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Review Article

Use of enzyme immunoassay as treponemal screening test in syphilis diagnosis

密螺旋體酶免疫測試於梅毒感染診斷中的應用

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Serological methods play a major role in the diagnosis and treatment monitoring of syphilis infection. With the development and commercial availability of well performing syphilis enzyme immunoassays (EIAs), many laboratories are switching from their traditional nontreponemal screening algorithm to new EIA treponemal screening strategy. Recent experiences have been gained on testing with different EIA antigens as well as amongst different at-risk populations e.g. pregnancy, HIV. This paper reviews these developments, evaluations and practices of treponemal EIAs and aims to provide an overview on its practical application for syphilis laboratory diagnosis in Hong Kong.

血清學方法在監測梅毒感染的診斷和治療中佔重要的一環。商業市場上已開發提供一些良好的梅毒酶免疫測定(EIAs)，因而許多實驗室把傳統的非密螺旋體(nontreponemal)篩選法轉換到密螺旋體(treponemal)篩選方案。最近不同的梅毒抗原在酶免疫測試上以及於不同風險人口(孕婦和HIV病人)中的表現得以分析和總結。我們回顧密螺旋體免疫測試的發展和評論，並為密螺旋體酶免疫測試在香港梅毒實驗室的診斷應用上提供一個概觀。

Keywords: Enzyme immunoassay, laboratory diagnosis, syphilis, *Treponema pallidum*

關鍵詞：酶免疫測定、實驗室診斷、 梅毒、 梅毒密螺旋體

Introduction

Syphilis is caused by infection with *Treponema pallidum* subspecies *pallidum* (*T. pallidum*) with a spectrum of clinical manifestations that change with duration of the illness. Classically, the disease is characterised by a primary lesion, a secondary eruption involving skin and mucous membranes, followed by a period of latency, and late lesions involving skin, bone, viscera, cardiovascular and central nervous systems. Corresponding to each of these developments, syphilis can be divided into primary, secondary, latent and tertiary stages that follow each other temporally in untreated patients. These stages have important implications regarding both diagnosis and treatment monitoring of the disease.

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T. pallidum is a human obligate parasite which is difficult to culture *in vitro*, therefore diagnosis can only be made either using direct detection of the spirochaetes from the exudates, body fluids or tissues or indirect serological tests. Currently there is no single ideal diagnostic test that can optimally detect the pathogen or host response across every stage of the disease.

Serological tests for syphilis can be divided into non-treponemal and treponemal tests. Non-treponemal tests, including Venereal Disease Research Laboratories (VDRL) or Rapid Plasma Reagin (RPR), detect non-specific antibodies and are good indicators of treatment response. On the other hand, treponemal tests measure specific treponemal antibodies in serum which include the *Treponema pallidum* particle agglutination (TPPA), *Treponema* haemagglutination assay (TPHA), native or recombinant antigen enzyme immunoassay (EIA) and fluorescent treponemal antibody absorption test (FTA-ABS).

There have been a number of recent reviews published with respect to serodiagnosis of syphilis[1,2] and a previous issue of this journal[3] provided update on syphilis laboratory testing. The focus in this article is on the use of enzyme immunoassay (EIA) for syphilis which has been adopted in Public Health Laboratory Centre in Hong Kong since 2004.

Development of enzyme immunoassays

In 1907, the first report of experimental inoculation of *T. pallidum* of human to the testicle of rabbit was published. It was not until 1949 that the first laboratory test identifying specific antitreponemal antibodies, the *T. pallidum* immobilisation test, was introduced.[4] Due to its complexity, however, this test has rarely been used for diagnostic purposes. Since 1957, with the development and launching of the fluorescent treponemal antibody test,[5] more treponemal tests were designed and adopted in diagnostic laboratories that are engaged with STD prevention and control programs on a population scale.

