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COMPARATIVE ANALYSIS OF SYPHILIS SCREENING TECHNIQUES IN A POPULATION OF PREGNANT WOMEN

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ABSTRACT

Syphilis is a Sexually Transmitted Infection, due to T. pallidum, it has increased considerably since the 2000s. Consequently, it represents a major activity for biological analysis laboratories. VDRL-charcoal and TPHA are the two main screening and serodiagnosis tests for syphilis. In the present work, we carried out a cross-sectional, monocentric study focusing on the search for anti-treponemal antibodies in serum samples from pregnant women consulting at the peripheral hospital in Casablanca, using four distinct syphilitic serology kits, in order to evaluate two kits from the firm PLASMATEC® (VDRL-charcoal and TPHA), compared to the kits used routinely and therefore that we considered as a reference by the laboratory of this peripheral hospital, this concerns the kits from the company BioSystems® for the VDRL-charcoal test and from the company OMEGA® for the TPHA test, with a the possibility of introducing them into routine analysis. The sample consisting of 100 sera from pregnant women was tested by the VDRL-charcoal and TPHA tests technique using the BioSystems® reference kit (RPR-charcoal) and the reference kit OMEGA® (TPHA), and the tested PLASMATEC® kit (VDRL-charcoal, TPHA). Both kits gave negative results for all samples analyzed, with 100% correlation, 0% sensitivity, 100% specificity, and 0% and 100% PPV and NPV, respectively. Despite the 100% correlation of the results of the kits tested against the reference kits. Their possible introduction to routine use remains subject to carrying out studies with samples that are more representative in number and quality (positive sera, sera from patients with confirmed clinical signs of syphilis).

KEYWORDS: VDRL-charcoal and TPHA.

INTRODUCTION

Sexually Transmitted Infections (STIs) constitute a real public health problem. They are transmitted mainly during sexual intercourse, but can also be transmitted by other routes, such as blood transfusion, transplacental passage from mother to child during pregnancy or at the time of childbirth.[1], [2]

Syphilis is a treponematosis caused by the bacterium Treponema pallidum (T. pallidum), it is a venereal species, only human-to-human.

Since the era of penicillin in the early 1940s, a considerable course of the disease has been marked[3],[4], before its resurgence from the year 2000 in Europe, the United States and Australia[3], this was favored by sex tourism, unprotected homosexuality, and blood contamination among drug addicts.[2] This upsurge has made syphilis a Notifiable Disease (ND) in France[5], and also in Morocco, according to royal decree n ° 554-65 of 17 rabii I 1387.[6]

Screening for syphilis is a major concern of biological analysis laboratories, hence the interest they confer on the usual assay techniques, in order to guarantee continuous reliability and reproducibility of the results provided. In this sense, the evaluation of new syphilis screening kits is essential in order to be able to introduce them for routine use in the future.

Our work aims to evaluate the kits from the PLASMATEC® company (VDRL-charcoal and TPHA), compared to the kits used routinely and considered as reference by the biological analysis laboratory of a peripheral hospital in Casablanca, BioSystems ® for the VDRL-charcoal test and OMEGA® for the TPHA test.

MATERIAL AND METHODS

This is a cross-sectional, single-center study on the search for anti-treponemal antibodies by four separate syphilitic serology kits, in serum samples from pregnant women, consulting at the peripheral hospital as part of systematic screening. as well as the follow-up assessment during pregnancy.

The sample for our study is made up of 100 sera from pregnant women, collected over a period of two months.

The principles of the tests used are different, there are.

- **Non-treponemal tests (TNT): VDRL, RPR**

These tests are non-specific, sensitive, they usecardiolipid antigens and become negative after treatment. They allow to affirm the active character of the disease.

- The VDRL (Veneral Disease Research laboratory) is a passive agglutination test of antibodies directed againsta cardiolipid Ag fixed on an inert support consisting of cholesterol crystals. This test is qualitative or semi-quantitative by carrying out successive dilutions
- RPR (Rapid Plasma Reagin) has the same characteristics; it is composed of charcoal particles coated with a mixture of lipid antigens. The reading of the agglutination is facilitated and non-specific reactions are eliminated.

