APPENDIX 1:2

History of Diagnostic Tests for Syphilis

Treponemal Detection Tests

The use of diagnostic tests for syphilis began in 1903\(^1\) with the first attempt to link the infection with a microorganism through animal inoculation. In 1905 Schaudin\(^2\) and Hoffmann\(^3\) were the first to associate *Spirochaeta pallida*, as *Treponema pallidum* was then known, with the disease syphilis. They used a modified Giemsa stain to examine lesion material from individuals with chancres. Others have attempted to use biological stains to identify *T. pallidum* without success. In 1909, Coles\(^4\) described the use of darkfield illumination, basing diagnosis on the motility of the organism. The direct microscopic examination of treponemes in stained tissue sections was described in 1910, and the use of darkfield microscopy became a routine diagnostic procedure in 1923. The application of the fluorescent antibody technique to lesion material was described in 1964\(^5\). Subsequently the DFA-TP technique was expanded for use with monoclonal antibodies and tissue sections\(^6,7\) (see Chapter 1). The PCR technique is the newest of the antigen detection methods and is discussed in Chapter 1. The PCR is applicable to specimens from lesions, CSF and tissue sections.\(^8-13\) The utility of PCR with samples from blood are under investigation.\(^14\)

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<th>Date</th>
<th>Author</th>
<th>Accomplishment</th>
</tr>
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<tbody>
<tr>
<td>1903</td>
<td>Metchnikoff and Roux(^1)</td>
<td>transferred syphilitic infection to chimpanzees</td>
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<tr>
<td>1905</td>
<td>Schaudin(^2) and Hoffmann(^3)</td>
<td>linked <em>Spirochaeta pallida</em> (<em>Treponema pallidum</em>) with syphilis</td>
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<tr>
<td>1909</td>
<td>Coles(^4)</td>
<td>described use of darkfield illumination</td>
</tr>
<tr>
<td>1964</td>
<td>Yobs, Brown and Hunter(^5)</td>
<td>developed the direct fluorescent antibody (DFA-TP) test</td>
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<tr>
<td>1991</td>
<td>Grimprel et al(^8)</td>
<td>described polymerase chain reaction (PCR) for the diagnosis of congenital syphilis</td>
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<td></td>
<td>Noordhoek et al(^9)</td>
<td>applied PCR to the diagnosis of neurosyphilis</td>
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Nontreponemal Serologic Tests (Table 1:2A)

The first nontreponemal serologic test for syphilis was developed in 1906. Wassermann, Neisser, and Brück\(^15\) adapted the complement-fixation test, previously introduced by Bordet and Gengou in 1901\(^16\), to serologic testing for syphilis. The Wassermann antigen was derived from extracts of liver from newborns who had died of congenital syphilis. Later Landsteiner demonstrated that tissues from animals without syphilis, particularly beef heart extracted in alcohol, could be used equally well as antigens.\(^17\) Cholesterol and lecithin were added to
increase the sensitivity of the antigens.\textsuperscript{17} Although the complement-fixation tests contributed immensely to the diagnosis of syphilis, the complement-fixation tests were complicated to perform, required many reagents and 24 h to complete. Michaelis\textsuperscript{18} and Meinicke,\textsuperscript{19} using water or sodium chloride to extract antigen from a syphilitic liver, developed the first precipitation tests that did not require complement. In 1922, Kahn\textsuperscript{20} introduced a macroscopic flocculation test without complement. The results of this test could be read in a few hours. Many modifications of the Kahn test appeared, each identified by the name of its developer and each claiming greater degrees of sensitivity or specificity.\textsuperscript{21} The major drawback of all these tests was the lack of a standardized antigen. Standardization of the tests was difficult and relied on comparison with stored serum samples and antigen controls to determine the differences in the sensitivity and specificity of the test. In 1941, with Pangborn\textsuperscript{22} successful precipitation of the phospholipid cardiolipin from beef heart, the active antigenic component of the nontreponemal tests, standardization was possible. In contrast to the crude tissue extract antigens, the pure cardiolipin-cholesterol-lecithin antigens could be standardized chemically as well as serologically. With the advent of these new purified antigens, microflocculation tests, such as the venereal disease research laboratory (VDRL)\textsuperscript{23} (Chapter 8) test, were developed. These flocculation tests, in which standardized reagents were used, yielded reproducible results, could be rapidly performed, gave acceptable levels of sensitivity and specificity, and were soon converted to methods suitable for mass screening. The addition of choline chloride and EDTA to the VDRL antigen enhanced the reactivity of the test and stabilized the antigen suspension.\textsuperscript{24} In the resulting unheated serum reagin (USR) test (Chapter 9), as the

