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Research paper

Performance evaluation of nine different syphilis serological tests in comparison with the FTA-abs test

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ABSTRACT

Background: Serological methods have great importance for the detection of *Treponema pallidum* antibodies in syphilis diagnosis. The goal of the present study is to evaluate various commercially available screening assays in comparison with the FTA-abs test.

Methods: A total of 363 serum samples were enrolled in the study. Following routine testing including RPR and TPHA tests, each sample was tested by treponemal immunoassays (Chorus Syphilis Screen Recombinant, Architect Syphilis TP, Syphilis Virclia Monotest, Siemens Advia Centaur Syphilis, Euroimmun *Treponema pallidum* Screen ELISA, Vircell Syphilis ELISA IgG + IgM, SD Bioline Syphilis). The result obtained from each test was compared with the confirmatory FTA-abs test. Kappa (κ) coefficients were used to compare the concordance of the tests.

Results: When the various tests were evaluated in comparison with the FTA-abs test, the sensitivity, specificity and percent agreement of each test were as follows: Architect Syphilis TP, 92.3%, 94.5%, 92.8%; Chorus Syphilis Screen Recombinant, 87.9%, 91.2%, 88.7%; Syphilis Virclia Monotest, 80.5%, 97.8%, 84.9%; Siemens Advia Centaur Syphilis, 87.5%, 89%, 87.9%; Euroimmun *Treponema pallidum* Screen ELISA, 87.5%, 85.7%, 87.1%; Vircell Syphilis ELISA IgG + IgM, 73.2%, 62.6%, 70.5%; TPHA, 89%, 63.7%, 82.6%; SD Bioline Syphilis, 58.1%, 94.5%, 67.2%; RPR test, 57.7%, 57.1%, 57.6%.

Conclusion: The results of the present study show that *Treponema pallidum* specific immunoassays with a performance similar or better than TPHA test generally performed well with the confirmatory FTA-abs test and may be an alternative for screening total antibodies in syphilis infection.

1. Introduction

Syphilis remains a significant health problem worldwide. Accurate and rapid diagnosis is very important for disease control and patient management (Bazzo et al., 2017). The disease has several clinical manifestations which makes laboratory testing a critical aspect of diagnosis (Ratnam, 2005). Two types of antibodies, treponemal and nontreponemal, are generated during the infection. As well as our country, many developing countries oftenly use nontreponemal Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory (VDRL) tests for the detection of nontreponemal antibodies (cardiolipin, lipoidal or reagin) (Castro et al., 2003). These tests are simple, cheaper and the sensitivity is generally high in the early stage whereas low in the late phases of the disease (Naidu et al., 2012). The other antibody type, treponemal antibodies, can be detected by *T. pallidum* Hemagglutination Assay (TPHA), Enzyme Linked Immunosorbent Assay (ELISA) and Fluorescent Treponemal Antibody-Absorption (FTA-abs) tests. TPHA which is one of the tests mostly preferred for screening in routine use is sensitive in the late period however the sensitivity is lower in the early stages of the infection (Larsen et al., 1995). On the other hand, as the lipoidal tests and the TPHA test can not be completely automated, the results of the tests are subjectively evaluated and recorded manually (Li et al., 2016). Although the other treponemal test, FTA-abs test is highly sensitive and specific, it is laborious, time-consuming and the interpretation is difficult, therefore alternative methods have been introduced. Furthermore whenever possible in order to reduce costs, there is a trend towards automation and the automated tests are typically ELISA tests (Woznicová and Valisová, 2007). In resource limited settings, treponemal ELISA tests are of great value and are considered as the reference tests by WHO (WHO, 2003). So far, manual tests were widely used in syphilis laboratory diagnosis but due to the various factors affecting test performance ELISAs have overcome many

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problems and therefore they are most commonly used nowadays (Liu et al., 2014). The use of the immunoenzymatic methods in syphilis diagnosis begins in the mid 1970s (Sambri et al., 2001) and different types of ELISA tests are available in the market currently. Various automated ELISA tests use either whole cells or 15TpN, 17TpN, 47TpN antigens derived from *T. pallidum* Nichols strain detecting antibodies against *T. pallidum*. Initially *T. pallidum* specific antibody ELISA tests and later chemiluminescence based immunoassays (CLIAs) have been developed (Park et al., 2016).

