

Review on Serologic Diagnosis of Syphilis

Dr. K. K. Ho

Social Hygiene Service (Venereology), Department of Health, Hong Kong

ABSTRACT

*In the era of HIV infection, there is a resurgence of syphilis. Despite the advancement of molecular technique and the whole genome of *Treponema pallidum*, the causative agent of syphilis, has been sequenced, the diagnosis and treatment response of syphilis still rely on serologic tests. This review article will summarize most of the commonly used serologic tests for syphilis and briefly discuss the diagnostic dilemma in various clinical situations such as neurosyphilis, congenital syphilis and syphilis in HIV infected patients.*

Keywords: *Review, serologic diagnosis, syphilis*

INTRODUCTION

Syphilis, a chronic and systemic sexually transmitted infection, is caused by the spirochaete *Treponema pallidum* subspecies *pallidum*. It has the nickname of great imitator in the history of medicine because of its diverse clinical manifestations that occur in different stages of the disease. Shortly after the discovery of causal relationship between syphilitic infection and *Treponema pallidum* by Schaudinn and Hoffmann with a modified Giemsa stain in 1905, researchers were trying to develop different methods to diagnose syphilis at various stages. *Treponema pallidum* cannot be cultured *in vitro*¹ and it can only be demonstrated by specific laboratory stains or dark ground microscopy.² Since the organism is not easily accessible in early stage of infection, serologic tests are still the main and essential tools in making the diagnosis of syphilis.

NATURAL HISTORY OF SYPHILIS

A detailed knowledge on the natural history of syphilis is crucial because serologic test of syphilis is

an indirect method to recognize *Treponema pallidum*. The serologic response varies between different stages of syphilis. The natural history of syphilis is variable and usually divided into early and late stages by an arbitrary time of one year. It is more infectious in the early stage of syphilis comparing with the late stage. Early syphilis is further divided into primary, secondary and early latent syphilis. Late syphilis includes late latent syphilis and different forms of tertiary syphilis namely gummatous, cardiovascular syphilis and neurosyphilis.

Our understanding of the natural history of syphilis without treatment was based on several large studies in the nineteenth century and is outlined in Figure 1. Oslo study was the largest prospective study of patients conducted in Oslo from 1890 to 1910 by Professor Boeck. In 1890, Professor Boeck considered mercurials, the standard treatment of syphilis in the nineteenth century, was more toxic than the disease itself. A total of 1978 patients with clinical diagnosis of early infectious syphilis were recruited and hospitalized till they were considered not infectious. Years later Gjestland followed those patients and determined the natural outcome of untreated syphilis in 1949 to 1951.³

The average incubation period of syphilis is about three weeks after exposure to infected partner. Primary syphilis is recognized by painless papule that ulcerates. Classic chancre of primary syphilis is one to two centimetres painless solitary ulcer with a raised indurated margin. The ulcer has a non-exudative base and is usually associated with mild to moderate regional

Correspondence address:

