DF) microscopy

The oldest, simplest, and still quite reliable method for identification of intact *T. pallidum* is darkfield microscopy. An experienced microscopist can identify *T. pallidum* from syphilitic lesions by morphology and movements of the organism. This method of diagnosing syphilis is particularly important when antibodies are not yet detectable in early syphilis or in patients with immunodeficiency. However, this technique has limitations in sensitivity (Table II). Furthermore, at a time when DF might prove useful, lesions may not be visible (located in rectum or genital tract), or appropriate for DF examination, i.e., in the mouth. Failure to detect *T. pallidum* by DF examination does not indicate that the patient is free of syphilis. There are also a number of technical factors that may cause negative results: too little material on the slide may cause quick drying of the preparation, too much fluid may cause difficulty in observing the movements of treponemes, refractile elements in specimens may obscure identification of treponemas, and improper thickness of the microscopic cover glass may cause difficulty in focusing. This technique should not be used for examination of suspicious lesions in areas (mouth and anal regions) where nonpathogenic treponemes are frequently present. Morphology and motility of nonpathogenic spirochetes may cause false-positive results. A detailed description of material collection, proper preparation of the film, calibration of the microscope, mode of examination, and differentiation from other microorganisms were described [21].

2.1.2. Direct fluorescent antibody test for *T. pallidum* in body fluids (DFA-TP) and tissues (DFAT-TP)

Whereas in the DF examination the identification of the spirochetes is based on the morphology and movements of the organism, in the DFA-TP or DFAT-TP tests fluorescent isothiocyanate (FITC)-labeled anti *T. pallidum* antibody identifies the organism. This antibody, however, does not differentiate between the pathogenic treponemes of syphilis and *T. pallidum* subsp. *pertенue* causing yaws, *T. pallidum* spp endemicum causing endemic syphilis, or *T. careteum* causing pinta. For the DFA-TP or DFAT-TP techniques, polyclonal anti *T. pallidum* or monoclonal antibodies against *T. pallidum* or *T. pallidum* components, labeled with FITC, are used. Due to the fact that *T. pallidum* shares antigens with a variety of microorganisms [11, 24], whether polyclonal or monoclonal antibodies are used in these tests, it is crucial that they be rendered treponema-pathogen-specific by adsorption with non-pathogenic spirochetes. Pathogen-specific antibodies may be used in a direct or indirect immunofluorescent method. The number of organisms in the fluid or tissue that can be detected by fluorescent antibody tests is similar to that for DF (Table II). The sensitivity of these methods is only slightly better than DF. This test has the advantage over DF

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**Table I.** Estimated global occurrence of new cases of syphilis in 1995 in people age 15 to 49.

<table>
<thead>
<tr>
<th>Geographical region</th>
<th>Number of new cases in millions – both sexes</th>
<th>Population (15–49 years old) in millions – both sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Europe</td>
<td>0.200</td>
<td>211 360</td>
</tr>
<tr>
<td>North America</td>
<td>0.144</td>
<td>152 910</td>
</tr>
<tr>
<td>Latin America</td>
<td>1 260</td>
<td>250 780</td>
</tr>
<tr>
<td>Eastern Europe &amp; Central Asia</td>
<td>0.100</td>
<td>157 600</td>
</tr>
<tr>
<td>East Asia &amp; Pacific</td>
<td>0.560</td>
<td>802 530</td>
</tr>
<tr>
<td>South &amp; South East Asia</td>
<td>5 790</td>
<td>942 540</td>
</tr>
<tr>
<td>Asia</td>
<td>0.010</td>
<td>11 500</td>
</tr>
<tr>
<td>North Africa &amp; Middle East</td>
<td>0.610</td>
<td>162 580</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>3 530</td>
<td>254 400</td>
</tr>
<tr>
<td>Total</td>
<td>12 204</td>
<td>2 946 200</td>
</tr>
</tbody>
</table>

Adapted from [13] with permission.
because it allows detection of organisms in both exudates of lesions and in fresh or formalin-fixed tissues. Principles of the techniques, preparation of films or tissues, staining, and interpretation were described in detail [25, 26].

2.1.3. Silver staining

This procedure is widely used by pathologists, but it is not specific for *T. pallidum*. Silver nitrate will impregnate a number of different organisms and allows only for identification of the morphology of the organisms. Tissue artifacts are a potential hazard for misidentification. Pathologists would be better served using fluorescent antibody tests [25, 26].

2.1.4. Polymerase chain reaction (PCR)

The PCR technique has been used for identification of *T. pallidum* in various body fluids and fresh and paraffin-embedded tissues by a few investigators [7–9, 29, 31]. In certain situations, PCR is the most practical, sensitive, and specific technique available. However, PCR is not standardized. Interlaboratory differences are found in the source of primers and in the method of DNA extraction. In spite of differing methods, the sensitivity of PCR has been reported from several laboratories to be approximately 1 to 5 organisms per specimen [7–9, 29–31]. Application of PCR in an experimental animal model of syphilis has yielded useful information [32–34]. It was found that PCR of whole blood is preferential to PCR of serum. Of 18 serum samples from animals whose whole blood was PCR-positive, only one serum gave positive PCR [32]. Grimprel et al., however, reported that six (30%) of 20 neonates’ sera were positive by PCR [29]. In that report, however, what was considered to be ‘serum’ by the authors was likely plasma. We have found that clotting of whole rabbit blood spiked with treponemes trapped the organisms. All 15 blood clots examined by PCR were positive, but sera were PCR-negative (K.W. unpublished observation). Furthermore, when swabs from early skin syphilitic lesions were immersed in TE buffer and kept at room temperature for 90 days, 90% (7/8) were PCR-negative. Swabs from the same lesions, but kept at room temperature dry (without diluent) for 60 days, were still 100% (3/3) PCR positive. Swabs from healing lesions are less suitable for PCR examination. Punch biopsy (3 mm) of healing skin lesions kept in TE buffer for 90 days were still positive in approximately 85% (10/12) of specimens [32]. It should be stressed that in experimental conditions in rabbits, even after the skin lesion has spontaneously healed, biopsies from the site of infection taken four months after infection were still (10%) PCR-positive [34], suggesting that the organism may persist for a long period of time at the site of infection. It may be expected that similar conditions are found in natural infection.