Enzyme immunoassay was introduced in the mid-1970's, and has rapidly become a widely adopted immunological assay for the diagnosis of both infectious and noninfectious stages of the disease. Engvall and Perlmann,[6,7] who pioneered the development of enzyme immunoassays, employed antigens or antibodies conjugated to enzymes in a way that the immunological and enzyme activity of each moiety can be measured spectrophotometrically. Since then, these assays took over much of the place of radioimmunoassay in many diagnostic laboratory settings as the former offers both objective results and comparable sensitivity, while at the same time avoided the problems of disposal and short half-lives with radioactive materials.

Subsequently, Veldkamp and Visser[8] published EIA for syphilis using an ultrasonicate *T. pallidum* as antigen with promising results. Following that work, numerous new products and studies on syphilis EIAs had since been designed and adopted according to different laboratory settings.

Different EIAs formats are usually used, which may include one- or two-step sandwich assay, competitive assay, capture assay,...etc. The theory and practice of EIA has been well described by Voller et al.[9] Over the years, the development platform of EIA testing has become relatively standard, and the performance of each new syphilis EIA test is mostly dependent upon the specific antigen that is chosen.

In brief, there are two categories of EIAs for syphilis laboratory testing, viz: immunoassays for nontreponemal antibodies, and assays for specific treponemal antibodies. The antigens used in nontreponemal EIAs mostly are cardiolipin antigens or cardiolipin-lecithin-cholesterol (VDRL antigen). Several nontreponemal EIAs are also under development with performance comparable to classical nontreponemal tests.[10,11]

The early syphilis EIAs used antigens derived from sonicated or solubilised native *T. pallidum*. Evaluations found that these produced comparable sensitivity and specificity to other treponemal tests.[12-16] Later on, recombinant proteins were designed and developed that utilised cloned antigens specific for *T. pallidum*.[17] Recombinant antigen-based enzyme immunoassay for syphilis has become the main stream of EIA testing products used in most high throughput laboratories. A number of commercial recombinant EIAs are available and the evaluation studies that followed or as on-going projects, showed promising results that are comparable with other treponemal tests.[18-24] At the present stage, depending on the testing algorithm adopted for a specific epidemiological situation, it is now quite conclusive that EIA can serve both as a screening or confirmatory treponemal test in syphilis laboratory testing.[12,25-31]

Syphilis clinical staging with serology findings

In primary syphilis, clinical presentation includes typical chancriform ulcer commonly found in the genital tract and perineal areas, with the exception of intra-oral ulcers and typical spirochetes in serous discharge of the ulcers best examined under dark ground microscopy (DGM).[32] Reactive serology of EIA, TPPA, FTA-ABS, or VDRL supports clinical diagnosis. Quantitative VDRL test reflects disease activity and essential for treatment monitoring. In secondary syphilis, typical clinical features of secondary syphilis include condylomata lata, mucous patches, papulosquamous skin eruption with involvement of palms and soles, moth eaten alopecia, fever, malaise, joint pain, periostitis, hepatitis, meningitis, uveitis and general lymphadenopathy. Laboratory diagnosis include demonstrable typical spirochetes by DGM from serous discharge of lesions or positive EIA and QVDRL plus either reactive TPPA or FTA-ABS. Again, quantitative VDRL test at this stage reflects disease activity and useful for treatment monitoring. In one study[32] comparing DGM and treponemal serological tests in early syphilis, EIA had a sensitivity of 57% in primary syphilis when compared to DGM where the latter was performed (the reason stated for not performing was that herpes was the presumed diagnosis). Therefore, DGM remains a rapid and sensitive test in primary syphilis, but requires physician alertness as well as trained staff to perform on all anogenital ulcers and suspected syphilitic lesions.