Dr. K. K. Ho

Cheung Sha Wan Dermatological Clinic

3/F, West Kowloon Health Centre

303 Cheung Sha Wan Road

Kowloon

Hong Kong

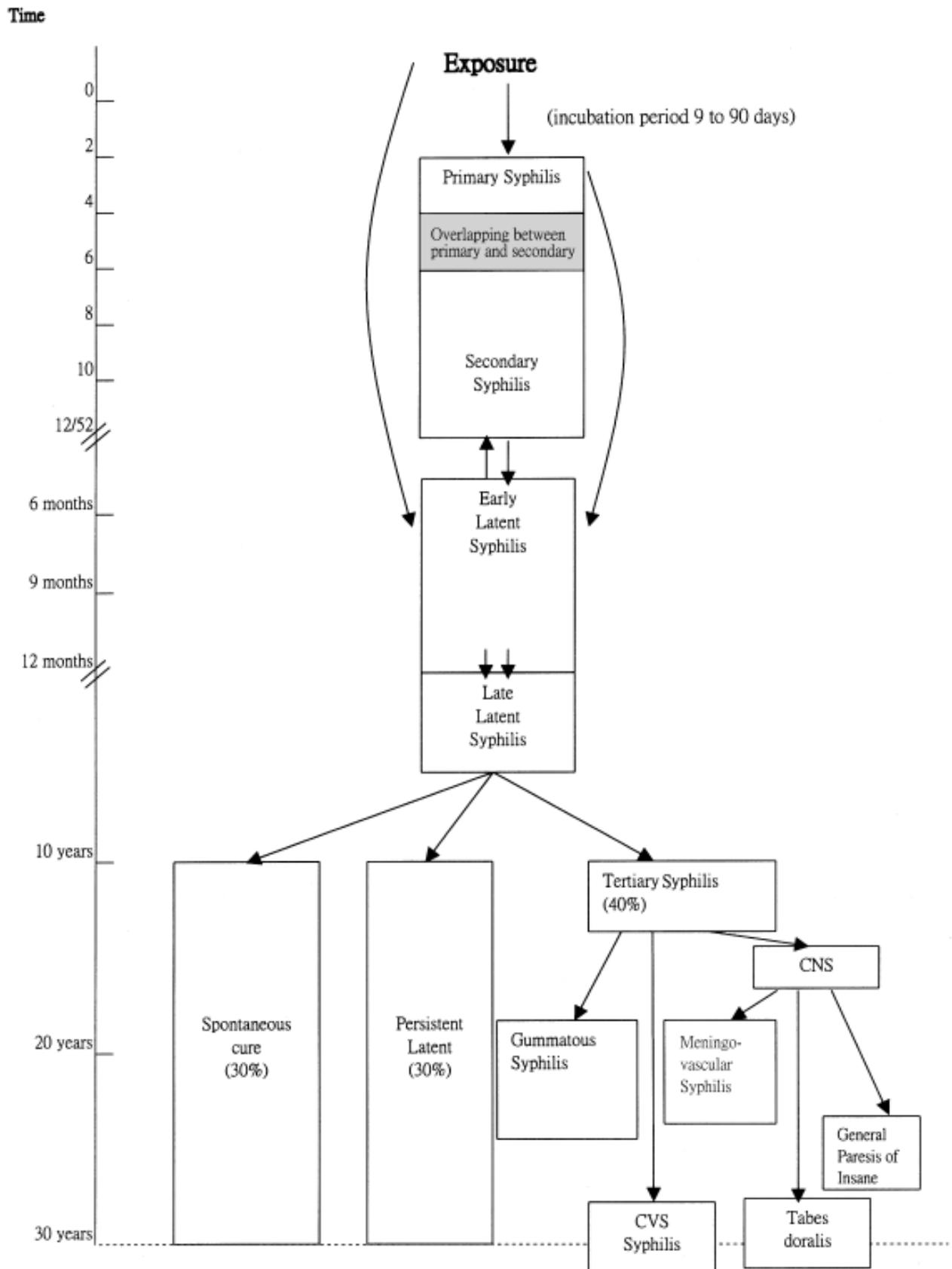


Figure 1: The natural course of syphilis

lymphadenopathy. Even without treatment, chancre heals spontaneously in three to six weeks. Weeks to months later, untreated syphilis may develop systemic illnesses that represent secondary syphilis. It is characterized by skin rash, diffuse lymphadenopathy, alopecia and systemic symptoms of fever, weight loss, headache, malaise and myalgia. It is not uncommon for a patient to present with both chancre and systemic illness especially in HIV infected patient, as there may be an overlapping period between primary and secondary syphilis.⁴ Similar to primary syphilis, secondary syphilis resolves spontaneously without treatment. Occasionally, one quarter of untreated secondary syphilis runs a relapsing course up to five years. Over 90% of the relapses occur in the first year.

Latency is defined as the absence of any clinical signs or symptoms of syphilis with historical or serological evidence of syphilis. Latent syphilis is usually divided into early latent and late latent syphilis by an arbitrary time of one year. After a certain period of latency, 40% of untreated syphilis will develop tertiary syphilis namely gummatous, cardiovascular syphilis and neurosyphilis. Gummatous syphilis, the most common tertiary syphilis in pre-antibiotic era, may present with cutaneous or visceral gumma with the typical histology of granuloma formation in about one third of tertiary syphilis. The mean duration between the infection and development of gummatous syphilis are 15 years. Cardiovascular syphilis classically involves the ascending aorta resulting in aortic aneurysm, aortic valve regurgitation and angina pectoris. The clinical symptom of cardiovascular syphilis is insidious in onset and presents with an average of 30 years after infection. There are many forms of neurological involvement. They are typically grouped as asymptomatic neurosyphilis, meningovascular syphilis, general paresis of insane and tabes dorsalis.