It has been presumed that the shortcoming of PCR is that it cannot differentiate between live and dead organisms. This problem has been evaluated in experiments in rabbits using PCR and the rabbit infectivity test (RIT, 34). Heat-killed *T. pallidum* were eliminated within 10 days from testes and within 15 days from the skin. However, when chromosomal DNA from *T. pallidum* was injected (10 fg to 100 ng) into multiple sites in the skin or testes, it was eliminated within 24 to 48 h [34]. In view of this information we believe that in untreated *T. pallidum*-infected individuals, positive PCR indicates active infection. In a treated patient, when taking material for examination two to three weeks after treatment, positive PCR suggests treatment failure. PCR correlated almost 100% with the RIT [34].

2.1.5. Multiplex PCR (M-PCR)

Genital ulcers may be caused by *T. pallidum* as well as by herpes simplex virus (HSV) or *Haemophilus ducreyi*. Because of the correlation between transmission of HIV infection and genital ulcers, effective early treatment for ulcerogenital diseases is important. However, treatment differs for each of these microorganisms, so that identification of the organism(s) is critical for appropriate therapy.

Roche Molecular Systems in California, USA, developed a simultaneous PCR system detecting *T. pallidum*, herpes simplex virus type 1 and 2, and *H. ducreyi* [35]. The M-PCR applies three different sets of primers for detection of the target organisms. The M-PCR assay, when evaluated independently for each organism, has a sensitivity of 1 to 10 organisms. When all three targets were present in the same reaction tube, the sensitivity was 10 organisms. The concordance of M-PCR with confirmatory PCR for *T. pallidum* was 99.3% [35]. This test, however, is not yet commercially available.

2.1.6. Reverse transcriptase PCR (RT-PCR)

This assay, to date, has been used only in an experimental setting in which pooled human cerebrospinal fluid was spiked with *T. pallidum* [36]. The 16S rRNA gene from *T. pallidum* was used for the RT-PCR and compared with the TpN47 DNA PCR. The 16S rRNA RT-PCR was more sensitive (reproducibly detected 1 organism) than the DNA
PCR (10 organisms detected). This procedure still must be evaluated using clinical specimens. The authors rightfully noted that RNA is rapidly degraded once *T. pallidum* organisms are dead, limiting the use of this technique.

2.1.7. Rabbit infectivity test (RIT)

The RIT remains a research tool of academic interest for detection of virulent organisms in clinical specimens because of the need for animals, the very long incubation time after infection (several weeks to months), and variation in rabbit susceptibility to infection. The RIT, using susceptible rabbits, has a sensitivity (10 to 50 organisms) similar to that of DNA PCR [30, 34].

2.2. Indirect confirmation of syphilis: antibody detection

In the course of infection with treponemes, two types of antibodies are produced, nontreponemal and treponemal antibodies. The nontreponemal antibodies are referred to as phospholipid or VDRL or cardiolipin antibodies. They are also referred by some as nonspecific antibodies.

The term ‘nonspecific’ is, in our opinion, a misnomer in syphilis. Not knowing the source of the antigen responsible for stimulating antiphospholipid antibodies does not have a bearing on the specificity of the antibodies. The time of their appearance in an infected host is similar to that of treponemal antibodies, which are considered specific, in spite of the fact that those antibodies cannot differentiate between *T. pallidum* and other pathogenic treponemes, and cross react with many nonpathogenic treponemes and other microorganisms. Phospholipid antibodies should be considered as specific for syphilis as are the treponemal antibodies.

Prior to 1945, phospholipid antibodies were detected by complement fixation or precipitation tests using alcohol extract of organs, mostly heart. Mary Pangborn from the New York State Department of Health in Albany, NY, USA in 1941 isolated a phospholipid from beef heart which she termed cardiolipin [37]. This hapten required lecithin and cholesterol to be active in a complement fixation test with sera from syphilis patients. Frank and Elizabeth Maltaner from the same institution ‘fine-tuned’ the concentrations of the components, creating a working antigen for a complement fixation test [38], at that time the major test for syphilis. Rachel Brown, also from the same institution, developed a precipitation test using the cardiolipin antigen [39]. By 1945 the cardiolipin antigen was ready for widespread use. In 1946, Harris, Rosenberg and Riedel [3], from the Venereal Disease Research Laboratory, US Marine Hospital, Staten Island, NY, USA, adapted the cardiolipin-lecithin-cholesterol antigen for a microflocculation test and called the antigen and the test, VDRL.

2.3. Phospholipid antibody tests

Phospholipid or VDRL antibodies parallel the pathologic process of the infected host. They do not, however, bear any relationship to the development of immunity. These antibodies are detected by flocculation tests and by enzyme-linked-immunosorbent assay (ELISA). The Centers for Disease Control and Prevention (CDC)-approved standard tests for phospholipid antibodies in the USA are the VDRL slide test, the rapid plasma reagent (RPR) card test, the unheated serum reagent (USR) test, and the toluidin red unheated serum test (TRUST) [18].

2.3.1. VDRL test

This is a slide microflocculation test using a cardiolipin-lecithin-cholesterol mixture as antigen. The antigen, which is an alcohol solution containing 0.03% cardiolipin, 0.21% lecithin, and 0.9% cholesterol, is suspended in a buffered saline solution. When combined with antibodies, it forms flocculates that are visible using the microscope’s low magnification. Both IgM and IgG antibodies react in this test. The VDRL test may be used as a qualitative test for detection of syphilis, as well as a quantitative test for evaluation of treatment. False negative reactions may be encountered due to improper technique, or because of a prozone effect due to an excess of antibodies. However, the prozone effect appears infrequently (1 to 2%) and may be corrected by diluting the examined serum [18]. It is the test of choice for examination of cerebrospinal fluids (CSF) in suspected neurosyphilis. The antigen for CSF examination requires a slightly different preparation than that for examination of serum. Technical details are in [40].

2.3.2. The RPR test

The rapid plasma reagin card test is a microscopic flocculation test. (Reagin is an old term used in the past to denote the phospholipid antibodies.) It is a modification of the VDRL test. The test is done on plastic cards having multiple 18-mm circles onto which serum and modified VDRL antigen are placed and gently rotated. The VDRL antigen for the RPR contains choline chloride (to eliminate the inactivation of tested serum), ethylenediaminetetraacetic acid (to enhance the stability of the suspension), and charcoal particles (for visualization of the reaction). In the presence of antibodies a flocculation reaction takes place, and the charcoal particles are entrapped in the antigen-antibody aggregates, causing visible agglutination. For this test, serum or plasma may be used, active or inactivated. It may be used for qualitative as well as quantitative purposes. The sensitivity and specificity of these flocculation tests are shown in table III. The RPR has a slightly higher sensitivity compared with the VDRL test, especially in primary syphilis. But the VDRL has a slightly better specificity, which in practical sense means fewer nonspecific reactions that must be confirmed by other tests. Technical details are in [41].

