Original research article

INTERNATIONAL JOURNAL OF STD & AIDS

International Journal of STD & AIDS
2014, Vol 25(1) 52–56

DOI: 10.1177/0956462413491938
std.sagepub.com
SAGE

Use of the INNO-LIA syphilis score assay in the resolution of discordant positive screening enzyme immunoassay results for the serological diagnosis of syphilis

TK Lam[1], HY Lau[1], YP Lee[1], SM Fung[1], WL Leung[1] and KM Kam[2]

Abstract

We studied the use of the INNO-LIA syphilis score assay in the resolution of discordant positive screening results of the Murex ICE Syphilis enzyme immunoassay (EIA) with the confirmatory results of both the Serodia *Treponema pallidum* particle agglutination (TPPA) and the fluorescent treponemal antibody-absorption (FTA-Abs) assays, for the serological diagnosis of syphilis. This was an observational study on the serum samples received by the Syphilis Laboratory, Hong Kong, during the period from January 2006 to December 2012. A total of 801 serum samples with discordant positive screening EIA results were used. Consensus results of such serum samples were derived from results of the EIA, TPPA and FTA-abs assays. The age range of the individuals was 14 to 104 years (median of 52). There were 369 males and 432 females. Of 378 serum samples, 139 showed agreement among positive results, 23 of 310 showed agreement among indeterminate results and 277 of 465 showed agreement among negative results. The proportions of agreement among positive, indeterminate and negative results were 0.37 (95% CI 0.32–0.42), 0.07 (95% CI 0.05–0.11) and 0.60 (95% CI 0.55–0.64), respectively; kappa 0.55 (95% CI 0.49–0.60). There were 60 serum samples with positive consensus results but negative INNO-LIA syphilis score results and 10 with negative consensus results but positive INNO-LIA syphilis score results. Although the INNO-LIA syphilis score assay can be considered a valid alternative confirmatory test for the serological diagnosis of syphilis, the present study showed that its use in the resolution of discordant positive screening EIA results was moderate. A more extensive characterization of serum samples with discordant reactive screening treponemal test results is necessary.

Keywords

Syphilis, sexually transmitted infection, serology, diagnosis, agreement, immunoblot assay, INNO-LIA

Date received: 1 February 2013; accepted: 18 April 2013

Introduction

Syphilis is an infectious disease caused by the spirochete bacterium *Treponema pallidum* subspecies *pallidum*. Laboratory diagnosis of syphilis depends heavily on serology because the causative organism is not able to grow on any artificial culture media and direct detection of the organism by dark-field microscopy is possible only in the presence of active lesions in the early stages of the disease. The serological tests for syphilis fall into two categories: (1) treponemal tests, which detect specific antitreponemal antibody, e.g. enzyme immunoassay (EIA), *Treponema pallidum* particle agglutination (TPPA) assay, *Treponema pallidum* haemagglutination (TPHA) assay, fluorescent

[1]Microbiology Division, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong
[2]Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong

Corresponding author:
TK Lam, Room 731, Public Health Laboratory Centre, 382 Nam Cheong Street, Shek Kip Mei, Kowloon, Hong Kong.
Email: smo_phls4@dh.gov.hk

treponemal antibody-absorption (FTA-abs) assay and immunoblot assay and (2) non-treponemal tests, which detect non-specific antilipoidal antibody, e.g. Venereal Disease Research Laboratory (VDRL) assay and rapid plasma reagin (RPR) assay. In general, treponemal tests are used to provide the serological evidence of syphilis because they are more sensitive and specific.[1] On the other hand, non-treponemal tests are used in treatment monitoring because the titres correlate with disease activity.

The INNO-LIA syphilis score (LIA) assay is an immunoblot assay methodologically distinct from the EIA, TPPA and FTA-abs assays. The LIA assay uses three recombinant antigens (TpN47, TpN17 and TpN15) and one synthetic peptide (TmpA) electrophoretically separated and blotted to nitrocellulose strips. The antitreponemal antibodies in patients' sera bind to the antigens and are then detected by enzyme-labeled anti-human immunoglobulin. Enzyme and substrate reaction then produces visible discrete bands. The interpretation of results is based on the number and intensity of reactive bands.

Recently, the LIA assay has been validated and evaluated for use as a confirmatory treponemal test for the serological diagnosis of syphilis.[2,3] The LIA assay was shown to have sensitivities of $\geq 92\%$ in detecting the different stages of syphilis.[4] The present study explored the use of the LIA results in the resolution of discordant positive screening EIA results for the serological diagnosis of syphilis.

