Clinica Chimica Acta 433 (2014) 84–87

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Syphilis detection using the Siemens ADVIA Centaur Syphilis treponemal assay

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ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form 21 January 2014
Accepted 24 January 2014
Available online 8 February 2014

Keywords:
Automated immunoassay
Syphilis
Reverse algorithm
Treponemal testing
Performance evaluation

ABSTRACT

Background: Treponemal tests for detecting syphilis should be sufficiently sensitive and specific, especially when used as the first-line method in reverse-algorithm testing. We compared the Siemens ADVIA Centaur® Syphilis assay to 2 other commercial assays in use by the Star-MDC laboratory to evaluate its performance and usability.
Methods: Agreement between the Siemens ADVIA Centaur Syphilis assay, Siemens IMMULITE® 2000 Syphilis Screen, and Biokit bioelisa Syphilis 3.0 assay was evaluated using 1251 patient samples (50 from known positives, 701 from patients referred for syphilis testing, and 500 from pregnant women). Reactive samples (i.e., reactive according to at least two of the three treponemal methods) were further evaluated using Western blot IgG and IgM, and Venereal Disease Research Laboratory (VDRL) testing.
Results: Overall, positive and negative agreement was 100% between the Centaur and IMMULITE assays. In this study, overall agreement was 99.92% between either of the Siemens assays and the Biokit assay; positive agreement was 99%, and negative agreement was 100%. Overall, 0.88% (11/1251) of the samples were interpreted as positive/reactive based on the combined positive results by the ADVIA Centaur, IMMULITE 2000, and bioelisa assays; a positive Euroline anti-*Treponema pallidum* IgM blot; and a VDRL result of ≥1:8. In this study, no false-reactive samples were identified using this method.
Conclusion: The Centaur Syphilis assay performance is comparable to the other 2 commercial assays.

1. Introduction

Syphilis is a sexually transmitted disease caused by the bacterium *Treponema pallidum* subspecies *pallidum*. This disease is also passed from infected pregnant women to their offspring in utero. Syphilis can be classified into three infectious stages (primary, secondary, and early latent) and 2 noninfectious disease stages (late latent and tertiary) [1–3]. Because *T. pallidum* is capable of infecting a wide variety of tissues [1–4], the disease can mimic a variety of conditions in its clinical presentation. Thus, laboratory analysis in combination with clinical presentation plays an important role in the diagnosis of syphilis.

There are 2 types of serological tests for syphilis: nontreponemal and treponemal tests. Nontreponemal tests are not specific for treponemal antigen. Instead, these tests detect antibodies to cardiolipin, which is a component of both the treponemal membrane and the eukaryotic mitochondrial membrane. Reactivity to mitochondrial cardiolipin stems from host cellular debris, which may result from the immunological response to treponemal infection but can also arise due to nonrelated cellular damage. Two common nontreponemal methods in standard use are the Venereal Disease Research Laboratory (VDRL) test and the rapid plasma reagin (RPR) card test. In contrast, treponemal tests detect specific treponemal antibodies. These include the *Treponema pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination assay (TPPA), fluorescent treponemal antibody-absorbed test (FTA-ABS), and most enzyme immunoassays (using antibodies created against native and/or recombinant antigens) [2,4].

Two primary approaches to testing are currently in use [1,3,5]. In the traditional testing algorithm, a nontreponemal test is performed first. If reactive, it is followed by treponemal testing. Nontreponemal testing requires manual pipetting, which can be very time-consuming and labor-intensive; it is also more likely to generate a large number of samples requiring follow-up testing due to nonspecific detection of cardiolipin. Another approach that is becoming increasingly popular is to perform a treponemal test first and then, if reactive, perform a nontreponemal test to confirm the results and establish state of infection (acute vs. remote). The popularity of this method—termed reverse algorithm—continues to grow for two primary reasons: Increasing syphilis infection rates in many parts of the world, including the U.S. and Europe (where 12 million new infections are anticipated annually), are driving

Abbreviations: VDRL, Venereal Disease Research Laboratory; RPR, rapid plasma reagin; TPHA, *Treponema pallidum* hemagglutination assay; TPPA, *Treponema pallidum* particle agglutination; FTA-ABS, fluorescent treponemal antibody-absorbed test; Tp15, *T. pallidum* p15 antigen; Tp17, *T. pallidum* p17 antigen; AE, acridinium ester; RLUs, relative light units; S/CO, signal-to-cutoff ratio.

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http://dx.doi.org/10.1016/j.cca.2014.01.041

the need for higher-volume and less labor-intensive testing methods. In addition, test volume is increasing because many countries are now requiring screening of all pregnant women to prevent maternal transmission. Popularity of the reverse-algorithm method has grown because it can be conducted more efficiently using fully automated treponemal tests. Not only are such tests less time-consuming and less labor-intensive, but they typically also have higher sensitivity than nontreponemal tests in the primary and latent disease stages [1–5]. Regardless of the algorithm applied, both treponemal testing and nontreponemal testing are required to confirm a syphilis infection and determine its stage.

2. Materials and methods

2.1. Patient samples

A total of 1251 patient samples were tested for the presence of *T. pallidum* antibodies at Star-MDC (Rotterdam, The Netherlands). All samples were drawn sequentially at the Star-MDC center as requested by a treating physician. Fresh samples were taken from 500 pregnant women in the course of regular obstetric care, and 701 samples were drawn from at-risk individuals suspicious for syphilis; these samples were either tested immediately or stored between 2 and 7 h at 4 °C until tested. The other 50 samples were remnant samples (stored at −25 °C) from known positives previously diagnosed and treated for syphilis at our institute. Per Star-MDC's policy, all blood samples used for studies were fully de-identified and used with patient consent.