DIAGNOSTIC TESTS FOR SYPHILIS

The basic principle of serologic test for syphilis is to measure or demonstrate either the specific or non-specific anti-treponemal antibody, hence inferring that the examined patient has been exposed to *Treponema pallidum*. Not surprisingly, this methodology has its inherent limitation. Knowledge of its advantages and

limitations are important in interpreting the serologic results. In fact, it is an indirect method to document the presence of *Treponema pallidum* or history of exposure to the organism in a patient with an intact immune system that generates sufficient amount of antibodies. The first demonstrable humoral immunologic response is the production of specific anti-Treponemal IgM at the end of second week, and IgG at about four weeks after exposure to *Treponema pallidum*.⁵

The diagnostic tests of syphilis are broadly divided into three categories: (1) direct microscopic examination in the early stage of syphilis where the lesion is present; (2) indirect treponemal and non-treponemal serologic tests, and (3) molecular biology-based method.

Direct microscopic examination

Because the width of *Treponema pallidum* are too narrow and too slender to be seen under the ordinary microscopy, dark ground microscopic examination is still the most productive and invaluable method during primary, secondary and early congenital syphilis. Briefly, a specimen of serous fluid free of erythrocyte should be examined immediately through a reflecting dark-field condenser because viability is crucial for examiner to distinguish *Treponema pallidum* from other non-pathogenic saprophytic spirochaetes. The tightness of the spirals, 10 to 13 coils with 6 to 20 μm in length, and the characteristic movements of angulation, rotation, undulation, compression and expansion distinguish *Treponema pallidum* from other spirochaetes.⁶ However, even experienced observer may find it difficult or impossible to differentiate *Treponema pallidum* from other spirochaetes in oral cavity, hence it is not recommended to take specimen from oral lesion for dark ground microscopic examination.

In mid-1960s, the direct fluorescent-antibody (DFA) test for *Treponema pallidum* had been developed.⁷ To make it simple, specific monoclonal antibody to *Treponema pallidum* are used to conjugate with the pathogenic spirochaete in the specimen and to be read under the fluorescence microscope equipped with a dark-field condenser. Because the specific nature of the antibody used, *Treponema pallidum* in the specimen do not need to be motile and viable. DFA for *Treponema pallidum* is applicable to any lesion including oral and rectal lesions.

Syphilitic serology

The first serologic technique to diagnose syphilis was first described by Wasserman in 1906. Since then, a lot of works have been done to refine and to take advantage of new developments. Serologic tests for syphilis divide into two categories, non-treponemal and treponemal serologic tests. They are further divided into different tests based upon different methodology. Serologic tests are indirect measurement of the antibodies produced by the host to *Treponema pallidum*.

In non-treponemal serologic test, such as Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR), the antibodies to be measured are non-specific treponemal antibodies. It is based upon the reactivity, both IgM and IgG, of sera from patients with syphilis to non-specific cardiolipin-cholesterol-lecithin antigens. The limitations of non-treponemal serologic tests are lack of sensitivity in dark ground microscopic examination positive primary syphilis and in late syphilis,⁸ the possibility of biological false positive reactions (Table 1) and prozone phenomenon in secondary syphilis. Prozone phenomenon, false negative in undiluted serum, occurs in 1 to 2% of patients with secondary syphilis where the antibodies are in excess to block the normal antigen-antibody

complex formation.⁹ However, it has the advantages of mass screening, monitoring the response to treatment or re-infection as positive result is reported as titre of antibodies.

Treponemal serologic tests are more complex and based upon the detection of specific antibodies to cellular component of *Treponema pallidum*. It is further divided by different methodology such as Fluorescent Treponemal Antibody absorption (FTA-ABS), *Treponema pallidum* haemagglutination assay (TPHA) and enzyme immunoassay (EIA). The FTA-ABS test is an observer dependent technique to detect indirectly the presence of anti-treponemal antibodies on microscopic slide pre-fixed with *treponema pallidum* antigen by a fluorescence microscope. Before microscopic examination, patient's serum is first diluted in sorbent, an extract from nonpathogenic Reiter treponeme, to remove group specific treponemal antibodies. Because sorbent is added to absorb the non-specific antibodies to enhance the specificity, the word absorption (ABS) is added after FTA test to differentiate this test from less specific fluorescent treponemal antibody test. Although false positive is less common in FTA-ABS comparing with non-treponemal serologic test, it has been documented in the literature (Table 2).