Materials and methods

Study design and serum samples

This was an observational study on the serum samples received for the serological testing of syphilis by the Syphilis Laboratory, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong, during the period from January 2006 to December 2012. The sources of the serum samples included the attendees of social hygiene clinics (servicing patients with sexually transmitted infections), maternal & child health centres (caring for pregnant women and newborns) and an integrated treatment centre (caring for patients with human immunodeficiency virus [HIV] infection) of the Department of Health in Hong Kong, as well as referred serum samples submitted by clinical microbiology laboratories of public hospitals in Hong Kong. Duplicate serum samples were excluded and only the first serum sample of each individual was included. Neither clinical stages of syphilis nor treatment history were detailed on the request forms.

Serological testing of syphilis

All serum samples were screened with the Murex ICE Syphilis EIA (Murex Biotech Limited, Dartford, UK) assay. Negative EIA results were regarded as indicating no serological evidence of syphilis. Where the EIA results were positive, the serum samples were further tested with both the Serodia TPPA (Fujirebio Inc., Tokyo, Japan) and FTA-abs (TrepoSpot IF; bioMérieux, Marcy l'Etoile, France) assays. The positive screening EIA results were regarded as concordant when both the TPPA and FTA-abs results were reactive (i.e. $\geq 1+$). Otherwise, the positive screening EIA results were regarded as discordant and the serum samples were further tested with the LIA (Innogenetics NV, Ghent, Belgium) assay. The testing algorithm was detailed in Figure 1.

The treponemal tests were performed according to the manufacturers' instructions and the standard operating procedures[5] at the Syphilis Laboratory, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong. The technical staff who performed the tests were blinded to the clinical details of the serum samples as well as the results of individual tests. All test results were counter-checked by another technician.

Statistical analysis

The consensus result of each serum sample with discordant positive screening EIA results was derived from the most predominant results of the EIA, TPPA and FTA-abs assays. As each serum sample had a positive screening EIA result, the consensus result of a serum sample was positive if either the TPPA or FTA-abs result was reactive, negative if both the TPPA and FTA-abs results were nonreactive and indeterminate if the TPPA result was indeterminate and the FTA-abs result was nonreactive. The strength of agreement of the LIA and consensus results was expressed by the kappa coefficient with quadratic weighting as the categories of the results were ordinal. Furthermore, the proportions of specific agreement were calculated. The 95% confidence intervals (CIs) of the proportions were calculated using the score method.

Results

A total of 801 serum samples with discordant positive screening EIA results were used in the present study. The age range of the individuals was 14 to 104 years (median of 52). There were 369 male and 432 female sera. The serum samples came from 305, 140 and 33 attendees of social hygiene clinics, maternal & child health centres and an integrated treatment centre, respectively, as well as 323 referred serum samples