In the USA the RPR test is generally used as a screening test, followed by a test detecting treponemal antibodies (usually TP-passive agglutination test or FTA-ABS). This strategy is dictated by cost, the simplicity of the RPR test, and by allowing serial evaluations of the disease activity after treatment. In some countries of Europe, RPR and one of the treponemal tests are used. However, in other countries an enzyme immunoassay for treponemal antibodies is used for screening [42]. This is most likely dictated by higher sensitivity, especially in the early stages of syphilis. However, treponemal antibodies are not a good indicator for monitoring disease activity.
2.3.3. Enzyme immunoassay for cardiolipin antibodies (EIA-VDRL)

The EIA or ELISA, introduced in the mid-1970s has become a widely used immunologic assay for diagnosis of infectious and noninfectious diseases. The principle of this technique is based on a reaction between antigen fixed onto a solid phase (most frequently wells of a microhemagglutination plate or strip) and antibodies in tested serum. The antibodies that bind to the antigen are identified by enzyme-labeled antiglobulin serum and a substrate that reacts with this enzyme. The enzyme-substrate interaction causes a color reaction, the intensity of which is measured by spectrophotometry. The intensity of the color is proportional to the concentration of antibodies in tested serum. An EIA for detection of cardiolipin antibodies has been developed [43]. The VDRL antigen is used for the test in which both IgM and IgG antibodies are detected.

2.3.4. Spirotek Reagin II test

The EIA for phospholipid antibody detection is designed as a screening test. The EIA lends itself to automation and computerized reporting. A test under the name Spirotek Reagin II (formerly Visuwell Reagin II [44] is commercially available (Organon Teknika, Durham, NC, USA). In Europe this product is distributed under the name Teponostika. The sensitivity and specificity of some of the cardiolipin tests are listed in table III. Because EIA's are in general much more sensitive (detect lower levels of antibodies) than flocculation tests, the EIA for syphilis has higher sensitivity than flocculation tests. On a provisional basis, the Spirotek Reagin II was approved by the CDC as a screening test for syphilis [45]. The technical details of the Spirotek Reagin II test are described [45].

2.3.5. Capture-S Cardiolipin test

More recently, a new test for phospholipid antibody detection was introduced [46]. It is a solid-phase erythrocyte adherence assay. It is designed primarily for screening blood donors (Immucor, Inc. Norcross, GA, USA). The wells of a microtiter plate are coated with modified VDRL antigen, and human anti-lipid antibodies are detected by indicator erythrocytes sensitized with anti-human IgG and IgM. To make this test economically feasible for large number of samples, it requires equipment for reagent application, washing, and reading. The sensitivity and specificity of this test are comparable to those of the RPR test [46].

2.4. Biological false-positive reactions (BFP)

Antibodies to phospholipids are produced not only as the result of *T. pallidum* infection, but also, in lower percentages, after infection with other microorganisms, in diseases associated with inflammation such as autoimmune disorders, during pregnancy, and in aging non-*T. pallidum*-infected individuals (table IV). These antibodies were detected in the past by the complement fixation test and presently are detected by the flocculation tests used for syphilis diagnosis. This has led to the introduction of the term biological false-positive (BFP) reaction or reactors. Evidence suggests that the so-called BFP reaction is an antigen-antibody reaction in which nothing is ‘biologically false’. However, in some situations the triggering antigen is not known. It would be more correct to refer to these reacting antibodies as nontreponemal-infection associated phospholipid antibodies (NTPA). In the past two decades the development of sensitive solid-phase immunoassays, utilizing various phospholipids as antigens (including cardiolipin without lecithin and cholesterol) has permitted identification of antibodies associated with connective tissue disorders, patients with anticardiolipin syndrome, and differentiation of these antibodies from VDRL antibodies appearing in active syphilis [47]. More details on this topic may be found in [48].

### Table III. Sensitivity and specificity of the cardiolipin antibody tests of untreated syphilis based on CDC studies.

<table>
<thead>
<tr>
<th>Source of sera</th>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Percent</td>
<td>Range</td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>VDRL</td>
<td>78</td>
<td>74–87</td>
</tr>
<tr>
<td>Secondary</td>
<td>100</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Latent</td>
<td>95</td>
<td>88–100</td>
<td>–</td>
</tr>
<tr>
<td>Late</td>
<td>71</td>
<td>37–94</td>
<td>–</td>
</tr>
<tr>
<td>Nonsyphilis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>RPR</td>
<td>86</td>
<td>77–99</td>
</tr>
<tr>
<td>Secondary</td>
<td>100</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Latent</td>
<td>98</td>
<td>95–100</td>
<td>–</td>
</tr>
<tr>
<td>Late</td>
<td>73</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Nonsyphilis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>Spiro</td>
<td>93</td>
<td>–</td>
</tr>
<tr>
<td>Secondary</td>
<td>100</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Latent</td>
<td>100</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Non syphilis</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

From [40, 41, 45] with permission. * The Visuwell Reagin ELISA of White and Fuller [44] demonstrated a sensitivity of 97.3% in untreated early syphilis and 85.3% in treated early syphilis. In nonsyphilis sera the overall specificity was 78.5%.
Nelson, working in the laboratory of Manfred M. Mayer in the Department of Bacteriology of The Johns Hopkins School of Hygiene and Public Health in Baltimore, MD, USA, together with Judith A. Diesendruck and John T. Eagen, developed a test for treponemal antibodies based on immobilization of T. pallidum in the presence of anticomplementary fixation and precipitation tests. The TPI was in use until the 1980s. Due to its complexity, difficulty in reproducibility, cost, and requirement for animals, it was replaced in many laboratories by the classic fluorescent treponemal antibody absorption test (FTA-ABS). The TPI, most likely for sentimental reasons, is still used in some laboratories in Europe.

There are several tests that are used for detection of treponemal antibodies. Herein, we will discuss only those most commonly used and the new tests, which promise improvements over older tests. It should be noted that the serodiagnosis of syphilis is constantly evolving, suggesting that no test is ideal for all stages of syphilis.