Table 1. Biological false positive reactions of non-treponemal serologic test

Acute (<6 months)	Chronic (>6 months)
Physiological state:	Physiological state:
Pregnancy	Old age
Infection:	Chronic infection:
<i>Bacterial infection</i>	<i>Mycobacterial infection</i>
Pneumonococcal pneumonia	Tuberculosis
Scarlet fever	Leprosy
Infective endocarditis	<i>Others</i>
<i>Mycobacterial infection</i>	Infective endocarditis
Tuberculosis	Malaria
Leprosy	Connective tissue disease:
<i>Other STI</i>	SLE
Chancroid	Maligancy:
Lymphogranuloma venerum	Myeloma
<i>Other spirochaetal infection</i>	Injection drug user
Relapsing fever	Multiple transfusion
Leptospirosis	
Viral infection:	
HIV infection	
Infectious mononucleosis	
Measles	
Mumps	
Chickenpox	
Viral hepatitis	

Table 2. Documented false positive FTA-ABS serologic tests

Chronic infection:

- Leprosy
- Malaria

Other spirochaetal infection:

- Relapsing fever
- Leptospirosis

Connective tissue disease:

- SLE

In TPHA, purified *Treponema pallidum* antigens are attached to red cells in order to detect the specific anti-treponemal antibodies in patient's serum. A reactive result is defined as passive agglutination of red cells. If particle is used instead of red cell, it is called Treponemal Pallidum Particle Agglutination (TPPA) test.

EIA is a test using reaction indicators that employ enzyme acting on an antigen-antibody complex to produce measurable chromogenic end products. The chormogenes can be read by automated spectro-

photometric device. The antigen used in EIA can either be cardiolipin, purified treponemal antigen or recombinant treponemal antigen. If cardiolipin is used instead of *Treponema pallidum* antigen, this test will become a non-treponemal serologic test. Purified treponemal antigen and recombinant treponemal antigen are used in most of the commercially available EIA tests. The advantages of EIA serologic test are more objective, less labour intensive and automated.

Figure 2 shows the level of antibodies versus with time of *Treponema pallidum* exposure. If an arbitrary line is drawn to represent the level of detectability by serologic test, FTA-ABS will be the first to become positive in around four weeks followed by VDRL and TPHA at six and eight weeks respectively. As EIA is a relatively new test and different commercial kits use different antigens to detect specific treponemal antibodies, it is not included in Figure 2.