In our department RPR, TPHA, ELISA and FTA-abs tests are employed for syphilis laboratory diagnosis. Generally screening can be performed by ELISA, TPHA, RPR tests or a combination of these tests according to clinicians' request. However, no comparative evaluation of the performance of different methods is available in Turkey. This study aims to assess the performance characteristcs of various commercially available screening (Chorus Syphilis Screen Recombinant, Architect Syphilis TP, Syphilis Virclia Monotest, Siemens Advia Centaur Syphilis, Euroimmun *Treponema pallidum* Screen ELISA, Vircell Syphilis ELISA IgG + IgM, SD Bioline Syphilis 3.0, RPR, TPHA) tests with the gold standard treponemal FTA-abs test.

2. Material and method

Totally 363 sera submitted to the Public Health General Directorate, Sexually Transmitted Diseases Reference Laboratory between January 2016–2017 for routine syphilis diagnosis were enrolled in the present study.

Routine testing included the RPR (Omega Diagnostic, UK) and TPHA (Omega Diagnostic, UK) tests. Following routine testing, each sample was tested by all different immunoassays. All kinds of *T. pallidum* tests evaluated included, Chorus Syphilis Screen Recombinant (DIESSE Diagnostica Senese, Italy), Architect Syphilis TP (Abbot Diagnostics, USA), Syphilis Virclia Monotest (Vircell, Spain), Advia Centaur Syphilis (Siemens Healthcare Diagnostics, USA), *Treponema pallidum* Screen ELISA (Euroimmun, Germany), Vircell Syphilis ELISA IgG + IgM (Vircell, Spain) and SD Bioline Syphilis 3.0 (Standard Diagnostics, Korea) tests. All the samples were tested according to the manufacturer recommendations. The ELISA tests were performed under blinded conditions. Equivocal results of any of the ELISA tests were repeated and the final result was accepted. Repeated eqivocal results were considered as positive in order to calculate sensitivity and specificity. The results obtained were compared with a second treponemal FTA-abs test (Euroimmun, Germany) which is considered as the gold standard test in our study.

2.1. Serological methods

2.1.1. Architect syphilis TP assay

The Architect syphilis TP assay uses paramagnetic particles coated with recombinant TpN15, TpN17 and TpN47 to bind antibodies (IgG and IgM) specific for *T. pallidum*. After the incubation period of microparticles with serum samples, murine acridinium labelled antibody conjugate was added and chemiluminescence produced was measured.

2.1.2. Syphilis virclia monotest

The Syphilis Virclia Monotest is an indirect chemiluminescence immunoassay (CLIA) to test IgG + IgM antibodies against *T. pallidum* antigens. The CLIA method is based upon the reaction of antibodies in the serum sample tested with the antigen adsorbed on the polystyrene surface. Anti-human IgG + IgM peroxidase conjugate binds the antigen-antibody complex and a chemiluminescent substrate solution that will generate a glow-type luminescence can be read with a luminometer.

2.1.3. Chorus syphilis screen recombinant

The Chorus Syphilis Screen Recombinant is a sandwich based solid phase qualitative EIA, coated with recombinant *T. pallidum* antigens. During incubation, the antibodies present in the sample are bound to the solid phase antigen as well as to the antigen labelled with horseradish peroxidase (HRP), creating an antigen-antibody-antigen-HRP "sandwich". The unbound conjugate is eliminated by washing and the bound enzymatic activity is determined by adding a chromogen substrate. The intensity of the colour is proportional to the anti-*Treponema pallidum* antibody concentration in the sample.

2.1.4. Advia centaur syphilis assay

The Advia Centaur syphilis assay is a sandwich based chemiluminescence EIA (IgG). Acridinium ester labelled *T. pallidum* antigens are added to samples and controls. The solid-phase reagent, containing biotinylated *T. pallidum* recombinant antigen preformed to streptavidin-coated magnetic latex particles, is added to the sample mix. These particles capture the *T. pallidum* antigen-antibody complexes. Antibody-antigen complex form if syphilis antibodies are present in the sample.

2.1.5. Euroimmun Treponema pallidum screen ELISA

The wells are coated with a mixture of *T. pallidum* recombinant antigens. In case of positive samples, specific IgG and IgM antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG/IgM catalysing a colour reaction. The test is performed manually with wash steps automated on the Tecan hydrospeed microplate washer (Tecan Diagnostics, Switzerland) and absorbance values read on the Labsystem Multiskan microplate reader (Thermo Fisher Scientific, USA).