During latent syphilis, there are typically little or no clinical symptom and sign, and manifesting only as positive EIA with non reactive or low tire VDRL, plus either TPPA or FTA-ABS positive.[28,33] Quantitative VDRL test can also reflect disease activity and used for treatment monitoring for those VDRL-reactive cases. When neurosyphilis occurs, clinical presentations include Argyll Robertson pupil, tabes dorsalis, and features of general paralysis of insane. At this stage, there will be positive EIA plus either TPPA or FTA-ABS positive. CSF-VDRL titre is also typically higher than serum VDRL titre. In cardiovascular syphilis, clinical presentations are that of aneurysm of ascending aorta, aortic incompetence, and atypical angina. Syphilis laboratory findings are positive EIA plus either positive TPPA or FTA-ABS.[33]

Application of enzyme immunoassays for syphilis laboratory testing

A presumptive diagnosis of syphilis infection is possible with the use of a single syphilis serology test. However, the use of only one type of serologic test is not adequate for diagnosis as false-positive nontreponemal test results are well known to be associated with a number of medical diseases and conditions unrelated to *T. pallidum* infection. In one case control study comparing cases at Sexual Health Centre with two control groups, a manual review of 22 isolated positive syphilis EIA (all other syphilis serology is negative) showed that 32% had clinical grounds for suspecting that the EIA signified syphilis.[34]

For many years, the diagnostic approach to syphilis had been based upon the traditional testing algorithm, viz: screening by a non-treponemal test (VDRL or RPR), and then confirmation of reactive results and/or clinical observations with a treponemal test such as TPPA, FTA-ABS (Figure 1). This strategy is still in use in some of the public health laboratories and clinical laboratories in the United States.[25] In Hong Kong and elsewhere where clinical laboratories have much smaller volume of testing work, this approach for syphilis laboratory diagnosis is currently still used.

Recent development

Laboratory diagnostic approach for syphilis has changed gradually in recent years. First in Europe and then in the United States, the availability of syphilis EIA tests became widespread since the turn of the century.[26-28] EIA format is ideally fitted for automation whereby screening of large numbers of specimens is required, and results can be read objectively with reports generated electronically to minimise problems of manual transcriptions. Blood banks, high-volume reference or clinical laboratories had generally chosen to switch to treponemal EIA screening. As experiences gathered, however, there are a few points that should be observed while interpreting EIA test results. EIA screening identifies persons with treated, untreated or incompletely treated syphilis.[35] It should be remembered that this is a test for patient's antibodies and, like other antibody tests, is dependent on host immune response and false positives do occur.[36-42]

A new laboratory testing algorithm for screening with treponemal test has been recommended.[26-28] For all reactive EIA test results from the initial screening, there should be confirmation with a nontreponemal test.[27] If the nontreponemal test result is also reactive, and there is no reliable clinical history and/or treatment, then the patient could be presumed having syphilis infection and