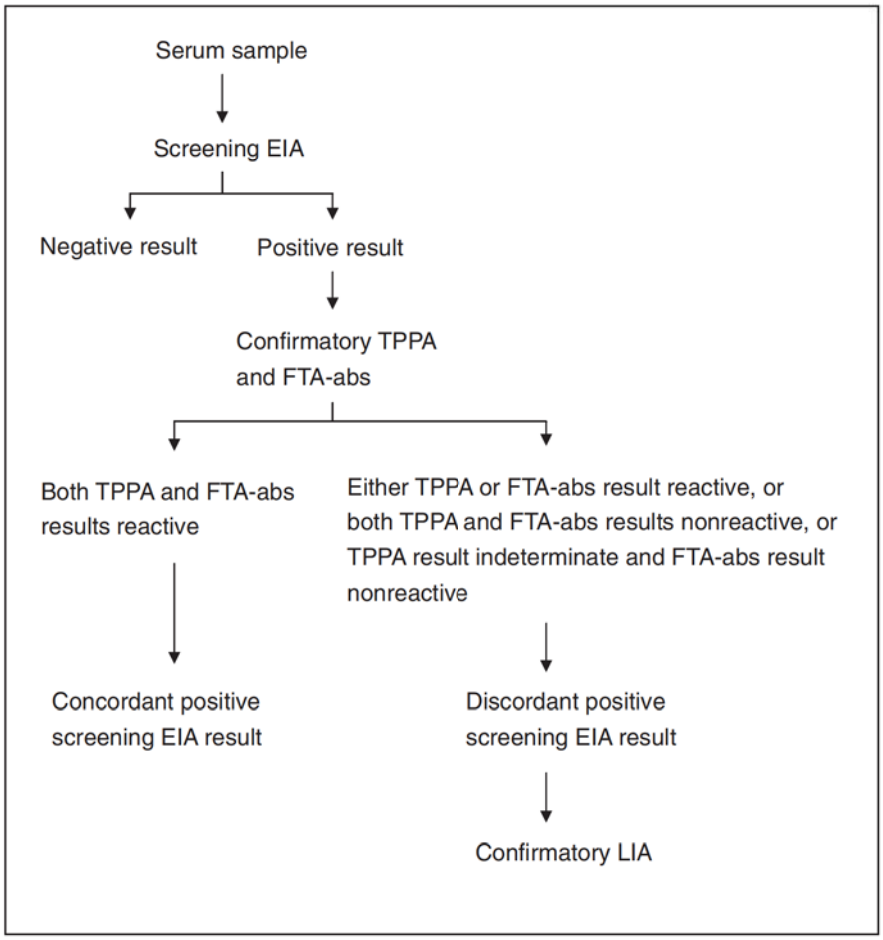

Figure 1. Syphilis serological testing algorithm used in the present study.

submitted by clinical microbiology laboratories of public hospitals in Hong Kong.

With regard to the serum samples with discordant positive screening EIA results, the distribution of TPPA and FTA-abs results is summarized in Table 1. There were 345 serum samples with positive consensus results, 100 with indeterminate consensus results and 356 with negative consensus results. Among the 345 serum samples with positive consensus results, there were 49 with reactive TPPA results but nonreactive FTA-abs results and 129 with nonreactive TPPA results but reactive FTA-abs results.

With regard to the serum samples with discordant positive screening EIA results, the distribution of LIA and consensus results is summarized in Table 2. Three serum samples with positive consensus results but invalid LIA results, and two with indeterminate consensus results but invalid LIA results, were excluded in the calculation of the proportions of specific agreement and the kappa coefficient; 139 out of 378 ($[139+143+60]+[139+26+10]-139$) serum samples showed agreement among positive results, 23 out of 310 ($[26+23+49]+[143+23+69]-23$) showed agreement among indeterminate results and 277 out of 465 ($[10+69+277]+[60+49+277]-277$) showed agreement among negative results. The proportions of agreement among positive, indeterminate and negative results were 0.37 (95% CI 0.32–0.42), 0.07 (95% CI 0.05–0.11) and 0.60 (95% CI 0.55–0.64), respectively. The kappa coefficient with quadratic weighting was 0.55 (95% CI 0.49–0.60). There were 98 and 235 serum samples with indeterminate consensus results and indeterminate LIA results, respectively. There were 60 serum samples with positive consensus results but negative LIA results and 10 with negative consensus results but positive LIA results.

Discussion

An important principle of the serological diagnosis of syphilis is the detection of antitreponemal antibody by a screening treponemal test, followed by confirmation of a reactive screening test result by additional testing.[1] The confirmatory treponemal test or tests should have

Table 1. Distribution of TPPA and FTA-abs results among serum samples with discordant positive screening EIA results.

Number of serum samples	FTA-abs results		
	Reactive (i.e. ≥1+)	Nonreactive	Total
TPPA results			
Reactive (i.e. ≥1+)	[a]	49[b]	49
Indeterminate	167[b]	100[c]	267
Nonreactive	129[b]	356[d]	485
Total	296	505	801

EIA: enzyme immunoassay; FTA-abs: fluorescent treponemal antibody-absorption; TPPA: *Treponema pallidum* particle agglutination.
[a]Concordant positive screening EIA results.
[b]Positive consensus results.
[c]Indeterminate consensus results.
[d]Negative consensus results.

Table 2. Distribution of LIA and consensus results among serum samples with discordant positive screening EIA results.

Number of serum samples	Consensus results			
	Positive	Indeterminate	Negative	Total
LIA results				
Positive	139	26	10	175
Indeterminate	143	23	69	235
Negative	60	49	277	386
Total	342[a]	98[b]	356	796

EIA: enzyme immunoassay; LIA: INNO-LIA syphilis score.
[a]Three serum samples with positive consensus results but invalid LIA results were excluded.
[b]Two serum samples with indeterminate consensus results but invalid LIA results were excluded.

similar sensitivity but greater specificity when compared to, and should be methodologically distinct from, the screening test, in order to reduce the chance of coincident false-positive reactions. However, the situation of discordant reactive screening test results with nonreactive confirmatory test results may arise.