2.1.6. Vircell syphilis ELISA IgG + IgM

The Vircell Syphilis ELISA is an indirect immunoenzyme assay to test IgG + IgM antibodies against *T. pallidum* antigens. The ELISA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. An anti-human peroxidase conjugate dilution binds the antigen antibody complex and bound conjugate is developed with the aid of a substrate solution (TMB) which turns into yellow after adding the acid stopping solution. The test is performed manually with wash steps automated on the Tecan hydrospeed microplate washer (Tecan Diagnostics, Switzerland) and absorbance values read on the Labsystem Multiskan microplate reader (Thermo Fisher Scientific, USA).

2.1.7. SD bioline syphilis 3.0

The SD BIOLINE Syphilis 3.0 test is a solid phase immunochromatographic assay for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) against *T. pallidum*. The test contains a membrane strip pre-coated with recombinant *T. pallidum* antigens on the test band region. The recombinant *T. pallidum* antigens-colloid gold conjugate, patient sample and sample diluent move along to the test region (T) chromatographically and form a visible line as the antigen-antibody complex forms. Therefore, the formation of a visible line in the test region (T) indicates a positive result. If specific antibodies are absent in the sample, no visible colour band will appear in the test region (T).

Details of the characteristics of the different assays are summarized in Table 1.

2.2. Statistical analysis

IBM SPSS version 23 statistical program was used for statistical evaluation and descriptive information was shown by number and percentage distributions. Various tests were evaluated for sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), percent agreement and Cohen's Kappa value, according to 363 suspected syphilis patients. The kappa (κ) coefficients were calculated to analyze the agreement between the tests. The agreement according to κ values were categorized as perfect (0.81–1.00), substantial (0.61–0.80), moderate (0.41–0.60), fair (0.21–0.40), slight (0–0.20)

Table 1
Characteristics of the various tests evaluated according to the information provided by the manufacturers.

Characteristics	Abbot	DIESSE	Vircell	Siemens	Euroimmun*	Vircell*	Standard
Kit used	Architect Syphilis TP	Chorus Syphilis Screen Recombinant	Syphilis Virclia Monotest	ADVIA Centaur Syphilis (SYPH) assay	*Treponema pallidum* Screen ELISA	Syphilis ELISA IgG + IgM (Manual)	SD Bioline Syphilis 3.0
Instrument used	Architect 2000i	Chorus Trio	ThunderBolt	ADVIA Centaur XP	–	–	–
Principle	Chemiluminescent microparticle immunoassay	Sandwich solid phase immunoassay	Chemiluminescent immunoassay	Sandwich chemiluminescent immunoassay	Indirect solid phase immunoassay	Indirect solid phase immunoassay	Solid phase immunochromatographic assay
Quantitative or qualitative	Qualitative	Qualitative	Qualitative	Qualitative	Qualitative or quantitative	Qualitative	Qualitative
Antigen used	Recombinant antigens; 15 kDa, 17 kDa, 47 kDa	Recombinant antigens; 15 kDa, 17 kDa	Recombinant antigens; 15 kDa, 17 kDa, 47 kDa	Recombinant antigens; TpN17 and TpN15	Recombinant antigens; 15 kDa, 17 kDa, 47 kDa, 42 kDa (TmpA)	Recombinant antigens; 15 kDa, 17 kDa, 47 kDa	Recombinant *Treponema pallidum* antigens; 17 kDa, 15 kDa
Sample type	Plasma, serum	Serum	Plasma, serum	Serum	Plasma, serum	Plasma, serum	Plasma, serum
Sample volume (μl)	30	50	5	100	10	5	10
Assay time approx. (min)	28	< 50 min	50	28.5	105	95	5–20

* Assay time is calculated as the incubation time.

Table 2
Evaluation of various *Treponema pallidum* assays in comparison with the FTA-abs assay (n = 363).

	Chorus Syphilis Screen Recombinant (DIESSE Diagnostica Senese, Italy)		Architect Syphilis TP (Abbot Diagnostics, USA)		Syphilis Virclia Monotest (Vircell, Spain)		Advia Centaur Syphilis (Siemens Healthcare Diagnostics, USA)		*Treponema pallidum* Screen ELISA (Euroimmun, Germany)		Vircell Syphilis ELISA IgG + IgM (Vircell, Spain)		TPHA (Omega, UK)		SD Bioline 3.0 Syphilis (Standard Diagnostics, Korea)		RPR (Omega, UK)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sensitivity	239/272	87.9	251/272	92.3	219/272	80.5	238/272	87.5	238/272	87.5	199/272	73.2	242/272	89.0	158/272	58.1	157/272	57.7
Specificity	83/91	91.2	86/91	94.5	89/91	97.8	81/91	89.0	78/91	85.7	57/91	62.6	58/91	63.7	86/91	94.5	52/91	57.1
PPV*	239/247	96.8	251/256	98.0	219/221	99.1	238/248	96.0	238/251	94.8	199/233	85.4	242/275	88.0	158/163	96.9	157/196	80.1
NPV**	83/116	71.6	86/107	80.4	89/142	62.7	81/115	70.4	78/112	69.6	57/130	43.8	58/88	65.9	86/200	43.0	52/167	31.1