Molecular-based method

By far, Polymerase Chain Reaction (PCR) is the

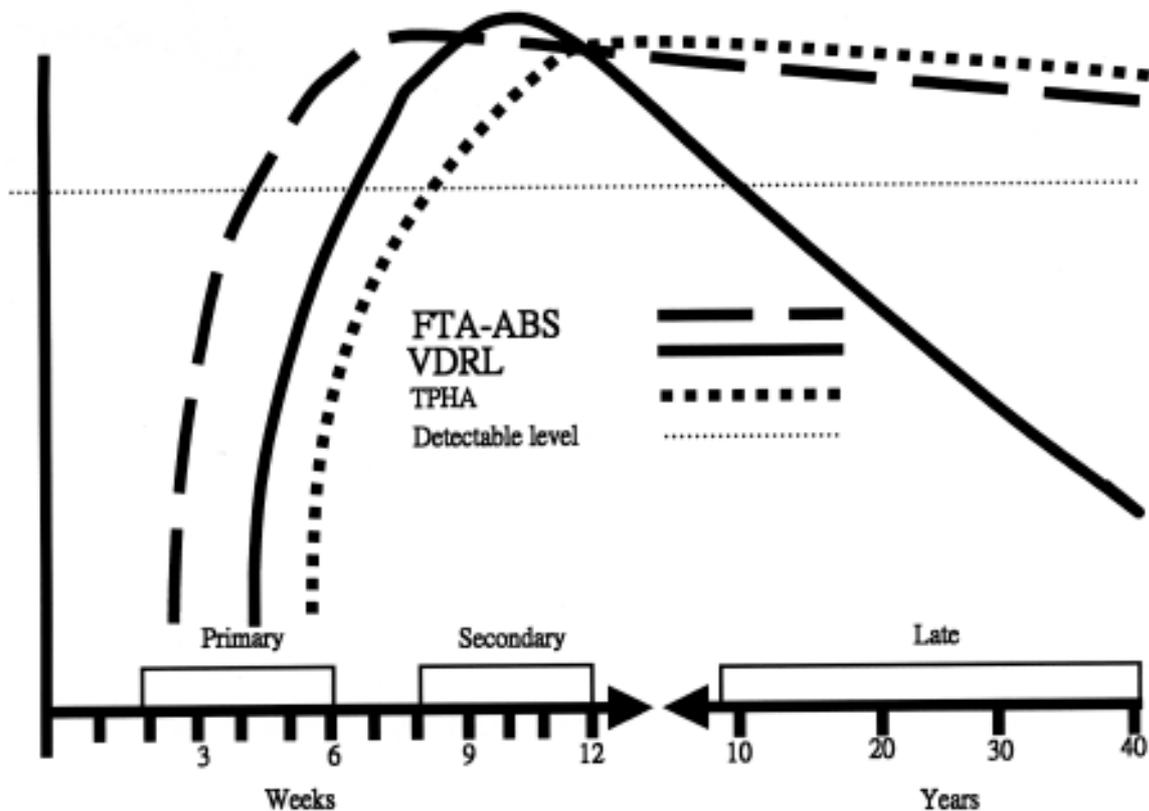


Figure 2: The relationship between levels of antibodies versus time

most commonly used molecular-based method to detect treponemal DNA or antigen. Because the genetic stability,¹⁰ treponemal membrane lipoproteins are the target for PCR in early primary syphilis, early congenital syphilis and neurosyphilis where the application of treponemal serologic tests are limited. However, it is found that the overall sensitivity in various tissue fluids examined was 78% comparing with rabbit infectivity test because of the presence of non-specific inhibitors to PCR, especially in CSF.^{11,12} False positive results caused by improper handling and contamination did not seem to be a problem.¹³ The potential clinical application of PCR is a multiplex PCR assay that differentiates syphilis, herpes simplex and chancroid in genital ulcer.¹⁴

In diagnosing syphilis, apart from detailed clinical history and physical examination, syphilitic serology is still the most important test in diagnosis, monitor the response to treatment and classification of stage by anti-treponemal IgM. The use of a single serologic test alone to diagnose syphilis is inadequate and dangerous as biological false positive reactions are common in non-treponemal serologic test and false negative tests further complicate the issue.

The algorithm of syphilis serology is to use a non-treponemal antibody test as a screening tool followed by a confirmatory serologic test of treponemal antibody test like FTA-ABS or TPHA. VDRL is the commonly used screening test in Hong Kong. In theory, a confirmatory serologic test should have as least as equivalent sensitivity but greater specificity than the screening test that use a different methodology. In clinical practice, the logic is patient's symptom, clinical history, and physical examination followed by investigation. However, for the sake of this review article the order is reversed and Table 3 summarized how to interpret the serologic test with respect to different syphilitic serology, direct microscopic examination and previous history of syphilitic treatment.

SEROLOGIC DIAGNOSIS IN VARIOUS CLINICAL CONDITIONS

Neurosyphilis

The major morbidity of syphilis occurs in its tertiary phase, in particular neurosyphilis. A detailed description of the clinical features of neurosyphilis that can easily be found in standard textbook is beyond the scope of this review article. A correct diagnosis is

important because it requires prolonged and preferably parenteral therapy to reduce the long-term sequel.

However, the diagnosis of neurosyphilis is challenging, as up to 25% of non-treponemal serologic screening test in serum may become non-reactive.¹⁵ Therefore, cerebrospinal fluid (CSF) examination is essential if there is any clinical evidence to suggest neurosyphilis. Other indications for CSF examination are listed in Table 4. Cell count, protein analysis and CSF-VDRL titre must be done in patients suspected to have neurosyphilis. The number of lymphocytes in CSF are in the range of 10 to 100 cells per ml and CSF protein is elevated at around 0.5 to 1 gm per litre in neurosyphilis. However, it may be a diagnostic dilemma if only cell count and protein analysis are suggestive, but with a non-reactive CSF-VDRL. A reactive CSF-VDRL is considered diagnostic of neurosyphilis but a non-reactive test does not exclude neurosyphilis because only 30% of individual suffered from neurosyphilis are CSF-VDRL reactive.^{15,16} Treponemal serologic test is generally not recommended for CSF samples because the standardization process is difficult to control. However, treponemal serologic test is highly sensitive for neurosyphilis that a non-reactive test almost always exclude neurosyphilis.¹⁷

Congenital syphilis

Universal antenatal VDRL screening and the availability of effective treatment accomplish the control of congenital syphilis. Over the past five years, only one case of early congenital syphilis was recorded by the Government Social Hygiene Service.¹⁸ It is hard to comment the above figure, but the important message is that there are still rooms for improvement. Unlike other stages of syphilis, the interpretation of syphilitic serology is complicated by the passive transfer of maternal treponemal IgG antibodies to neonate. It is difficult to interpret the result as most of the serologic tests detect both IgG and IgM antibodies. Apart from difficulty in interpreting the serologic results, some inherent features must be noted before ordering a test.