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<th>Date</th>
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<tr>
<td>1906</td>
<td>Wassermann, Neisser and Brück \textsuperscript{15}</td>
<td>developed complement-fixation test</td>
</tr>
<tr>
<td>1907</td>
<td>Michaelis \textsuperscript{18}</td>
<td>developed first precipitation test without need for complement</td>
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<tr>
<td>1922</td>
<td>Kahn \textsuperscript{20}</td>
<td>introduced a flocculation test that required no complement</td>
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<tr>
<td>1941</td>
<td>Pangborn \textsuperscript{22}</td>
<td>isolated and purified cardiolipin</td>
</tr>
<tr>
<td>1946</td>
<td>Harris, Rosenberg and Riedel \textsuperscript{23}</td>
<td>developed Venereal Disease Research Laboratory (VDRL) test</td>
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<tr>
<td>1957</td>
<td>Portnoy, Carson and Smith \textsuperscript{24}</td>
<td>modified the VDRL to create the unheated serum reagin (USR)</td>
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<td>1961</td>
<td>Portnoy et al. \textsuperscript{25}</td>
<td>modified the USR to create the rapid plasma reagin (RPR)</td>
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<tr>
<td>1980</td>
<td>March and Stiles \textsuperscript{26}</td>
<td>developed reagin screen test (RST)</td>
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<td>1983</td>
<td>Pettit et al \textsuperscript{27}</td>
<td>modified USR to create toluidine red unheated serum test (TRUST)</td>
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<tr>
<td>1987</td>
<td>Pedersen et al \textsuperscript{28}</td>
<td>developed nontreponemal enzyme-linked immunosorbent (ELISA)</td>
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name implies, the need for heating serum was eliminated and plasma was also found to be an acceptable sample source.\textsuperscript{24} The next modification in the late 1950s, was the incorporation of charcoal particles into the USR antigen to aid in reading the reaction.\textsuperscript{25} The resulting test was the rapid plasma reagin (RPR) test (Chapter 10) performed on a plastic coated card. Additional modifications of the RPR card test resulted in the reagin screen test (RST)\textsuperscript{26} and the toluidine red unheated serum test (TRUST)\textsuperscript{27} (Chapter 11), and numerous other variations on the RPR test. In 1987, Pedersen, Orum and Mouritsen\textsuperscript{28} developed an enzyme-linked immunosorbent assay (ELISA) with the VDRL test antigen to detect IgG antibodies. The test is now available commercially as the VISUWELL reagin test (Chapter 16).

**Treponemal Serologic Tests (Table 1:3A)**

A serologic test using *T. pallidum* as the antigen was not described until 1949.\textsuperscript{29} The *Treponema pallidum* immobilization (TPI) test uses *T. pallidum* (Nichols strain) grown in rabbit testes as the antigen and is based on the ability of patients' antibody and complement to immobilize living treponemes. The results are read by darkfield microscopy. Although the TPI test was rapidly accepted as a specific test for syphilis, the TPI test was complicated, technically difficult, time-consuming, and expensive to perform. A simpler procedure was soon sought and an array of treponemal tests was developed. In 1953, D'Allesandro and Dardanoni\textsuperscript{30} prepared an antigen from *T. phagedenis*, the Reiter treponeme, a nonpathogenic organism, which, unlike subspecies *pallidum*, can be easily cultivated *in vitro*. The Reiter antigen was thought to be treponeme specific not only to the Reiter treponeme but also to subsp. *pallidum*. Complement-fixation tests based on the Reiter antigen detected an antibody different from that detected by nontreponemal tests, but a significant number of false positive results occurred. The most widely used tests employing the Reiter treponeme or an extract of the organism were the Reiter protein complement-fixation (RPCF) test and the one-fifth volume Kolmer test with Reiter protein antigen (KRP) test. Subsequent evaluation of these tests proved them to be less specific and less sensitive than the TPI test.\textsuperscript{31}

In 1957, a major breakthrough in treponemal antigen tests occurred with the development of the fluorescent treponemal antibody (FTA) test.\textsuperscript{32} The original FTA procedure used a 1:5 dilution of the patient's serum in saline solution, reacted with a suspension of killed treponemes. A fluorescein-labeled antihuman immunoglobulin was used as the conjugate, and the test was read under a microscope with an ultraviolet light source. When an improved fluorescein compound (fluorescein isothiocyanate) was used to prepare the labeled antihuman globulin conjugate, nonspecific reactions were encountered in approximately 25% of normal serum specimens.\textsuperscript{31} To eliminate these false-positive reactions, the test was modified by diluting the patient's serum 1:200, the FTA-200.\textsuperscript{33} However, the FTA-200 test, although highly specific, was not very sensitive. The nonspecific reactions of the original FTA test were found to arise because of shared antigens common to *T. pallidum* and the nonpathogenic treponemes that occur as part of the normal bacterial flora of humans.\textsuperscript{34} Deacon and Hunter\textsuperscript{34} prepared a sonicate from cultures of the Reiter spirochete and removed, by absorption, the common antigens. Their work led to the development of the more specific and sensitive fluorescent treponemal antibody absorption (FTA-ABS) test\textsuperscript{35} (Chapter 12). The FTA-ABS and its counterpart, the FTA-ABS double-
staining (DS) test (Chapters 12 and 13), viewed with incident light microscopes, remain the standard treponemal tests for syphilis today.