* Positive Predictive Value.
** Negative Predictive Value.

(Landis and Koch, 1977). A p value below 0.05 was considered as statistically significant.

3. Results

The performance characteristics of nine different syphilis serological tests were evaluated. When the sensitivities of different tests were determined in comparison with the FTA-abs test, it was detected that Architect TP ELISA test has the highest and RPR test has the lowest sensitivity. The sensitivities of Chorus Syphilis Screen Recombinant, Siemens ADVIA Centaur Syphilis, Euroimmun *Treponema pallidum* Screen ELISA and TPHA tests were similar and detected to be lower than the Artitect TP ELISA test. Regarding specificity, it was determined that Syphilis Virclia Monotest has the highest specificity and Architect TP ELISA and SD Bioline Syphilis tests followed respectively. The lowest specificity was determined in the RPR test. NPV was the highest in Architect TP whereas lowest in RPR test and PPV was the highest in Syphilis Virclia Monotest followed by Architect Tp Syphilis test. A comparison of the main characteristics of each test is shown in Table 2.

The percent agreement was the highest in Architect TP Syphilis and the lowest in RPR test. The kappa values ranged from 0.12 (RPR) to 0.82 (Architect TP Syphilis). Only the Architect TP Syphilis kappa test result showed perfect agreement with the reference method. The performance parameters for each test are summarized in Table 3.

4. Discussion

Early and accurate diagnosis of syphilis is critical not only for the prevention of the disease but for treatment delays as well (Tao et al., 2017). Serological tests are most commonly used for the diagnosis of syphilis; however the interpretation phase of the tests has shown conflicting results in the literature (Liu et al., 2014). Variations in treponemal assay performance in low and high prevalence populations underline the need to verify the performance of treponemal screening tests (Gratzer et al., 2014; Morshed and Singh, 2015). In consistent with the increase in the number of patients requiring syphilis testing in Turkey (laboratory pending results), a critical need exists for an accurate, rapid and high throughput test for syphilis diagnosis. This study has determined the diagnostic capacity of nine different treponemal and nontreponemal tests using samples from 363 syphilis suspected patients.

According to our results, when the internationally accepted assays, mainly the TPHA and RPR tests were compared in comparison with the FTA-abs test, the sensitivity, specificity and the percent agreement were detected as 89%, 63.7%, 82.6% and 57.7%, 57.1%, 57.6% respectively. The overall sensitivity of the tests performed in this study varied between 57.7 and 92.3%. Compatible with the study of Castro et al., sensitivity of the tests can vary from 48.5% to 100% according to the type of tests performed, with the phase of the disease and disease prevalence in the population (Castro et al., 2003). On the other hand, other authors have reported a higher percent agreement between TPHA and FTA-abs tests compared to our findings (Bradford and Larsen, 1985). In the present study no clinical data were obtained from the suscepted patients and therefore it was not possible to correlate the laboratory results with the clinical presentation. As TPHA test is not quite sensitive in the early phases of the disease, it is considered that lower percent agreement may have been resulted from the clinically unknown early syphilis cases among our tested sera. According to the data shown so far, TPHA and FTA-abs tests are comparable in all categories of syphilis except in the primary and early secondary stages (Bradford and Larsen, 1985).

Nowadays ELISA tests are the most preferred methods for the detection of antibodies against *T. pallidum* in both clinical settings and blood banking (Janier et al., 2014). All the ELISA tests included in the present study which showed similar or better performance compared with the TPHA results appear to be suitable for screening syphilis antibodies with their advantages over sensitivity, specificity, percent agreement and simple operation. The exception were the Vircell syphilis ELISA IgG + IgM test, SD Bioline Syphilis immunochromatographic test and RPR agglutination tests which demonstrated lower performance compared with the TPHA test. Hence higher diagnostic value was determined with automated assays and manual *Treponema pallidum* Screen ELISA (Euroimmun, Germany) compared to the results of other tests. Although the sensitivity of Syphilis Virclia Monotest was lower than TPHA test, the specificity and the percent agreement were higher so it is suggested that it may be used instead of TPHA test. Moreover the performance of Architect TP Syphilis was superior to other methods and is favoured over the TPHA because of its ability to run on an automated system and remove subjectivity in the evaluation phase.