- **Treponemal tests (TT) : TPHA, TPPA, WB**

More specific, these tests remain positive after treatment. They make it possible to make the diagnosis of syphilis, but not to distinguish an active syphilis from a serological scar.

- The TPHA (*Treponema Pallidum Hemagglutination Assay*) is a manual agglutination technique using alysate of pathogenic treponemes attached to erythrocytes. Easy to carry out, it is qualitative or semi-quantitative after successive dilutions of the serum.
- TPPA (*Treponema Pallidum Particle Agglutination*) is an agglutination technique close to TPHA; erythrocytes are replaced by inert particles.
- FTA (*fluorescent Treponemal Antibody absorption test*) this technique is called absorbed FTA because the serum is first absorbed by a lysate of commensal treponemes in order to eliminate false positives due to group antigens. This test is also qualitative or semi-quantitative.
- Western blot (WB) IgG or IgM is a confirmation technique with high specificity and sensitivity. It looks for antibodies directed against 4 immunodominant Ag of T. pallidum: TpN47 (47 kDA), TmpA (transmembrane protein or Tp45), TpN17 (17 kDA) and TpN15 (15 kDA). These 4 proteins inducing a strong immune response play an important role in pathogenesis and diagnosis. TpN47, strongly immunogenic, is present mainly in the primary phase; TpN15 induces humoral and cellular immunogenicity; TpN17, very abundant and the most immunogenic, is involved in the transmission of syphilis; Tp45 generates the highest levels (IgG and IgM) and is present during primary syphilis. WB positivity requires the presence of at least 2 bands. In the absence of bands, it is negative, in the presence of a single band, it is incomplete (request a 15-day check). The WB has an interest in the event of questionable, dissociated screening, or false positive (autoimmune diseases)

To perform this study, we used two different kits for the VDRL test, and two different kits for the TPHA test.

RPR-COAL BioSystems®: the RPR test (rapidplasma reagin) uses the principle of agglutination, by demonstrating syphilitic reagin (Ac formed during syphilitic infection), the latter are directed against phospholipids of T. pallidum, the antigenicity of which is crossed with cardiolipid antigens[8], these techniques recommend the use of cardiolipid Ag carried by charcoal particles as an inert support, the agglutination of which is easy to demonstrate.

VDRL ANTIGEN CARBON PLASMATEC®: is a modified form of VDRL containing microparticles of charcoal. It is intended for use in flocculation tests for the serological diagnosis of syphilis. The charcoal particles help the microscopic reading of the results. Weak positive results can be clearly distinguished from negative profiles showing a macroscopically smooth and uniform appearance.

OMEGA IMMUTREP® TPHA: is composed of formalin red blood cells sensitized by T. pallidum, unsensitized formalin red blood cells, a dilution buffer. Mixing the diluted positive sample with the red blood cells causes the cells to agglutinate, thanks to the specific antibodies. Agglutinated red blood cells exhibit a characteristic profile at the bottom of microtiter wells. In the absence of antibodies, the red blood cells gather in a pellet in the form of a button in the well. This reagent has been calibrated with the WHO reference serum for serodiagnosis of treponema infections (Ref 3-1980) with a 1/2 dilution to ensure correct sensitivity.

TPHA TEST KIT PLASMATEC® : This kit uses avian erythrocytes preserved and coated with antigens of T. pallidum (Nichols strain), binding specific antibodies present in the patient's serum or plasma. The cells are suspended in a medium containing components which suppress non-specific reactions. Positive reactions are detected by the agglutination of cells, while negative reactions are represented by settling of cells in the form of a button or a small ring. Although the kit is intended for primarily qualitative use, antibody levels can be titrated by doubling the dilution. Hemagglutination profiles are interpreted with the naked eye, or using a plate reader capable of reading hemagglutination patterns.