Based on the success of the FTA-ABS test for the diagnosis of acquired syphilis, variations on the test were attempted for the diagnosis of congenital syphilis and of neurosyphilis. By replacing the IgG conjugate with a IgM conjugate, the FTA-IgM was the first test produced, followed by the FTA-ABS IgM. Both were reported to be both nonspecific and lacking in sensitivity as is the FTA-ABS 19S IgM test (Chapter 1).

The other variation on the FTA-ABS procedure for the diagnosis of neurosyphilis is the FTA-ABS CSF test. FTA tests used for CSF examination have included techniques that used undiluted CSF (the CSF-FTA) and tests that used CSF diluted 1:5 with sorbent (the CSF-FTA-ABS test). FTA tests on CSF are more sensitive than the CSF-VDRL slide test. However, the clinical significance of this greater sensitivity remains to be established because the test may be reactive as a result of antibodies remaining in the CSF of patients who had been adequately treated for early or latent syphilis rather than as a result of neurosyphilis. False-positive results have been reported with the CSF-FTA tests; these appear to be eliminated by diluting the CSF in sorbent. The results of three studies appear to support the use of a nonreactive result with the FTA-ABS CSF test to rule out a diagnosis of neurosyphilis.

In 1965 another treponemal test for the diagnosis of syphilis was described by Ratlev. This hemagglutination test was initially performed in a tube test. Later, in an attempt to automate the test, a microtiter version of the test was developed -- the microhemagglutination assay for antibodies to T. pallidum (MHA-TP) (Chapter 14). The automation of the test was abandoned until the development of the PK-TP (Chapter 1).

The enzyme linked immunosorbent assay (ELISA) was first applied to syphilis serology in 1975. Since that time many other tests using the ELISA format have been developed and evaluated as treponemal tests. One of the ELISAs uses a sonicate of T. pallidum as the antigen, the Captia Syphilis-G test. This test is described in Chapters 1 and 18. A variation on the ELISA test is the Captia Syphilis-M test for the detection of congenital syphilis in the newborn. This test is also described in Chapters 1 and 15.

Many of the ELISA tests are based on the use of cloned antigens. The first cloned antigen to be used in the development of a serologic test for the diagnosis of syphilis was the tmpA protein. This protein has since been shown to be a membrane-localized lipoprotein closely associated with another membrane protein (tmpB). When used for an ELISA the tmpA protein proved to be both sensitive and specific; results were almost exactly the same as those seen with the MHA-TP and FTA-ABS tests. Because the authors found a significant correlation between the antibody titer against this antigen and the efficacy of treatment of patients, they suggested that the test could be used to monitor the outcome of treatment for syphilis with antibiotics. The test is not available on the U.S. market, to date.

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<tr>
<th>Table 1:3A</th>
<th>History of serologic tests for syphilis: Treponemal tests</th>
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Nelson and Mayer developed the Treponema pallidum immobilization (TPI) test in 1949.

D'Allesandro and Dardanoni developed the Reiter complement fixation test in 1953.

Deacon, Falcone and Harris developed the fluorescent treponemal antibody (FTA) test in 1957.

Hunter, Deacon and Meyer modified the FTA by the addition of sorbent, FTA-ABS test in 1964.

Rathlev developed the hemagglutination test for syphilis (TPHA) in 1965.

Cox et al. modified TPHA to a micromethod (MHA-TP) in 1969.

Veldekamp and Visser developed treponemal enzyme-linked immunosorbent test (ELISA) in 1975.

Hanff et al. applied the Western blot technique to the diagnosis of syphilis in 1982.

Schouls et al. first used a cloned antigen in a serologic test in 1989.

Several other recombinant proteins have been used to develop ELISAs since; the protein on which the greatest amount of work has been done is the 47-kDa protein. The ELISA based on the 47-kDa protein is described in Chapter 17.

The application of Western blotting (Chapter 19) techniques for the diagnosis of syphilis was first described by Hanff. The Western blot technique can be used to detect either IgG or IgM antibodies. The test using IgG conjugate appears to be at least as sensitive and specific as the FTA-ABS tests. Efforts to standardize the procedures for Western blot continue today.

References

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69. Weigel LM, Radolf JD, Norgard MV. The 47-kDa major lipoprotein immunogen of Treponema pallidum is a penicillin-binding protein with carboxypeptidase activity. Proc Natl Acad Sci USA 1994;91:11611-5.