2.5.1. Fluorescent techniques FTA-ABS

The principle of the fluorescent antibody technique is the reaction of antibodies with a T. phagedenis Reiter preparation fixed on a microscope slide. In the FTA-ABS test, sera are diluted 1:5 with sorbent before they are applied to slides. The sorbent is a heated, concentrated culture filtrate of T. phagedenis Reiter, which reacts with antibodies to common or group-specific treponemal antigens. Because the FTA-ABS test requires the participation of two antibodies (patients’ serum and conjugate), of which only the second is under the control of laboratory staff, the quality of the conjugate is critical. The antibodies reacting in the FTA-ABS are the first to be detected after infection. Whether treponemal antibodies appear before phospholipid antibodies is not known. Their early detection may reflect a difference in sensitivity of the tests. During the primary stage of untreated syphilis, particularly in patients seronegative for cardiolipin antibodies, most (75%) of the antibodies are IgM and approximately 25% are IgG [49]. The IgM antibodies disappear in treated patients or even when the disease progresses untreated, generally during the latent period [50]. Persistence of IgM and IgG phospholipid antibodies and IgG antitreponemal antibodies may indicate reactivated syphilis and potential development of late symptomatic disease. The sensitivity and specificity of the FTA-ABS assay are shown in Table V. The technical description is in [51].

<table>
<thead>
<tr>
<th>Source of sera</th>
<th>Test</th>
<th>Percent</th>
<th>Range</th>
<th>Percent</th>
<th>Range</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Primary syphilis</td>
<td>FTA-ABS</td>
<td>84</td>
<td>70–100</td>
<td>–</td>
<td>–</td>
<td>51</td>
</tr>
<tr>
<td>Secondary</td>
<td></td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Latent</td>
<td></td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Late</td>
<td></td>
<td>96</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Nonsyphilis</td>
<td></td>
<td>–</td>
<td>–</td>
<td>97</td>
<td>94–100</td>
<td></td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>Spirotek</td>
<td>93</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>71</td>
</tr>
<tr>
<td>Secondary</td>
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<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Latent</td>
<td></td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Nonsyphilis</td>
<td></td>
<td>–</td>
<td>–</td>
<td>94</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>Immunoblot</td>
<td>90</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>78</td>
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<td>Early latent</td>
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<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Non syphilis</td>
<td></td>
<td>–</td>
<td>–</td>
<td>99</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

With permission from [51, 71, 78].

Table IV. Some common causes of BFP reactions.

<table>
<thead>
<tr>
<th>Long-term persistence</th>
<th>Short-term persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Infection with various microorganisms</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Vaccination against smallpox</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>Infectious mononucleosis</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Infective hepatitis</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>Viral pneumonia</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>Chickenpox</td>
</tr>
<tr>
<td>Thyroiditis</td>
<td>Measles</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>Viral encephalitis</td>
</tr>
<tr>
<td>Various neuropathies</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>Hepatic cirrhosis</td>
<td>Malaria</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Narcotic addiction (injections)</td>
</tr>
<tr>
<td>Erythema nodosa</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>Apparently healthy with undetermined causes</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td></td>
</tr>
<tr>
<td>Waldenström’s macroglobulinemia</td>
<td></td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td></td>
</tr>
<tr>
<td>Leprosy</td>
<td></td>
</tr>
<tr>
<td>Narcotic addiction (injections)</td>
<td></td>
</tr>
<tr>
<td>Apparently healthy (increase with age)</td>
<td></td>
</tr>
</tbody>
</table>


With permission from [51, 71, 78].

Table V. Sensitivity and specificity of treponemal antibody tests in untreated syphilis based on CDC studies.

With permission from [51, 71, 78].
2.5.2. Fluorescent technique-double staining (FTA-ABS DS)

Negative FTA-ABS results are difficult to evaluate. One cannot be sure whether the negative results are due to a lack of antibodies or a lack of treponemes on the slide. This difficulty may be obviated by using the FTA-ABS DS, which applies two conjugates to the slide. The first, tetramethyl-rhodamine isothiocyanate-conjugated anti-human immunoglobulin (μ or γ specific) detects the presence and isotype of antitreponemal antibodies. The second, FITC-conjugated anti- T. pallidum globulin, stains all treponemes. A two-filter system is needed for reading the reactions.

The specificity of the fluorescent tests is very high. However, false-positive reactions may occur in approximately 1% of normal individuals and 3% of pregnant women [18]. The frequency of false-positive reactions is higher in sera from patients with various immunological disorders such as systemic lupus erythematosus (SLE), drug-induced lupus, rheumatoid arthritis, scleroderma, and others. Sera of patients with SLE may show an atypical, uneven granular staining of organisms. However, some SLE-patients' sera may present homogenous staining which does not differ from a true positive FTA-ABS test. The false-positive reaction is presumably due to anti-DNA antibodies or other antibodies to nuclear proteins reacting with the proteins on the surface of the fixed T. pallidum. Technical details are in [52].

2.5.3. FTA-ABS 19S IgM test

This test was designed for diagnosis of congenital syphilis [53]. The predecessor of this test was the FTA-ABS IgM test, which had unsatisfactory specificity. In the latter, the conjugated antibody reacted not only with infants' IgM, but also with rheumatoid factor (RF) consisting of neonates' IgM bound to placentally transmitted maternal IgG antibodies. In congenital syphilis, RF may be found in 80% of sera of newborns [54]. To eliminate this interference, the new FTA-ABS 19S IgM requires the removal of all IgG, free or bound to IgM (RF), in the infant serum. This is achieved by passing the neonatal serum through a small column containing protein G. The serum IgM-rich fraction is then examined with conjugated antibody specific for IgM. This test has a sensitivity of 92% and a specificity of 93% compared with 90 and 75%, respectively, for the FTA-ABS IgM test using unfractionated serum for the diagnosis of congenital syphilis. This is a screening as well as confirmatory test.