First of all, the non-treponemal antibody titres remaining from previous treatment of syphilis tend to persist or even increase non-specifically in pregnancy. Therefore, relapse or re-infection is considered only in case of fourfold increase in titre, positive dark ground examination from typical lesion or a recent sexual contact with a person with infectious syphilis.

Table 3. Interpretation of syphilitic serology

Non-treponemal (VDRL)	FTA-ABS	TPHA	DGE/ Histology	History of syphilis with adequate treatment	Interpretation
Non-reactive	Non-reactive	Non-reactive	Negative	No	• No syphilis
Non-reactive	Non-reactive	Non-reactive	Positive	No	<ul style="list-style-type: none"> • No syphilis with false positive DGE with saprophytic treponema • Early primary syphilis with false negative FTA-ABS, suggest repeat serologic test later • Genuine syphilis with abnormal immunologic response in severe immunodeficiency patient such as AIDS patient
Non-reactive	Non-reactive	Reactive	Negative	No	• No syphilis with false positive TPHA
Non-reactive	Non-reactive	Reactive	Negative	Yes	• Treated syphilis with seroconverted FTA-ABS
Non-reactive	Reactive	Non-reactive	Negative	No	• No syphilis with false negative FTA-ABS
Non-reactive	Reactive	Non-reactive	Positive	No	• Early primary syphilis
Non-reactive	Reactive	Reactive	Negative	No	• Latent syphilis
Non-reactive	Reactive	Reactive	Negative	Yes	<ul style="list-style-type: none"> • Serologic scar after previous syphilis • Repeat VDRL or check anti-treponemal IgM if clinically indicated to look for relapse or re-infection
Non-reactive	Reactive	Reactive	Positive	No	• Secondary syphilis with prozone phenomenon, repeat VDRL after dilution
Reactive	Non-reactive	Non-reactive	Negative	No	• No syphilis with biological false positive VDRL
Reactive	Non-reactive	Non-reactive	Positive	No	• Syphilis with false negative FTA-ABS
Reactive	Non-reactive	Reactive	Negative	No	• Syphilis with false negative FTA-ABS
Reactive	Non-reactive	Reactive	Negative	Yes	• Re-infected or relapse syphilis with false negative FTA-ABS, if fourfold increase in VDRL titre or anti-treponemal IgM positive, suggest repeat DGE and VDRL if clinically indicated
Reactive	Non-reactive	Reactive	Positive	No	• Syphilis with false negative FTA-ABS
Reactive	Reactive	Reactive	Negative	No	• Syphilis
Reactive	Reactive	Reactive	Negative	Yes	<ul style="list-style-type: none"> • Serologic scar of previous treated syphilis if asymptomatic and VDRL are not fourfold increase, suggested further follow up • Re-infected or relapse syphilis if fourfold increase in VDRL titre or anti-treponemal IgM positive
Reactive	Reactive	Reactive	Positive	No	• Early syphilis
Reactive	Reactive	Reactive	Positive	Yes	• Re-infected or relapse syphilis, it is more suggestive if fourfold increase in VDRL titre or anti-treponemal IgM positive

Table 4. Indications for CSF examination

1. Patients with late syphilis
2. Any signs or symptoms of neurosyphilis
3. Evidence of active tertiary syphilis
4. Treatment failure (clinical or serological relapse)
5. Concomitant HIV infection

Secondly, up to 50% of congenital syphilis are asymptomatic at birth and clinical manifestations such as hepatosplenomegaly, cutaneous lesions, osteochondritis and snuffles are usually not observed until three weeks to six months old.¹⁹ The definitive diagnosis of congenital syphilis can be made by the demonstration of *Treponema pallidum* in the tissue such as umbilical cord, placenta, cutaneous lesions or even nasal discharge by means of Warthin Starring stain.

Thirdly, as few as 22% of congenital syphilis had infant's non-treponemal titre higher than mother's titre.²⁰ Therefore, examination of paired sera from both infant and mother is highly specific but not sensitive enough to rule out congenital syphilis.