Generally as treponemal assays offer improved sensitivity and specificity and remove subjectivity, an increasing number of laboratories have switched from the nontreponemal to treponemal tests recently (Buono et al., 2017). Nontreponemal antibodies appear later than treponemal antibodies in *T. pallidum* infection. As nontreponemal antibodies may decrease after treatment or in the late stages of the disease, they are no longer suggested for syphilis screening (Liu et al., 2014). On the other hand, the non treponemal tests are commonly used to determine serological activity and to monitor the therapeutic effects (Sonmez et al., 2018). Despite their advantages such as they are widely available in the market, cheaper and simple to perform, the results require further confirmatory screening. In our study the only nontreponemal test performed was RPR test. When the results were analyzed, the lowest performance capacity test seems to be RPR test with a percent agreement of 57.6% compatible with another study (54.1%) of Sonmez et al. (2018). Tests with better sensitivity, specificity and percent agreement including Vircell Syphilis ELISA IgG + IgM and SD Bioline Syphilis may be used instead of RPR for screening purposes as they are treponemal and the evaluation phase is not as subjective as RPR test. It is known that nonsyphilitic treponemas can be reactive in serological tests resulting in false positivities. Nevertheless none of the serological laboratory methods can distinguish between infections

Table 3
Agreements between assays in comparison with the FTA-abs assay (n = 363).

Test	Percent Agreement	Cohen's Kappa Test		
		Kappa Coefficient	p value	Interpretation
Chorus Syphilis Screen Recombinant (DIESSE Diagnostica Senese, Italy)	88.7	0.73	P < .001	Substantial agreement
Architect Syphilis TP (Abbot Diagnostics, USA)	92.8	0.82	P < .001	Perfect agreement
Syphilis Virclia Monotest (Vircell, Spain)	84.9	0.66	P < .001	Substantial agreement
Advia Centaur Syphilis (Siemens Healthcare Diagnostics, USA)	87.9	0.70	P < .001	Substantial agreement
Treponema pallidum Screen ELISA (Euroimmun, Germany)	87.1	0.68	P < .001	Substantial agreement
Vircell Syphilis ELISA IgG + IgM (Vircell, Spain)	70.5	0.31	P < .001	Fair agreement
SD Bioline Syphilis 3.0 (Standard Diagnostics, Korea)	67.2	0.38	P < .001	Fair agreement
TPHA (Omega, UK)	82.6	0.53	P < .001	Moderate agreement
RPR (Omega, UK)	57.6	0.12	P = .014	Slight aggrement

caused by closely releated spirochetes (*T. pallidum* subsp. *perteneu*, *T. pallidum* subsp. *endemicum*, *T. carateum*) (Li et al., 2016). This feature makes serology based assays potentially less specific. However, as nontreponemal tests or the treponemal screening tests with a low specificity can give false positive results (Morshed and Singh, 2015; Silletti, 1995), FTA-abs test is generally overused. Since the manual and subjective nature of the FTA-abs test, it requires a considerable technical ability and experience. A reduction in use would be of great benefit to the laboratories (Silletti, 1995). With the availability of a combination of *T. pallidum* antigens produced with recombinant DNA technology, new tests have the potential for improving the specificity of serological assays (Schmidt et al., 2000).

The nontreponemal tests such as the RPR and the treponemal tests such as the TPHA Assay and the FTA-abs tests are highly operator dependent (Sena et al., 2010). In recent years automated enzyme immunoassays (EIAs) and chemiluminescence immunoassays (CIAs) have been developed for the serodiagnosis of syphilis. These assays, using one or more of the recombinant *T. pallidum* proteins TpN15 (Tp0171), TpN17 (Tp0435), TpN44.5 (TmpA, Tp0768) and TpN47 (Tp0574) have gained interest in the field as they are objective, reproducible, automated and computerized (Xu et al., 2016; Hoover and Radolf, 2011). More importantly these tests have led some laboratories to use the reverse algorithm in various countries (CDC, 2011; Lee et al., 2013).