2.5.4. Passive agglutination test for T. pallidum antibodies

In the past, the microhemagglutination tests known as MHA-TP or TPHA were popular tests used in the laboratories. The MHA-TP reagents are no longer being manufactured and the TPHA, also using erythrocytes as carrier for the T. pallidum antigen, will, most likely, be replaced by a new passive agglutination test using a lyophilized preparation of colored gelatin particles as carrier for the T. pallidum antigen. The test is known as SERODIA-TP-P [55] and is produced by Fujirebio Inc., Chuo-ku, Tokyo 1030007, Japan. The principle of the test is similar to that of microhemagglutination tests. Colored gelatin particle carriers sensitized with sonicated T. pallidum (Nichols strain) are mixed with serial dilutions of plasma or serum in a microtiter plate (similar to TPHA). A diluent containing normal rabbit serum and rabbit testicular extract is used for specimen dilution. The test is incubated for two hours (or overnight) at room temperature and the reaction is read in a viewer. A compact button formed by the settling of the nonagglutinating particles indicates a negative reaction. Specimens containing antitreponemal antibodies will react with antigen and form a smooth mat of agglutinating particles that settle on the bottom of the well. This is a qualitative test. The sensitivity and specificity are very similar to those of the TPHA test. The test is less sensitive than the FTA-ABS test in untreated primary syphilis, but compares favorably in all other stages. The test may react nonspecifically in approximately 3% of normal human sera (male and female) or sera from pregnant women. Specimens from individuals with autoimmune diseases, toxoplasmosis, i.e. drug users, HIV infection, or Helicobacter pylori infection may be reactive approximately 11% of the time. The technique is described in the commercial company reagent insert [55]. The product requires more thorough evaluation using larger numbers of clinical specimens from treated and untreated patients at different stages of syphilis as well as other clinical conditions. This product has not yet been approved by the US Food and Drug Administration.

2.5.5. Competitive EIA

The commercially available BioElisa Syphilis (Biokit, Barcelona Spain) is a competitive assay for screening of antitreponemal antibodies [56]. T. pallidum whole antigen is fixed to the bottom of a plate and the treponemal antibodies in the test serum compete with peroxidase-conjugated human antitreponemal antibodies. Detection of the reaction is similar to that of regular EIA. This assay performed as well as the FTA-ABS and TPHA (sensitivity 99.5% and specificity 99.4%) at all stages of syphilis for both untreated and treated syphilis except primary syphilis, where the BioElisa demonstrated lower sensitivity. This assay, as most of the newer EIAs, requires further evaluation.

2.5.6. Captia® syphilis G

This EIA is an indirect test for detection of treponemal antibodies. It may be used as a screening test as well as a confirmatory test for syphilis. The principles are the same as described earlier for EIA. This test uses microtитration plates or strips coated with sonicated T. pallidum antigen. The reacting human IgG treponemal antibodies are detected by antihuman IgG monoclonal antibodies labeled with biotin and enzyme-labeled streptavidin. Biotin has a high labeling efficiency and a high affinity for streptavidin. Therefore, a strong signal is produced for each molecule of bound antibody. In some laboratories the test has performed as well as combined VDRL and MHA-TP tests [57, 62]. It also has been shown to be suitable in syphilis-HIV coinfected cohorts [63], but further exploration of this test is needed. However, the Captia Syphilis G also has given false-positive reactions with sera containing nontreponemal infection-associated phospholipid antibodies, sera from patients with Lyme borreliosis [64], and other diseases [65]. This test is commercially available (Trinity Biotech, Jamestown, NY, USA; Sanofi Diagnostic Pasteur, Chaska, MN, USA): under the name CAPTIA® Syphilis G
Table VI. Sensitivity and specificity of CAPTIA-EIA for treponemal antibody detection.

<table>
<thead>
<tr>
<th>Test</th>
<th>Source of sera</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPTIA-G</td>
<td>Selected, adults, neonates, untreated, treated (n = 178)</td>
<td>98.3</td>
<td>100</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Routine, adults, adolescent, neonatal (n = 585)</td>
<td>100</td>
<td>99.8</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Routine, adults (n = 964)</td>
<td>100</td>
<td>98.2</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Adults, past syphilis, HIV (+) (n = 28), HIV (-) (n = 31)</td>
<td>82 (HIV+) 97 (HIV-)</td>
<td>100 (HIV+) 99 (HIV-)</td>
<td>63</td>
</tr>
<tr>
<td>CAPTIA-M</td>
<td>Selected, adults, neonates, treated, untreated (n = 178)</td>
<td>82–94</td>
<td>-</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Neonatal (n = 116)</td>
<td>88</td>
<td>97–100</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>asymptomatic congenital syphilis (n = 18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>asymptomatic congenital syphilis, probably infected (n = 60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>asymptomatic probably uninfected (n = 38)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The sensitivity and specificity of CAPTIA-G were compared to FTA-ABS test and the CAPTIA-M to 19S (IgM) FTA-ABS [57, 68] or to reverse enzyme-linked immunospot (RELISPOT) detecting immunoglobulin-secreting cells [68].

and is being used more and more frequently, especially in Europe. The sensitivity and specificity are given in Table VI. Technical details are in [66].

A new CAPTIA Select Syph-G EIA (Trinity BioTech, Jamestown, NY, USA) is in the process of evaluation. This test does not yet have Federal Drug Administration (USA) approval. Sonicated treponemal reagent is used as antigen. It differs from the CAPTIA Syph-G assay in several ways: it uses plate button well (C wells), and different reagents and the incubation time is shorter, and at room temperature.

2.5.7. Captia® syphilis M

This EIA is specifically designed for detection of IgM treponemal antibodies in sera of congenitally infected babies. The principle is similar to that of an EIA. However, instead of treponemal antigen, antihuman μ chain specific antibodies are fixed onto a well of a microplate or strip. These fixed antibodies indiscriminately bind IgM present in serum. Added sonicated extract of T. pallidum (Nichols strain) antigen will react only with the IgM having combining sites for treponemal antigens. A monoclonal antibody to the T. pallidum antigen, (axial filament) conjugated with horseradish peroxidase, is used to detect the IgM-antigen complex. An enzyme substrate yields a colored product when cleaved, and the intensity of the color is read by an ELISA reader. The intensity of the color is proportional to the concentration of the antibodies. The CAPTIA® Syphilis M assay’s sensitivity in symptomatic congenital syphilis is similar to that of the FTA-ABS IgM 195 test, but the specificity is higher [57, 67, 68]. This test still awaits more extensive evaluation in asymptomatic congenital syphilis. The Captia Syphilis-M assay is also suitable for early syphilis detection. The sensitivity was 94% for primary syphilis, 85% for secondary, and 82% for early latent syphilis [57]. This decrease in sensitivity is due to the disappearance of IgM antibodies in syphilis. The susceptibility and specificity of this assay are listed in Table VI. A detailed technical description of the test is provided in [69].