Lastly, because infected foetus can produce anti-treponemal IgM antibody in utero after 3 months; treponemal serologic test or Western blot that detect anti-treponemal IgM antibody may be the standard diagnostic test for congenital syphilis in the future.

Syphilis in HIV infected patient

In the era of Human Immunodeficiency Virus (HIV) epidemic, there is a resurgence of syphilis infection in United States and Africa where the prevalences of HIV infection are high because syphilitic ulcer enhances the acquisition and transmission of HIV infection by three to five fold and vice versa.²¹

In fact, HIV infection, like syphilis, is a protean disease that has a number of clinical presentations and they interact with each other in different ways from serology to clinical presentation. The documented serologic diagnostic confusion in HIV infected syphilis were (i) more biological false positive as a result of B-cell activation,²² (ii) lack of serologic response in clinically confirmed secondary syphilis,²³ (iii) rapid progress to neurologic involvement even after standard treatment in early syphilis,²⁴ (iv) failure of non-treponemal titre to decline after treatment²⁵ and

(v) disappearance of treponemal reactivity over time after treatment.²⁶

Nevertheless, most of the HIV infected patients who contracted syphilis should behave the same as non-HIV infected patients both clinically and serologically. Therefore, the guideline recommended by Centre of Disease Control (CDC) for serologic diagnosis and treatment of syphilis in HIV infected patient is not different from non-HIV infected patient.²⁷ The exceptions are CSF analysis is more liberal even in early stage, and whenever there is a discrepancy between clinical and serologic test result, direct microscopic examination by dark-field method or tissue biopsy with special stain of the lesion is suggested.

SEROLOGIC RESPONSE TO THERAPY

Once syphilis has been diagnosed, the response to treatment is monitored by the decline of non-treponemal titre as clinical sign and symptom may subside spontaneously even without treatment. It is important that a baseline non-treponemal serologic test must be done quantitatively before treatment and followed by the same testing method such as VDRL to monitor the response. In general, there would be a fourfold decline and eightfold decline by six and twelve months respectively after treatment in early syphilis.^{15,28} The rate of decline in non-treponemal titre after treatment in late syphilis is slower. A fourfold decline in twelve months is expected if the non-specific treponemal antibodies was reactive before treatment.

The CSF VDRL titre response to treatment is less predictable. It may take years for CSF VDRL titre to become non-reactive, although the titre should drop progressively. Apart from stage of infection, the rate of decline in non-treponemal titre also depends on initial titre, the number of prior episodes and HIV status.²⁹

CONCLUSION

Not until the HIV epidemic, people nearly forget the morbidity of syphilis in the era of penicillin as less and less tertiary syphilis is seen after the discovery of penicillin. Coincidence with the HIV epidemic and resurgence of syphilis, people become more concentrated on this obligate parasite, *Treponema pallidum*, because

of the enigmatic nature and its interaction with HIV infection. Despite the advancement of molecular technology, the diagnostic tool still mainly relies on indirect serologic test especially in the late stage, because *Treponema pallidum* is an extremely fastidious microaerophilic bacterium that cannot be cultured in vitro. Therefore, there is a large gap in the understanding of the physiology, antigenic structure, pathogenesis and genetic characteristics of this organism. As the sequence of the *Treponema pallidum* genome was completed in 1998,³⁰ it is hoped that these advances would lead to better improvement in in-vitro culture, diagnostic test and even the development of effective vaccine. Before this advancement, the author hoped this review article will enlighten our understanding on the basis of these serologic tests, their limitations and advantages in order to master the skill of when and how to order and interpret the results in an effective and meaningful way.