Recently the use of chemiluminescence immunoassays (CLIAs) to detect *T. pallidum*-specific IgG and IgM antibodies has been gradually increasing (Park et al., 2016). Chemiluminescence assays offer advantages in terms of high throughput, ease of use, automation, higher sensitivity and specificity which is important for early stage syphilis cases (Tao et al., 2017). In our present study Architect Syphilis TP, Syphilis Virclia Monotest and Advia Centaur Syphilis tests are the ones which were chemiluminescent based. When the kappa values were determined, it was detected that there was substantial agreement between the FTA-abs test, except from the Architect Syphilis TP test which showed perfect agreement. Moreover the percent agreement of Architect Syphilis TP test was the highest with 92.8%. In addittion to our study results, this test presented a good performance in other studies as well (Wellinghausen and Dietenberger, 2011; Young et al., 2009). Although Chorus Syphilis Screen Recombinant test is an automated sandwich based solid phase ELISA, it showed substantial agreement as the other automated chemiluminescence assays. Hence our results indicate that automated systems can be used for screening syphilis total antibodies in high capacity settings.

When the kappa value of the manuel tests, RPR, TPHA, SD Bioline Syphilis, Vircell IgG + IgM ELISA and Euroimmun *Treponema pallidum* Screen ELISA tests were determined Euroimmun *Treponema pallidum* Screen ELISA test showed substantial agreement while the others such as TPHA showed moderate, Vircell IgG + IgM ELISA and SD Bioline Syphilis 3.0 showed fair and the RPR test showed slight agreement. In these tests Euroimmun *Treponema pallidum* Screen ELISA test has a similar agreement with the automated assays. As the sensitivity of the test is similar and also specificity, percent agreement is better than the TPHA test, it is suggested that it may be used instead of TPHA test for screening. The sensitivity and specificity of the manuel Vircell IgG + IgM ELISA test and SD Bioline Syphilis 3.0 tests were considered to be low to implement the tests in a hospital laboratory. On the other hand smaller laboratories could benefit from running manuel Vircell IgG + IgM ELISA test and SD Bioline Syphilis 3.0 tests if they cannot afford automated ELISA tests instead of screening with RPR test. Rapid tests (RTs) like SD Bioline Syphilis 3.0 oftenly can be performed by staff with minimal training in less developed settings. Both SD Bioline Syphilis and manuel Vircell IgG + IgM ELISA tests have better sensitivity, specificity and percent agreement over RPR test. Consistent with the results of other studies (Herring et al., 2006), our findings revealed that although rapid tests such as SD Bioline Syphilis 3.0 test are generally less sensitive than TPHA, has a greater specificity suggesting that false positivity rates are low in these tests. Cost effectieveness studies indicate that a rapid test is cost effective if the sensitivity is higher than 88%. Otherwise the use of RPR is recommended (Bazzo et al., 2017; Drancourt et al., 2016). In our study the SD Bioline Syphilis rapid test demonstrated a sensitivity of 58.1% which was lower than TPHA however higher than RPR test. Mabey et al. reported the specificity of SD Bioline Syphilis rapid test as 95.5% to 99.4% for laboratory testing which is compatible with our study result (Mabey et al., 2006). Therefore as SD Bioline Syphilis is a treponemal test, it seems advantageous when considering the benefits of treponemal assays in primary settings instead of RPR testing for screening purposes.

The present study has the following limitations: (i) No clinical data has obtained, and therefore it was not possible to correlate the laboratory results with the clinical presentation, (ii) additionally the study is conducted in reference laboratory with trained staff performing the tests, it is expected that the results from health services will be comparable with those obtained in our study. However this study provides baseline information on the performance of commonly used syphilis screening tests in our country for the first time.

Syphilis laboratory diagnosis is not easy because of the different assay platforms presently used for screening in the reverse algorithm system ranging from FTA-abs, CIA, TPHA and Point of Care (POC) tests. Consequently it would be advisable for the laboratories to consider the selection of more sensitive screening and confirmatory treponemal assays to avoid having discrepant results.

In conclusion the results of the present study reveals that *T. pallidum* specific immunoassays with a performance similar or better than TPHA test generally performed well with the confirmatory FTA-abs test and may be an acceptable alternative for screening total antibodies in syphilis infection. It is nevertheless important to underline that the use of a confirmatory test remains a must in order to avoid false-positive results. A second treponemal test may lead to increased costs however can avoid unnecessary treatment caused by false positivity. It is worthy to remark that every laboratory should adapt their syphilis serological tests according to respective conditions and demands.

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