2.5.8. Treponemal antibody tests using recombinant T. pallidum antigens. EIA-TmpA

The membrane localized T. pallidum protein, TmpA, has shown promise for further diagnostic development for serodiagnosis of syphilis [70]. Evaluation of the EIA-TmpA has demonstrated similar sensitivity and specificity to the FTA-ABS test. Moreover, the levels of the antibodies to TmpA antigen decrease sharply within one year after antibiotic treatment of patients with syphilis, suggesting that EIA-TmpA may be suitable for monitoring treatment of syphilis. This assay was evaluated in the late 1980s in several laboratories. However, the literature survey did not show conclusive reports regarding its usefulness compared with other assays.

2.5.9. Spirotek syphilis antibodies

An EIA (Organon Teknika, Durham, NC, USA) for detecting treponemal antibodies has currently been recommended by the CDC to be used as a confirmatory test for syphilis. Similar to the Spirotek Reagin II test, the wells of a strip or microtiter plate are coated with a cloned component of T. pallidum, the 47-kDa antigen [71]. The antibodies in patient sera react with the antigen, and the antibodies are detected by biotinylated anti-human immunoglobulin labeled with streptavidin-peroxidase, causing a color reaction. The intensity of the color is proportional to the levels of antibody present in serum. Similar to other EIAs, the reaction is read in a plate reader, thereby eliminating the subjectivity of the human eye. Tested sera must not be cloudy or hemolized, or contain particles. The sensitivity of the Spirotek Syphilis test is the highest of all treponemal tests in diagnostic use especially in untreated primary syphilis. The specificity, however, is lower in comparison to other tests listed in Table V. Technical details are in [71].

2.5.10. Immune-capture EIA (ICE Syphilis)

A novel ICE Syphilis screening test, using three recombinant T. pallidum antigens, (TpN15, TpN17, and TpN47), was developed and compared with Captia Select Syph-G using sonicated T. pallidum antigen [42]. In the ICE Syphilis the microtiter wells are covered with unlabeled recombinant T. pallidum antigen (rTp) together with antihuman IgG and IgM. Serum or plasma to be tested are added into the wells. If antibodies to T. pallidum are present they are captured by the antigen on the plate. In addition, a proportion of total IgG and IgM of the tested specimen is captured by the anti-human antibodies. Unbound antibodies are washed off and the added conjugated rTp antigen is captured only by the specific antibodies already bound to the plate. Unbound rTp conjugate is washed off.
and the added enzyme-substrate cause a color reaction read by an ELISA reader. The intensity of the color is proportional to the concentration of antibodies reacting with rTp. An evaluation, done on a significant number of unselected and selected sera, concluded that both specificity and sensitivity were significantly higher in the ICE Syphilis test compared with the Captia Select Syph-G assay. The ICE Syphilis test was also significantly more sensitive than the FTA-ABS. The ICE Syphilis test was positive in all 15 HIV coinfected patients tested, whereas the Select Syph-G EIA detected treponemal antibodies only in 12/15 HIV-infected patients. The authors concluded that the ICE Syphilis test is suitable for automation and would make it an ideal screening test for syphilis in HIV-infected individuals [42].

2.5.11. Treponemal antibody test detected by Western blot (immunoblot, IB)

This assay provides a molecular characterization of the humoral immune response to T. pallidum based on detection of antibodies to a variety of T. pallidum proteins [72-77]. The technique is based on gel-electrophoretic separation of the solubilized T. pallidum proteins according to their molecular size. The separated proteins are transferred onto a nitrocellulose membrane and exposed to tested serum. The antibody-antigen complex is visualized by adding anti-human globulin-enzyme-substrate, causing a color reaction. The IB is a very helpful adjunct in the laboratory diagnosis of congenital syphilis. It has been demonstrated that IgM antibodies in congenital syphilis always react with certain T. pallidum peptides. However, due to cross-reactivity between spirochetes including Borrelia and other organisms, IB results must be interpreted critically, especially in Lyme disease endemic areas. This assay is still in the investigational category of the CDC. The sensitivity of the assay is shown in table V. Technical data and interpretation of results are in [78]. MarDx Diagnostic (5919 Farnsworth Ct. Carlsbad, CA) distributes T. pallidum Western blot kits through two distributors in Europe: Ingen, Sogaris 203, Rungis, cedex, France and Genzyme Virotech GmbH., Lowenplatz 5, Russelsheim, Germany.

3. Laboratory diagnosis in various clinical conditions

As indicated earlier, there are problems in the diagnosis of very early syphilis, neurosyphilis, and asymptomatic congenital syphilis. Problems also may arise in diagnosis of patients coinfected with T. pallidum and HIV, in intravenous drug users, and in patients infected with cross-reactive microorganisms.

3.1. Primary syphilis

In the very early stage of syphilis infection, when the lesion is still not well developed or appears in an unusual location of the body (mouth, anorectal region), or escapes the patient’s attention, the diagnosis of syphilis is extremely difficult to make. Such cases are rarely seen. In some instances, however, a patient seeks medical help who knowingly had or post factum learned of having had sexual contact with an untreated person with syphilis. In the first 2 to 3 weeks after infection, serology is positive in only 30% of cases (VDRL) or 50% using the FTA-ABS, test EIA, or Western blot [18]. If regional lymph nodes are enlarged and tender, needle biopsy material may be examined for the presence of T. pallidum by DF or DFA tests. It would be preferable to examine lymph node tissue by PCR because of PCR’s high sensitivity and specificity. However, PCR testing for syphilis is done in only a limited number of institutions, and needle biopsy for the diagnosis of syphilis is rarely done. When a suspicious lesion is visible and characteristic, it may be examined by any of the methods listed for the direct detection of T. pallidum. Usually, by this time, serologic tests are positive in a high percentage of cases (tables III, V, VI). The presence of a genital ulcer and the presence of a positive VDRL and treponemal antibodies does not always indicate primary syphilis. Very rarely, a patient may have latent syphilis (presence of antibodies) and chancroid, or another type of genital ulcer. Furthermore not every lesion in the urogenital region indicates syphilis. The differential diagnosis should include balanitis, trauma, erysipelas, genital herpes, and chancroid.