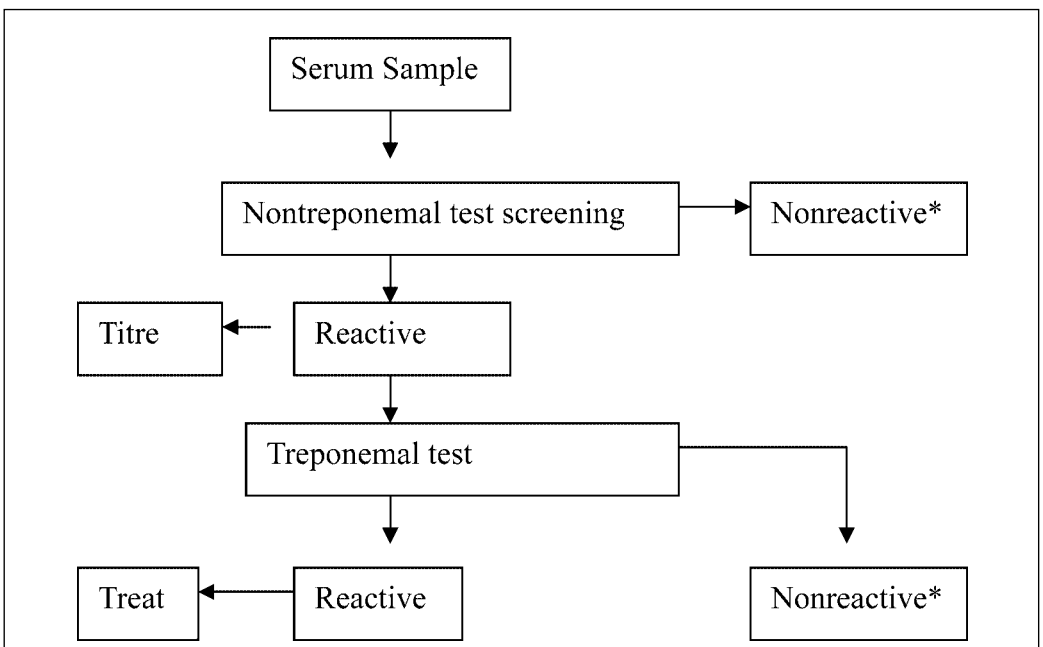

Figure 1. Traditional syphilis testing algorithm.

require treatment. If the nontreponemal test is negative, then another treponemal test (FTA-ABS, TPPA) that uses a different testing format than EIA, should be performed. If this second treponemal test is also positive, treatment could be initiated after discussion with a venereology specialist.

Serological diagnosis with treponemal EIA screening strategy

Considering the disease pattern and testing population that are presented to the Syphilis Laboratory in Public Health Laboratory Centre in Hong Kong, our current EIA treponemal screening strategy has been designed to be in line with international practice as well as local demographics. Specifically, we have included, for those EIA positives, confirmation with one more treponemal test on top (Figure 2). Although this practice increases the diagnostic specificity following a positive EIA test, there is a concomitant increase in laboratory workload which is justifiable because of improvement in patient diagnostic accuracy.

A point of note is that not all syphilis EIA tests perform alike. In a comparative study of three