References

- Norris SJ. In vitro cultivation of *Treponema pallidum*: independent confirmation. *Infect Immun* 1982;36:437-9.
- Coles AC. Spirochaeta pallida: methods of examination and detection, especially by means of the dark-ground illumination. *Br Med J* 1909;1:1117-20.
- Gjestland T. The Oslo study of untreated syphilis: An epidemiologic investigation of the Boeck-Bruusgaard material. *Acta Derm Venereol* 1955;35(Suppl(Stockh)34):1.
- Hutchinson CM, Hook EW 3rd, Shepherd M, et al. Altered clinical presentation of early syphilis in patients with human immunodeficiency virus infection. *Ann Intern Med* 1994;121:94-100.
- Luger AFH. Serologic diagnosis of syphilis. In: Young H, McMillan A, editors. *Immunological diagnosis of sexually transmitted disease*. New York: Marcel Decker 1988:249-74.
- Larsen SA. Syphilis. *Clin Lab Med* 1989;9:545-57.
- Ito F, Hunter EF, George RW, et al. Specific immunofluorescent staining of pathogenic treponemes with a monoclonal antibody. *J Clin Microbiol* 1992;30:831-8.
- Young H. Syphilis: new diagnostic direction. *Int J STD AIDS* 1992;3:391-413.
- Jurado RL, Campbell J, Martin PD. Prozone phenomenon in secondary syphilis: Has its time arrived? *Arch Intern Med* 1993;153:2496-8.
- Steiner BM, Wong GH, Sutrave P, et al. Oxygen toxicity in *Treponema pallidum*: deoxyribonucleic acid single-stranded breakage induced by low doses of hydrogen peroxide. *Can J Microbiol* 1984;30:1467-76.
- Grimprel E, Sanchez PJ, Wendel GD, et al. Use of polymerase chain reaction and rabbit infectivity testing to detect *Treponema pallidum* in amniotic fluid, foetal and neonatal sera, and cerebrospinal fluid. *J Clin Microbiol* 1991;29:1711-8.
- Sanchez PJ, Wendel GD Jr, Grimprel E, et al. Evaluation of molecular methodologies and rabbit infectivity testing for the diagnosis of congenital syphilis and neonatal central nervous system invasion by *Treponema pallidum*. *J Infect Dis* 1993;167:148-57.
- Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;339:237-8.
- Beyrer C, Jitwatcharanan K, Natpratan C, et al. Molecular methods for the diagnosis of genital ulcer disease in a sexually transmitted disease clinic population in northern Thailand: predominance of herpes simplex virus infection. *J Infect Dis* 1998;178:243-6.
- Hook EW 3rd, Marra CM. Acquired syphilis in adults. *N Engl J Med* 1992;326:1060-9.
- Lukehart SA, Hook EW 3rd, Baker-Zander SA, et al. Invasion of the central nervous system by *Treponema pallidum*: implications for diagnosis and treatment. *Ann Intern Med* 1988;109:855-62.
- Jaffe HW, Larsen SA, Peters M, et al. Tests for treponemal antibody in CSF. *Arch Intern Med* 1978;138:252-5.
- Hong Kong STD/AIDS Update - quarterly surveillance report 1997;4(3).
- Kaufman RE, Jones OG, Blount JH, et al. Questionnaire survey of reported early congenital syphilis: problems in diagnosis, prevention, and treatment. *Sex Transm Dis* 1977;4:135-9.
- Stoll BJ, Lee FK, Larsen S, et al. Clinical and serologic evaluation of neonates for congenital syphilis: a continuing diagnostic dilemma. *J Infect Dis* 1993;167:1093-9.
- Wasserheit JN. Epidemiological synergy. Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. *Sex Transm Dis* 1992;19:61-77.
- Rompalo AM, Cannon RO, Quinn TC, et al. Association of biologic false-positive reactions for syphilis with human immunodeficiency virus infection. *J Infect Dis* 1992;165:1124-6.
- Hicks CB, Benson PM, Lupton GP, et al. Seronegative secondary syphilis in a patient infected with the human immunodeficiency virus (HIV) with Kaposi sarcoma. A diagnostic dilemma. *Ann Intern Med* 1987;107:492-5.
- Berry CD, Hooton TM, Collier AC, et al. Neurologic relapse after benzathine penicillin therapy for secondary syphilis in a patient with HIV infection. *N Engl J Med* 1987;316:1587-9.
- Yinnon AM, Coury-Doniger P, Polito R, et al. Serologic response to treatment of syphilis in patients with HIV infection. *Arch Intern Med* 1996;156:321-5.
- Johnson PD, Graves SR, Stewart L, et al. Specific syphilis serological tests may become negative in HIV infection. *AIDS* 1991;5:419-23.
- Centres for Disease Control and Prevention: Guidelines for Treatment of Sexually Transmitted Disease. *MMWR* 1998;47 (No RR-1):38-40.
- Romanowski B, Sutherland F, Fick GH, et al. Serologic response to treatment of infectious syphilis. *Ann Intern Med* 1991;114:1005-9.
- Young H. Syphilis serology. *Dermatol Clin* 1998;16:691-8.
- Fraser CM, Norris SJ, Weinstock GM, et al. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* 1998;281:375-88.