3.2. Secondary syphilis

The secondary stage of syphilis commences at 6 to 8 weeks after the onset of infection. It is characterized by spirochetemia and widespread dissemination of T. pallidum. Early nonspecific symptoms may resemble an influenza-like syndrome. Generalized lymphadenopathy and a generalized or localized maculopapular rash involving the skin (including palms and soles), as well as mucocutaneous lesions, also occur frequently. T. pallidum are present in the lesions. When serology is positive, examination of the lesions for presence of T. pallidum is done only for academic curiosity. At this stage, in untreated patients, nontreponemal and treponemal antibody tests are positive nearly 100% of the time (tables III, V, VI). In immunocompromised patients, with very rare exceptions the results of these tests are as in immunocompetent patients [79]. The rash and symptoms of secondary syphilis, especially when not typical, may mimic a variety of nonsyphilitic diseases including pityriasis rosea, rosacea, erythema multiforme, and psoriasis.

3.3. Latent syphilis

The natural course of untreated disseminated syphilis is to resolve spontaneously. The patient is thereafter free of symptoms. In the pre-penicillin era, relapses with recurrent fulminant secondary syphilis occurred in 20% of patients. Such relapses generally occurred within the first two years of primary infection. Relapses after two years were rare. As a rule, when untreated patients have fulminating secondary syphilis, they develop a degree of immunity that helps in containing the infection and prevents further relapses. Between early latency and the onset of tertiary syphilis, untreated individuals enter a late latent period in which the patient is both noninfectious and immune to reinfection with T. pallidum. An exception is the pregnant woman, who can transmit infection to the fetus at any time during untreated disease, even when she is no longer infectious to her sexual partner.
3.5. Neurosyphilis

The presence of T. pallidum in the CNS. On the involvement of the central nervous system by T. pallidum, proposed to distinguish between invasion and infection. U.J. Wile, discussing the report of Moore and Hopkins, stated that early neurosyphilis is now more frequently encountered, often making the diagnosis of neurosyphilis difficult. Early neurosyphilis (< 2 years after primary infection) was uncommon in the pre-penicillin era. In contrast, early neurosyphilis is now more frequently encountered, especially in HIV-infected patients [81, 82]. Some time ago U.J. Wile, discussing the report of Moore and Hopkins, proposed to distinguish between invasion and involvement of the central nervous system by T. pallidum [83]. Invasion is a common phenomenon, indicating the presence of T. pallidum in the CNS. On the other hand, involvement suggesting pathological changes in the CNS, was less common. This concept seems to have been confirmed. In recent studies, Lukehart et al., using RIT, found T. pallidum in CSF in 12 of 40 (30%) patients with primary or secondary syphilis [84]. In this study, four patients whose CSF were negative by RIT had a reactive CSF-VDRL, strongly suggesting neurological involvement.

The high affinity of T. pallidum for brain tissue has also been demonstrated by PCR in the guinea pig experimental model [33]. T. pallidum DNA was detected in brain specimens of seven of 14 (50%) intradurally infected adult guinea pigs and in 12 of 33 (36%) congenitally infected animals [33].

The low percentage of patients with positive CSF-VDRL or CSF-FTA-ABS does not encourage the use of these tests for proving asymptomatic cases of neurosyphilis. The most reliable diagnostic modality for syphilis is identification of T. pallidum. Since T. pallidum cannot be cultured in vitro, PCR is a suitable substitute. A useful comparison of PCR with the ‘gold standard’ for syphilis (RIT) was done using materials from CS patients [29, 30]. Amniotic fluid from 11 serologically reactive pregnant women with untreated syphilis, and sera and CSF from seven asymptomatic and twelve asymptomatic infants with probable infection were examined by PCR, RIT, and IB for

3.4. Tertiary syphilis

Clinical manifestations of tertiary syphilis develop after a highly variable period of time. In studies conducted between 1890 and 1910, which were still being analyzed in the 1950s, it was observed that approximately 60% of untreated patients may go through life unharmed and approximately 30% will develop one or another form of active disease [80]. Benign syphilis, which involves non-vital structures such as skin, soft tissues, bones, cartilages or certain organs, became clinically apparent in approximately 15%, cardiovascular syphilis in 10 to 25%, and neurosyphilis in approximately 10% of untreated individuals [80].

Reactivation of syphilis is noticeable not only by clinical symptoms, but also by production of antcardiolipin antibodies (approximately 80% positive) and treponemal antibodies (> 90% positive) [18]. The lesions of tertiary syphilis are not suitable for direct examination by DF or DFA because they may contain undetectable numbers of treponemes. The presence of T. pallidum in the lesions of tertiary syphilis was disputed for decades. We have only recently documented by PCR the presence of T. pallidum in a brain graft of an HIV-syphilis coinfected subject [31].

3.5. Neurosyphilis

The protean neurologic and psychologic clinical manifestations, the lack of classic symptoms, and the lack of a single laboratory criterion as being absolutely diagnostic of neurosyphilis, often make the diagnosis of neurosyphilis difficult. Early neurosyphilis (< 2 years after primary infection) was uncommon in the pre-penicillin era. In contrast, early neurosyphilis is now more frequently encountered, especially in HIV-infected patients [81, 82]. Some time ago U.J. Wile, discussing the report of Moore and Hopkins, proposed to distinguish between invasion and involvement of the central nervous system by T. pallidum [83]. Invasion was a common phenomenon, indicating the presence of T. pallidum in the CNS. On the other hand, involvement suggesting pathological changes in the CNS, was less common. This concept seems to have been confirmed. In recent studies, Lukehart et al., using RIT, found T. pallidum in CSF in 12 of 40 (30%) patients with primary or secondary syphilis [84]. In this study, four patients whose CSF were negative by RIT had a reactive CSF-VDRL, strongly suggesting neurological involvement.

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IgM antibodies. Amniotic fluids from babies of seropositive mothers were positive by PCR in nine of eleven (82%) and by RIT in seven of nine (78%) specimens examined [29]. PCR correlated well with RIT in serum and CSF in the symptomatic group in which IgM antibodies were found in almost all cases and no additional tests were needed. However, in the asymptomatic group, neither RIT nor PCR were positive, except in one case. In asymptomatic CS, a comprehensive approach is advisable. Adequate assays are still lacking for diagnosis of asymptomatic CS, which accounts for > 60% of cases of CS. A stringent case definition is the only guideline for management [88]. One should not rely on results of one test alone, especially when it is negative in suspicious cases. Laboratory diagnoses in various clinical conditions were described earlier [20].