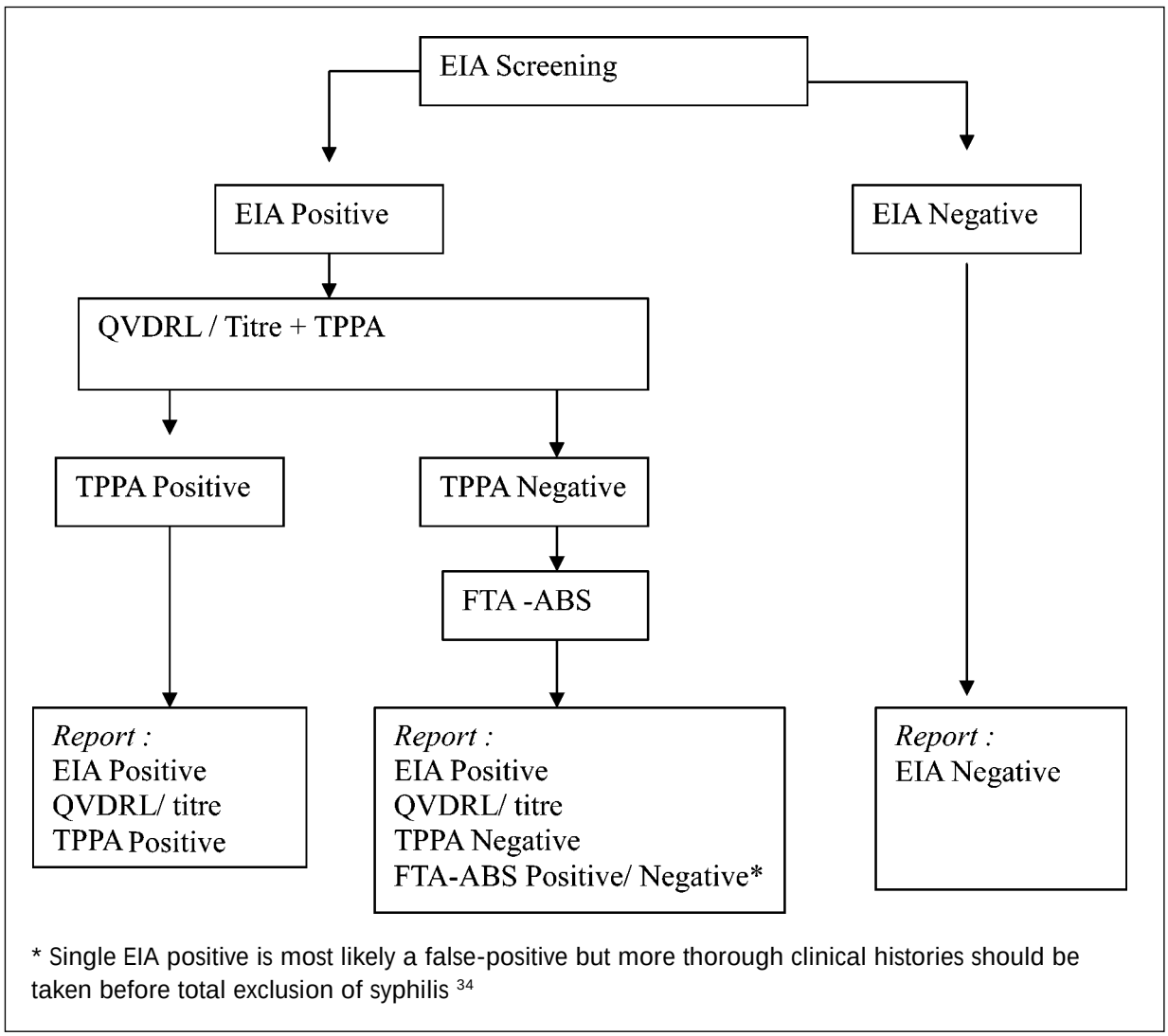

Figure 2. Syphilis testing algorithm in Public Health Laboratory Centre, Hong Kong.

commercially available syphilis EIA kits, the specificity (97.89%) of one is lowest amongst the three (the other two being 99.59% and 99.76%) but also the cheapest.[43] Therefore, the replacement of any existing test depends greatly on the purpose of the individual laboratory whereas performance characteristics considered with an appropriate economic evaluation. For HIV-positive patients, it should be remembered that each treponemal test (EIA-IgG, TPPA, FTA-ABS) gave a lower sensitivity (82%, 86%, 79% respectively) than in the HIV-negative group (97%), although the difference was significant only in the case of FTA-ABS test in that study.[44] With continued enhancement of the EIA testing platforms, therefore, the testing strategy can be reviewed when more experiences are gathered especially in relation to different subgroups.

Conclusion

The transition from nontreponemal test screening to EIA screening requires a paradigm change in the interpretation of serology results. Clinical findings with treatment history and risk factors should go together with the laboratory findings before making diagnosis and for treatment. It will be an on-going evaluation exercise after implementing the new syphilis screening strategy in terms of cost effectiveness from both patient care and public health perspectives. With rapid advancement in laboratory medicine, we should prepare ourselves to readily evaluate and accept new modalities in syphilis diagnosis.