The 2008 European guidelines on the management of syphilis recommend syphilis screening with either EIA or TPPA, followed by confirmation of a reactive screening test result by a different treponemal test (e.g. screening with EIA and confirmation with TPPA or vice versa). An immunoblot assay is recommended as an alternative confirmatory test when the first confirmatory test does not confirm the reactive screening test result. The FTA-abs assay is no longer recommended as an alternative confirmatory test. The FTA-abs assay is difficult to standardize across different laboratories and the fluorescence intensity of its results may be subjectively interpreted by different operators. However, it could be used as an alternative confirmatory test in certain circumstances, e.g. in highly specialized laboratories with a large volume of testing and a quality assurance system in place.[6]

In the present study, the EIA assay was used as the screening treponemal test. Both the TPPA and FTA-abs assays, and not just either one of them, were used as confirmatory tests so that the consensus results could be derived from the most predominant results of the EIA, TPPA and FTA-abs assays.

There is currently no single "gold standard" treponemal test available. There are inherent limitations in the serological diagnosis of syphilis. Firstly, characteristics of the individual, e.g. age, sex, pregnancy status, presence of co-morbidities and immune function status, may affect the individual's ability to produce antitreponemal antibodies and/or cross-reacting antibodies. Secondly, the disease process, e.g. the strain of the infecting organism, stage of syphilis and treatment history, may affect the kinetics of the production of antitreponemal antibodies. Thirdly, characteristics of the assay, e.g. the methodology, nature of antigens and type of conjugates, may affect the ability to detect antitreponemal antibodies in vitro. The EIA and LIA assays use recombinant treponemal antigens, whereas the TPPA and FTA-abs assays use native antigens. The EIA, TPPA and FTA-abs assays are capable of detecting both IgM and IgG antitreponemal antibodies, whereas the LIA assay detects only IgG antitreponemal antibody. Fourthly, characteristics of the operators, e.g. training, expertise and experience, may affect the quality of test results.

The kappa test quantifies the strength of agreement of pairs of measurement categories by removing the percentage of agreement that may have occurred by chance. A kappa coefficient with a large positive value (close to 1) suggests that the majority of agreement is not due to chance. A value close to zero suggests that little or none of the agreement is not due to chance. A negative value indicates disagreement. A value of 0.41–0.60 suggests a moderate level of agreement. However, interpretation of the value of the kappa coefficient requires caution. Firstly, even if the value is high, disagreement may have occurred, which may be clinically unacceptable.[7] Secondly, the value is affected by more than just agreement. It is also affected by the distribution of results across different categories by each assay. As a consequence, it is difficult to compare the values of kappa coefficient across different studies.

Although the LIA assay can be considered a valid alternative confirmatory test for the serological diagnosis of syphilis, the present study showed that its use in the resolution of discordant positive screening EIA results was moderate. However, it was acknowledged that validity of the consensus results, which were

derived from the most predominant results of the EIA, TPPA and FTA-abs assays, was uncertain. A more extensive characterization of the serum samples with discordant reactive screening treponemal test results is necessary.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Egglestone SI and Turner AJL. Serological diagnosis of syphilis. *Commun Dis Public Health* 2000; 3: 158–162.
2. Ebel A, Vanneste L, Cardinaels M, et al. Validation of the INNO-LIA syphilis kit as a confirmatory assay for *Treponema pallidum* antibodies. *J Clin Microbiol* 2000; 38: 215–219.
3. Hagedorn HJ, Kraminer-Hagedorn A, De Bosschere K, et al. Evaluation of INNO-LIA syphilis assay as a confirmatory test for syphilis. *J Clin Microbiol* 2002; 40: 973–978.
4. Lam TK, Lau HY, Lee YP, et al. Comparative evaluation of the INNO-LIA syphilis score and the MarDx *Treponema pallidum* immunoglobulin G Marblot test assays for the serological diagnosis of syphilis. *Int J STD AIDS* 2010; 21: 110–113.
5. Larsen SA, Pope V, Johnson RE, et al. *Syphilis: a manual of tests and supplement*, 9th ed. Washington, D.C.: American Public Health Association, 1999.
6. French P, Gomberg M, Janier M, et al. IUSTI: 2008 European guidelines on the management of syphilis. *Int J STD AIDS* 2009; 20: 300–309.
7. Kottner J, Andige L, Brorson S, et al. Guidelines for reporting reliability and agreement studies (GRRAS) were proposed. *J Clin Epidemiol* 2011; 64: 96–106.