4. Problems with sero-cross-reactivity

One particular diagnostic problem due to antigenic cross-reactivity has been raised regarding serologic differentiation of infection with Borrelia burgdorferi, the etiologic agent of Lyme disease, and T. pallidum. With current diagnostic assays, this antigenic cross-reactivity should not cause a major problem. Patients with syphilis may have a positive EIA for Lyme disease. However, when sera from patients with syphilis are tested by immunoblot for antibodies to B. burgdorferi, they generally do not meet the established criteria for Lyme disease [89]. There should be little problem of false serologic positivity for syphilis in the setting of Lyme diseases. Although sera from patients with Lyme disease may cross-react with antigens in treponemal assays, they are generally negative by nontreponemal tests.

5. Serologic response to antibiotic treatment

The effectiveness of cure in syphilis may be evaluated by remission of symptoms or seroreversion, or in extreme or experimental conditions by using RIT or PCR. Clinical improvements may not always represent cure of syphilis. Seroreversion may suggest a cure, but only if patients are monitored for long periods of time. The most certain test of cure is the one that demonstrates a lack of T. pallidum in organs. Traditionally, RIT was the only test to prove elimination of T. pallidum. In view of the fact that PCR correlates very well with RIT, it may replace RIT, which is impractical due to the length of time required to obtain results and because of cost.

The only reliable tests evaluating seroreversion are those detecting phospholipid antibodies. However, it must be stressed that even without treatment, a patient may become serologically negative after several years [80]. Seroreversion also may depend on various conditions, e.g., length of time of infection, severity of disease, titer of phospholipid antibodies, status of reactivation of the disease process, and HIV status. Retrospective studies have demonstrated that phospholipid antibodies in patients treated with standard doses of penicillin (2.4 million units, for early syphilis) decline four-fold after three months and eight-fold after six months [90]. To follow seroreversion, serum must be examined on average every three to six months for at least two years. Romanowski et al. retrospectively evaluated 800 patients treated with standard doses of antibiotics and found that a cumulative proportion of 63% seroverted by RPR [91]. Of note is the fact that after three years, patients with primary, secondary, and early latent disease seroverted 72, 56, and 26% of the time, respectively. However, patients with primary syphilis who had repeated episodes of infection became seronegative only 34% of the time after three years. A cohort of 13 patients with repeat infections in the secondary stage and two patients with early latent syphilis never seroverted during five years of observation.

Romanowski et al. also made another interesting observation [91]. There is a general notion that treponemal antibodies persist for years, if not for life, after treated infection. These authors observed seroreversion in FTA-ABS and MHA-TP testing in patients treated during first episodes of primary syphilis. Those treated during early latent syphilis never seroverted. The FTA-ABS test was more likely to become negative than the MHA-TP. This confirms the observations made by Jisselmaiden et al. that seroreversion occurred in treated patients whose sera were examined by EIA-TmpA [70]. It seems that when the host is not exposed to T. pallidum for long enough periods of time and the organisms have been eliminated rapidly after treatment, long-memory B-cell clones are unable to produce lifelong treponemal antibodies.

Tanaka and Suzuki, using an EIA assay with sonicated T. pallidum as antigen, reported that after treatment there was a decrease in treponemal antibodies – IgM (77%), IgA (69%) and one or more of the IgG sub-classes (77%) – within three months after treatment [92]. It is of note that HIV-infected patients with primary [93], secondary [93, 94], and late [93] syphilis appear to have slower, four-fold decreases in nontreponemal tests by six to twelve months than non-HIV-infected patients. Rolf et al. in a prospective treatment trial of 541 patients found that serologic treatment failures were more frequent in HIV-infected compared with non-HIV-infected patients with primary and secondary syphilis, but not early latent syphilis. However, clinical treatment failure was not more common in HIV-infected individuals [95]. Furthermore, as noted by Haas et al. [96], FTA-ABS and MHA-TP tests do not reliably identify prior syphilis in HIV-infected patients. The Captia EIA may be a better substitute.

A major problem for physicians is when to retreat patients who do not serovert after treatment. Patients with primary, secondary, and early latent syphilis generally serovert within one, two, and five years, respectively. Failure to serovert may indicate reinfection, reactivation, persistence of infection, or biologically false-positive reactions. If material is available for biopsy, PCR may be utilized to demonstrate persistence of infection. However, the decision for retreatment will frequently be based upon evidence of clinical disease, the level of persistent nontreponemal antibodies (lack of fall or actual rise), persistence of nontreponemal antibody slides be-
yond the time when they are expected to disappear (see text above), a positive PCR, and the exclusion of false-positive reactions. Patients with other treponemal infections such as *T. pallidum* subs. *endemicum* or *T. pallidum* subs. *pertenue* frequently do not serorevert after treatment such as *T. pallidum* subs. *endemicum* or *T. pallidum* positive reactions. Patients with other treponemal infections beyond the time when they are expected to disappear (see Review Wicher et al. 1999, 1035-1049). This work was supported (V.W.) by NIH grant # AI21833 from the National Institute of Allergy and Infectious Diseases, US Public Health Service.

### 6. Conclusion

Despite the significant advances made in microbiologic and molecular techniques, *T. pallidum* remains a noncultivable organism, and syphilis, for the most part, is diagnosed by direct clinical observation or serologic techniques. For most cases, a nontreponemal screening test using standard-quality VDRL antigen, followed by a highly sensitive and specific treponemal test, yields satisfactory results. Standardization of the treponemal test is a desired goal in the coming decade. Problems will remain, for some time, in the diagnosis of very early infection (when lesions are not evident or do not have the characteristic appearance), asymptomatic congenital syphilis, and neurosyphilis. Improving the diagnosis for these disease stages is of highest priority. Improved methods for the follow-up of syphilis disease activity, particularly in the setting of HIV infection, is another important priority. For both HIV-infected as well as non-HIV-infected patients, the results of serial serologic testing after infection are confusing enough, and the fear of late sequelae great enough, that patients are frequently needlessly treated with antibiotics multiple times. Laboratory methods for diagnosis of syphilis are not a factor in syphilis control. The methods of surveillance are. Unfortunately, due to lack of recognition of both the early and late stages of *T. pallidum* infection, in many instances infections are not suspected and therefore progress. Educational activities regarding the numerous clinical manifestations and need for testing of this ubiquitous infection must continue. In regions of the world where the incidence of *T. pallidum* remains high, mass screening, similar to that undertaken after World War II, will help decrease the incidence of this infection. Although it is unlikely that syphilis will be eliminated in the next century, there is hope that it can be brought to the verge of extinction.

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### References

Laboratory methods of diagnosis of syphilis

Review


