

APPENDIX 1:2

History of Diagnostic Tests for Syphilis

Treponemal Detection Tests

The use of diagnostic tests for syphilis began in 1903¹ with the first attempt to link the infection with a microorganism through animal inoculation. In 1905 Schaudin² and Hoffmann³ 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, Coles⁴ 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 1964⁵. 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

Table 1:1A **History of tests for syphilis: Direct treponemal detection tests**

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

Nontreponemal Serologic Tests (Table 1:2A)

The first nontreponemal serologic test for syphilis was developed in 1906. Wassermann, Neisser, and Brück¹⁵ adapted the complement-fixation test, previously introduced by Bordet and Gengou in 1901¹⁶, 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.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. Michaelis¹⁸ and Meinicke,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, Kahn²⁰ 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.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's²² 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, microfloculation tests, such as the venereal disease research laboratory (VDRL)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.²⁴ In the resulting unheated serum reagin (USR) test (Chapter 9), as the

Table 1:2A History of tests for syphilis: Nontreponemal tests

Date	Author	Accomplishment
1906	Wassermann, Neisser and Brück ¹⁵	developed complement-fixation test
1907	Michaelis ¹⁸	developed first precipitation test without need for complement
1922	Kahn ²⁰	introduced a flocculation test that required no complement
1941	Pangborn ²²	isolated and purified cardiolipin
1946	Harris, Rosenberg and Riedel ²³	developed Venereal Disease Research Laboratory (VDRL) test
1957	Portnoy, Carson and Smith ²⁴	modified the VDRL to create the unheated serum reagin (USR)
1961	Portnoy et al. ²⁵	modified the USR to create the rapid plasma reagin (RPR)
1980	March and Stiles ²⁶	developed reagin screen test (RST)
1983	Pettit et al. ²⁷	modified USR to create toluidine red unheated serum test (TRUST)
1987	Pedersen et al. ²⁸	developed nontreponemal enzyme-linked immunosorbent (ELISA)

name implies, the need for heating serum was eliminated and plasma was also found to be an acceptable sample source.²⁴ The next modification in the late 1950s, was the incorporation of charcoal particles into the USR antigen to aid in reading the reaction.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)²⁶ and the toluidine red unheated serum test (TRUST)²⁷ (Chapter 11), and numerous other variations on the RPR test. In 1987, Pedersen, Orum and Mouritsen²⁸ 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 1949.²⁹ 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 Dardanoni³⁰ 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.³¹

In 1957, a major breakthrough in treponemal antigen tests occurred with the development of the fluorescent treponemal antibody (FTA) test.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.³¹ To eliminate these false-positive reactions, the test was modified by diluting the patient's serum 1:200, the FTA-200.³³ 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.³⁴ Deacon and Hunter³⁴ 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) test³⁵ (Chapter 12). The FTA-ABS and its counterpart, the FTA-ABS double-

staining (DS) test (Chapters 12 and 13), viewed with incident light microscopes,³⁶ 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 sensitivity³⁷⁻⁴² 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)⁴³⁻⁵⁰ and tests that used CSF diluted 1:5 with sorbent (the CSF-FTA-ABS test).⁴³⁻⁵⁰ 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.⁴⁸ False-positive results have been reported with the CSF-FTA tests;^{44,49} these appear to be eliminated by diluting the CSF in sorbent.⁴⁹ 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.⁴⁸⁻⁵⁰

In 1965 another treponemal test for the diagnosis of syphilis was described by Ratlev.⁵¹ 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)⁵² (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 1975.⁵³ Since that time many other tests using the ELISA format have been developed and evaluated as treponemal tests.⁵⁴⁻⁶⁴ One of the ELISAs uses a sonicate of *T. pallidum* as the antigen, the Captia Syphilis-G test.⁶⁴ This test is described in Chapters 1 and 18. A variation on the ELISA test is the Captia Syphilis-M test⁶²⁻⁶⁵ 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.⁶⁶ 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.⁶⁶ 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.

Table 1:3A History of serologic tests for syphilis: Treponemal tests

Date	Author	Accomplishment
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1949	Nelson and Mayer ²⁹	developed the <i>Treponema pallidum</i> immobilization (TPI) test
1953	D'Allesandro and Dardanoni ³⁰	developed the Reiter complement fixation test
1957	Deacon, Falcone and Harris³²	developed the fluorescent treponemal (FTA) test
1964	Hunter, Deacon and Meyer ³³	modified the FTA by the addition of sorbent, FTA-ABS test
1965	Rathlev ⁵¹	developed the hemagglutination test for syphilis (TPHA)
1969	Cox et al. ⁵²	modified TPHA to a micromethod (MHA-TP)
1975	Veldekamp and Visser ⁵³	developed treponemal enzyme-linked immunosorbent test (ELISA)
1982	Hanff et al. ⁷⁰	applied the Western blot technique to the diagnosis of syphilis
1989	Schouls et al. ⁶⁶	first to use a cloned antigen in a serologic test

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.⁶⁷⁻⁶⁹ 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.⁷⁰ The Western blot technique can be used to detect either IgG⁷⁰⁻⁷³ or IgM^{74,75} antibodies. The test using IgG conjugate appears to be at least as sensitive and specific as the FTA-ABS tests.⁷³ Efforts to standardize the procedures for Western blot continue today.⁷⁶

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