References

1. Larsen SA, Steiner BM, Rudolph AH. Laboratory diagnosis and interpretation of tests for syphilis. Clin Microbiol Rev 1995;8:1-21.
2. Young H. Syphilis: new diagnostic directions. Int J STD AIDS 1992;3:391-413.
3. Kam KM. Current issues in laboratory investigations for syphilis. Hong Kong J Dermatol Venereol 2005; 13:67-8.
4. Nelson RA Jr, Mayer MM. Immobilization of Treponema pallidum in vitro by antibody produced in syphilitic infection. J Exp Med 1949;89:369-93.
5. Deacon WE, Falcone VH, Harris A. A fluorescent test for treponemal antibodies. Proc Soc Exp Biol Med 1957; 96:477-80.
6. Engvall E, Jonsson K, Perlmann P. Enzyme-linked immunosorbent assay. II. Quantitative assay of protein antigen, immunoglobulin G, by means of enzyme-labelled antigen and antibody-coated tubes. Biochim Biophys Acta 1971;251:427-34.
7. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay, Elisa. 3. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. J Immunol 1972;109:129-35.
8. Veldkamp J, Visser AM. Application of the enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of syphilis. Br J Vener Dis 1975;51:227-31.
9. Voller A, Bidwell DE, Bartlett A. Enzyme immunoassays in diagnostic medicine. Theory and practice. Bull World Health Organ 1976;53:55-65.
10. Pedersen NS, Orum O, Mouritsen S. Enzyme-linked immunosorbent assay for detection of antibodies to the venereal disease research laboratory (VDRL) antigen in syphilis. J Clin Microbiol 1987;25:1711-6.
11. White TJ, Fuller SA. Visuwell Reagin, a non-treponemal enzyme-linked immunosorbent assay for the serodiagnosis of syphilis. J Clin Microbiol 1989;27: 2300-4.
12. Pope V, Hunter EF, Feeley JC. Evaluation of the microenzyme-linked immunosorbent assay with Treponema pallidum antigen. J Clin Microbiol 1982; 15:630-4.
13. Farshy CE, Hunter EF, Larsen SA, Cerny EH. Double-conjugate enzyme-linked immunosorbent assay for immunoglobulins G and M against Treponema pallidum. J Clin Microbiol 1984;20:1109-13.
14. Muller F, Moskophidis M. Evaluation of an enzyme immunoassay for IgM antibodies to Treponema pallidum in syphilis in man. Br J Vener Dis 1984;60: 288-92.
15. Farshy CE, Hunter EF, Helsel LO, Larsen SA. Four-step enzyme-linked immunosorbent assay for detection of Treponema pallidum antibody. J Clin Microbiol 1985; 21:387-9.
16. Burdash NM, Hinds KK, Finnerty JA, Manos JP. Evaluation of the syphilis Bio-EnzaBead assay for detection of treponemal antibody. J Clin Microbiol 1987;25:808-11.
17. Ijsselmuiden OE, Schouls LM, Stolz E, Aelbers GN, Agterberg CM, Top J, et al. Sensitivity and specificity of an enzyme-linked immunosorbent assay using the recombinant DNA-derived Treponema pallidum protein TmpA for serodiagnosis of syphilis and the potential use of TmpA for assessing the effect of antibiotic therapy. J Clin Microbiol 1989;27:152-7.
18. Zrein M, Maure I, Boursier F, Soufflet L. Recombinant antigen-based enzyme immunoassay for screening of Treponema pallidum antibodies in blood bank routine. J Clin Microbiol 1995;33:525-7.