In order to compare the kits used in our study, we performed a statistical calculation according to the formulas mentioned below.

VP: true positive, number of positive tests by both techniques.
VN : true negative, number of negative tests by the two techniques.
FP : false positive, number of tests negative by the reference kits and positive by the kits tested.
FN: false negative, number of positive tests by the reference kits and negative by the tested kits.
T: Total number of samples.
The sensibility : the probability that the test will be positive if anti-treponemal antibodies are present in the patient's serum.

$$\text{Sensitivity} = \frac{VP}{VP+FN} \text{ x } 100$$

The specificity: the probability of obtaining a negative test in patients who do not have anti-treponemal antibodies in their sera.

$$\text{Specificity} = \frac{VN}{VN+FP} \text{ x } 100$$

Positive predictive value (PPV): the probability that anti-treponemal antibodies are present in the serum when the test is positive.

$$\text{PPV} = \frac{VP}{VP+FP} \text{ x } 100$$

Negative predictive value (NPV): the probability that anti-treponemal antibodies are not absent in the serum when the test is negative.

$$\text{NPV} = \frac{VN}{VN+FN} \text{ x } 100$$

Correlation between the 2 techniques.

$$\frac{VP+VN}{T} \text{ x } 100$$

RESULTS

The results of our study focused on the four kits used. Each serum was tested in duplicate, by the same operator and under the same production conditions, using the BioSystems reference kits. ® (RPR-charcoal) and OMEGA® (TPHA), and the tested PLASMATEC kits ® (VDRL-charcoal) and PLASMATEC®(TPHA).

1. The results of the VDRL-charcoal and RPR-charcoal tests of our series.

After validating the technique by carrying out positive and negative controls, the 100 sera tested by the two kits returned negative. The results obtained are summarized in the following table.

Table I: Results of the VDRL-charcoal serology by the two kits.

	RPR-CARBON BioSystems®	VDRL PLASMATEC®
Positive	0	0
Doubtful	0	0
Negative	100	100

The compatibility between the reference kit and the kit tested is elucidated in Table II:

Table II: Comparison of the results obtained by the two kits.

		RPR-CHARCOAL BioSystems®			Total
		Positive	**Doubtful**	**Negative**	
VDRL PLASMATEC®	Positive	0	0	0	0
	Doubtful	0	0	0	0
	Negative	0	0	100	100
Total		0	0	100	**100**

Based on the results obtained, we established the technical characteristics of the two boxes: VDRL-carbon PLASMATEC®, and RPR-carbon BioSystems®, by specifying the VP, FP, VN and FN (Table III), and in calculating the correlation, sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV), (Table IV).

Table III : Technical characteristics of the PLASMATEC® kit compared to the BioSystems® kit

		RPR-carbon BioSystems®	
		Positive	**Negative**
VDRL-carbon PLASMATEC®	**Positive**	VP (0)	FP (0)
	Negative	FN (0)	VN (100)

Table IV : Calculation of the characteristics of the PLASMATEC® kit compared to BioSystems®

Correlation (%)	Sensitivity (%)	Specificity (%)	PPV (%)	VPN (%)
100	0	100	0	100

2. TPHA test results from our series.

After carrying out the positive and negative controls, the 100 sera were negative for anti-treponemal antibodies for the two kits, the OMEGA reference kit® and the PLASMATEC tested kit® (table V).

Table V : Results of the TPHA test by the two kits.

	TPHA OMEGA®	TPHA PLASMATEC®
Positive	0	0
Doubtful	0	0
Negative	100	100

Based on the results obtained, we made a comparison between the two kits (table VI).

Table VI : Results of the TPHA test by the two kits.