19. Ebel A, Bachelart L, Alonso JM. Evaluation of a new competitive immunoassay (BioElisa Syphilis) for screening for Treponema pallidum antibodies at various stages of syphilis. J Clin Microbiol 1998;36:358-61.
20. Young H, Moyes A, Seagar L, McMillan A. vel recombinant-antigen enzyme immunoassay for serological diagnosis of syphilis. J Clin Microbiol 1998; 36:913-7.
21. Schmidt BL, Edjlalipour M, Luger A. Comparative evaluation of nine different enzyme-linked immunosorbent assays for determination of antibodies against Treponema pallidum in patients with primary syphilis. J Clin Microbiol 2000;38:1279-82.
22. Sambri V, Marangoni A, Simone MA, D'Antuno A, Negosamti M, Cevenini R. Evaluation of recomWell Treponema, a novel recombinant antigen-based enzyme-linked immunosorbent assay for the diagnosis of syphilis. Clin Microbiol Infect 2001;7:200-5.
23. Castro R, Prieto ES, Santo I, Azevedo J, Exposto Fda L. Evaluation of an enzyme immunoassay technique for detection of antibodies against Treponema pallidum. J Clin Microbiol 2003;41:250-3.
24. Aktas G, Young H, Moyes A, Badur S. Evaluation of the serodia Treponema pallidum particle agglutination, the Murex Syphilis ICE and the Enzywell TP tests for serodiagnosis of syphilis. Int J STD AIDS 2005;16: 294-8.
25. Centers for Disease Control and Prevention; Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines 2006. MMWR Recomm Rep 2006; 55(RR-11):1-94.
26. Young H, Moyes A, McMillian A, Patterson J. Enzyme immunoassay for anti-treponemal IgG: screening or confirmatory tests? J Clin Pathol 1992;45:37-41.
27. Pope V. Use of Treponemal tests to screen for syphilis. Infect Med 2004;21:399-404.
28. Egglestone SI, Turner AJL. Serological diagnosis of syphilis. Commun Dis Public Health 2000;3:158-62.
29. Lefevre JC, Bertrand MA, Bauriaud R. Evaluation of the Captia enzyme immunoassays for detection of immunoglobulins G and M to Treponema pallidum in syphilis. J Clin. Microbiol 1990;28:1704-7.
30. Moyer NP, Hudson JD, Hausler WJ Jr. Evaluation of the Bio-EnzaBead test for syphilis. J Clin Microbiol 1987;25:619-23.
31. Stevens RW, Schmitt ME. Evaluation of an enzyme-linked immunosorbent assay for treponemal antibody. J Clin Microbiol 1985;21:399-402.
32. Wheeler HL, Agarwal S, Goh BT. Dark ground microscopy and treponemal serological tests in the diagnosis of early syphilis. Sex Transm Infect 2004; 80:411-4.
33. Syphilis in Social Hygiene Manual. Social Hygiene Service, Public Health Services Branch, Centre for Health Protection, Department of Health, Government of the Hong Kong SAR, 2006:7-24.
34. Ooi C, Robertson P, Donovan B. Investigation of isolated positive syphilis enzyme immunoassay (ICE Murex) results. Int J STD AIDS 2002;13:761-4.
35. Baker-Zander SA, Roddy RE, Handsfield HH, Lukehart SA. IgG and IgM antibody reactivity to antigens of Treponema pallidum after treatment of syphilis. Sex Transm Dis 1986;13:214-20.
36. Nandwani R, Evans DT. Are you sure it's syphilis? A review of false positive serology. Int J STD AIDS 1995; 6:241-8.
37. Foged E, Voss Jepsen L, From E. Biological false positives to serological tests for syphilis in herpes genitalis. Ann Clin Res 1985;17:71-2.
38. Lobos P, Ortega R, Vera C, Poblete P, Saez C. Prevalence of false seropositivity for syphilis in a population of pregnant women. Rev Med Chil 1992; 120:1121-6.
39. Kaufman RE, Weiss S, Moore JD, Falcone V, Wiesner PJ. Biological false positive serological tests for syphilis among drug addicts. Br J Vener Dis 1974;50:350-3.
40. Conley CL, Savarese DM. Biologic false-positive serologic tests for syphilis and other serologic abnormalities in autoimmune hemolytic anemia and thrombocytopenic purpura. Medicine (Baltimore) 1989; 68:67-84.
41. Brauner A, Carlsson B, Sundkvist G, Ostenson CG. False-positive treponemal serology in patients with diabetes mellitus. J Diabetes Complications 1994;8: 57-62.
42. Joyanes P, Borobio MV, Arquez JM, Perea EJ. The association of false-positive rapid plasma reagin results and HIV infection. Sex Transm Dis 1998;25:569-71.
43. Viriyataveekul R, Laodee N, Potprasat S, Piyophirapong S. Comparative evaluation of three different treponemal enzyme immunoassays for syphilis. J Med Assoc Thai 2006;89:773-9.
44. Young H, Moyes A, Ross JD. Markers of past syphilis in HIV infection comparing Captia Syphilis G anti-treponemal IgG enzyme immunoassay with other treponemal antigen tests. Int J STD AIDS 1995;6: 101-4.