		IMMUTREP OMEGA®			Total
		Positive	**Doubtful**	**Negative**	
TPHA PLASMATEC®	Positive	0	0	0	0
	Doubtful	0	0	0	0
	Negative	0	0	100	100
Total		0	0	100	**100**

Likewise, for the VDRL-charcoal test, we established the characteristics of the two kits : TPHA from PLASMATEC®, and TPHA from OMEGA®, by specifying the VPs, FPs, VNs and FNs (Table VII), and by calculating the correlation, sensitivity, specificity, the Positive Predictive Value (PPV) and the Negative Predictive Value (NPV).

Table VII : Characteristics of the PLASMATEC® kit compared to the OMEGA® kit.

		TPHA OMEGA®	
		Positive	**Negative**
TPHA PLASMATEC®	**Positive**	VP (0)	FP (0)
	Negative	FN (0)	VN (100)

Table VIII: Characteristics of the PLASMATEC® kit compared to the OMEGA® kit.

Correlation (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
100	0	100	0	100

DISCUSSION

Syphilis is a real public health problem around the world. Treponemal and non-treponemal serological tests are crucial elements of any syphilis control program, they are used for screening for asymptomatic infections, and also remain the main diagnostic tool.[9]

The use of one type of serological test is insufficient for the diagnosis of syphilis.[11] Several studies have demonstrated the complementarity between non-treponemal tests and treponemal tests in the serodiagnosis of syphilis, as well as the advantages and disadvantages of each of them.

According to the literature study carried out, we find that the serological diagnosis of syphilis does not have a 100% reliable standardized test, whether for non-treponemal or treponemal tests.[10] In order to detect these antibodies, each manufacturer produces its own antigenic mixtures itself, consequently, no international standard is set up, and each manipulation is validated according to the results found by the positive and negative controls.

We report in our study, a comparison between kits considered as reference by the laboratory of biological analyzes of the peripheral hospital Casablanca, (OMEGA® for the TPHA, and BioSystems® for the RPR-charcoal), and newly tested kits (PLASMATEC® (VDRL-charcoal, and TPHA), in order to evaluate their characteristics.

Centers for Disease Prevention and Control (CDC) recommends a traditional screening algorithm starting with a non-treponemal test such as RPR or VDRL, for the identification of people with a possible untreated infection, this examination is followed by a confirmatory treponemal test such as TPHA or TPPA.[12] While ECDC recommends a primary treponemal screening test at first, followed by a second different confirmatory treponemal test.[10], [13]

In our study, we followed the algorithm recommended by the CDC, performing the non-treponemal tests followed by the treponemal tests.

Regarding the non-treponemal tests, the literature review that we carried out revealed a discrepancy in the description of the performance of RPR-charcoal and

VDRL-charcoal. Indeed, a study conducted at Stanley medical college in India by Dheepa, published in January 2017 reports that the VDRL-charcoal could detect low titers of antibodies more frequently than RPR-charcoal, in the same study, VDRL-charcoal detected 6 cases of low titers of antibodies which were positive by treponemal tests, this sensitivity is especially observed in the late latent phase where VDRL-charcoal also detected 8 cases, while RPR-charcoal only detected 5 cases.[14]

However, other studies give more support to the considerable sensitivity and specificity of RPR-charcoal compared to VDRL-charcoal, notably a study carried out in Ethiopia at Addis Ababa University by Afework in 2016, showing sensitivity and a specificity of RPR-charcoal of 62% and 99.6% respectively.[15] In the same way, a study carried out in Latvia at the CHU Pernavas Iela Riga by Ozoliņš et al in 2009, reported a sensitivity of 58.8% in favor of RPR-charcoal.[16] Likewise, another study conducted by the departments of dermatology and microbiology at Rize Hospital in Turkey in 2012 by Saral et al, reported almost the same results as the previous one, with a sensitivity of 58% for RPR-charcoal.[11]

In our study, the kit considered as reference BioSystems® based on the RPR-charcoal technique and the tested PLASMATEC® kit using VDRL-charcoal, both gave negative results, with a correlation between the two tests of 100%, specificity of 100%, sensitivity of 0% and PPV and NPV of 0% and 100% respectively. The zero value of the sensitivity is explained by the absence of seropositive samples in our series.

However, the sensitivity of treponemal tests, in particular TPHA, greatly exceeds that of VDRL-charcoal and RPR-charcoal as reported by a study conducted at Stanley Medical College in India by Dheepa, published in January 2017, that the TPHA detected 3 cases of syphilis. latent latent, while VDRL-charcoal and RPR-charcoal gave negative results, failure of detection by non-treponemal tests is probably due to the use of antibiotics by patients for other reasons.[14] These results were confirmed by the studies cited above, which demonstrated a sensitivity of 58% for RPR-charcoal and 98% for TPHA.[11], and a specificity of 66.7% versus 33.3% of TPHA and VDRL-charcoal respectively[16], this decrease in sensitivity of non-treponemal tests, especially during the late latent stages, can also be explained by technical errors.[14]

Furthermore, the principle of detecting antibodies specific to treponemes represents the major limitation of the treponemal tests used, and therefore does not allow the distinction between a recent infection and an old infection, the false positives resulting from these tests sometimes lead to a over-treatment of syphilis.[17]

Our results of the TPHA treponemal tests show a 100% correlation between the OMEGA® reference kit and the tested PLASMATEC® kit. Admitting that they use the same principle of dosage (hemagglutination in the presence of avian red blood cells), which explains the results obtained. Similarly for the VDRL test, the same results were obtained with the two kits. With 100% specificity, 0% PPV and VPN sensitivity of 0 and 100%. In this sense, we performed an analytical comparison of the technicality of the kits used in our study, referring to the data mentioned on their technical sheets.

- For the VDRL

The explanatory booklet accompanying the BioSystems reference kit®, indicates analytical sensitivity and specificity in the range of 98-100% and 99-100%, respectively. In addition, the brochure of the tested PLASMATEC kit®, does not indicate any data regarding sensitivity and specificity.

- For TPHA

The analysis of the performances mentioned by the suppliers, reports an identical analytical sensitivity between the OMEGA reference kit® 99.5%, and the PLASMATEC tested kit® 98.5%. This sensitivity gives them the ability to detect low levels of anti-treponemal antibodies in sick subjects. As for the analytical specificity, the two OMEGA kits®, and PLASMATEC®, have almost the same values, 100% versus 99.6% respectively, indicating a great performance in giving true negative results in healthy subjects.

We thus note, that the PLASMATEC® kits (VDRL-carbon and TPHA) present the same performances compared to the reference kits OMEGA® (TPHA) and BioSystems® (RPR-carbon), since they gave similar results and present the same performances on their technical data sheets, except the VDRL, several elements of which are not described in the specification of the kit tested.

Although these tests agree 100% with the reference kits, their introduction to daily practice remains subject to change. Given.

- The small size of the sample.
- The fact that the study was carried out on sera from asymptomatic pregnant women, as part of systematic screening during pregnancy, the probabilities of finding positive sera in our sample are minimal.
- The absence of clinical information on the population studied, which could point to a possible old infection not detected by the tests carried out.
- The evaluation of inter-operator reproducibility, to compare the reading of the results, with that of a health professional with more experience in terms of biological analyzes.

CONCLUSION

Syphilitic serology remains the mainstay of laboratory screening for T. pallidum infections. On the other hand, it constitutes the only means of diagnosis existing at the

present time, hence the interest in evaluating new kits before introducing them into current practice. Two types of tests are performed routinely for syphilis; non-treponemal tests and treponemal tests.

The results of our comparative study showed 100% agreement, 100% specificity, 0% sensitivity and 0% and 100% PPV and NPV, respectively, of the kits tested compared to the reference kits, these These values would allow the tested kits to be offered in the future for possible practical introduction.

Although the VDRL-charcoal and TPHA tests are the main laboratory tests for syphilis, their limits of use sometimes require the use of automated immunological techniques, distinguished by higher sensitivity and specificity, and which confer minimization considerable risk of errors.

Conflit of interest

The authors declare that they haven't known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval and consent to participate

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