2.2. Testing protocol (comparison of treponemal assay methods)

All samples were tested in duplicate using each of the three treponemal assays. Samples that were negative by all 3 assays were classified as negative. Samples that were positive according to 2 of the 3 assays or all 3 were considered positive. Our goals were to evaluate agreement between the ADVIA Centaur Syphilis assay and the other 2 assays and to determine its usability as a primary treponemal testing method. For these reasons, our protocol called for retesting all results that were reactive according to the Centaur Syphilis assay, even if samples were nonreactive according to both of the other two assays. However, no samples of this type were identified.

Presumed positive samples were then tested with the Euroline IgM and IgG assays (Medizinische Labordiagnostika AG) and the VDRL assay to establish the status of the infection. Active syphilis infections were confirmed on the basis of positive status assigned using the 3 treponemal assays in conjunction with the nontreponemal testing, i.e., a positive Euroline anti-*Treponema pallidum* IgM and/or IgG blot and a VDRL titer of ≥1:8 (Fig. 1).

2.3. Statistical analysis

The ADVIA Centaur assay was compared to each of the other 2 assays in terms of statistical positive and negative agreement. Agreement was chosen as the appropriate statistical method since none of the predicate tests—including the confirmatory Western blot and nontreponemal tests used in the study—constitute a definitive reference standard (in fact, no definitive reference standard has been defined for syphilis testing) [6]. Additionally, although positive agreement and negative agreement are analogous to sensitivity and specificity, the use of agreement clarifies that the comparison is made relative to an existing assay, and not to a clinical diagnosis [2,7–9]. Agreement was calculated along with the 95% confidence intervals [9]. Although overall agreement is reported, it is not as statistically sound as positive and negative agreement; we have reported it in this document for the sake of completeness.

3. Results

Using the algorithm described in the methods, we determined that 100 of the 1251 samples were treponemal antigen-positive. This included 50 newly identified syphilis-positive sera (beyond the 50 already-known positive sera). The large number of newly identified positives might seem unlikely, given that it indicates prevalence far greater than that of the general population. However, it is not unexpected, as 701 sera were taken from individuals practicing high-risk behavior and who were tested for a variety of sexually transmitted diseases. Of these 50 additional samples, 2 cases represented active syphilis, while the other 48 represented remote infections (3 of which were from pregnant women). In total, among the 100 treponemal antigen-positive samples, we identified 11 cases of active syphilis and 89 remote cases (Table 1).

Agreement between each of the 3 treponemal assays in this study was ≥99%. Positive agreement reflects the number of sera that were positive according to each assay, while negative agreement reflects the number of sera that were negative according to each assay. Total agreement is the combined number of positive and negative results for each assay (Table 2).

A single sample yielded discordant results between the Siemens and Biokit assays. This sample was negative according to both the IMMULITE 2000 and ADVIA Centaur assays but positive by the Biokit bioelisa assay. Upon further testing, this sample was also negative according to both

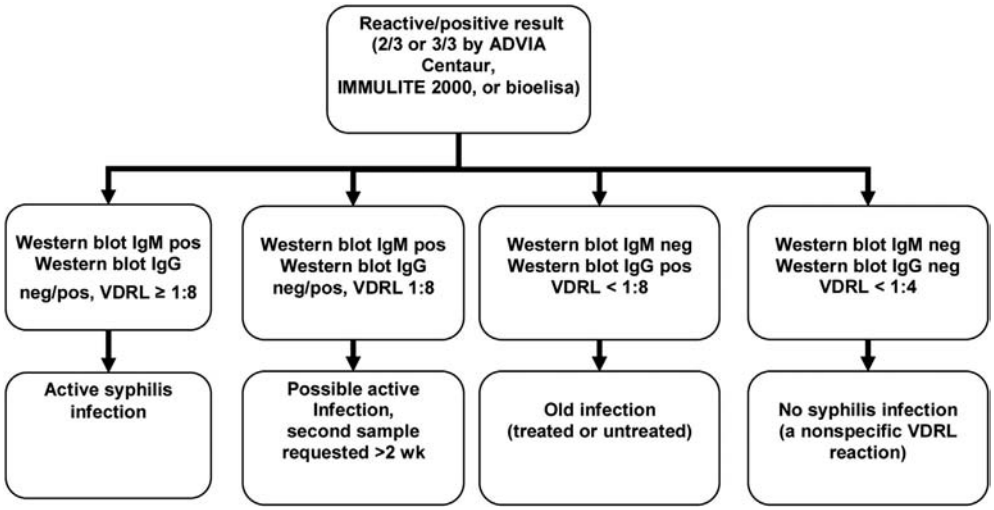

Fig. 1. The classification algorithm.

Table 1
Status of treponemal-positive samples according to the application of immunoassay, VDRL quantitative, and Euroline IgM and IgG assays combined results.

	No. samples	VDRL titer	IgM	IgG
Active syphilis	3	1:8	+	+
	5	1:16	+	+
	1	1:32	+	+
	2	1:64	+	+
Remote infection	8	1:1	−	+
	6	1:2	−	+
	75*	−	−	+

* One IgM-negative/IgG-positive sample was missing VDRL information. This sample was also positive according to the Centaur, IMMULITE, and ELISA assays, and was therefore included as a remote infection.

the VDRL and the Euroline IgM and IgG Western blot results, suggesting that this sample is truly negative (i.e., confirming the Centaur and IMMULITE 2000 assays is likely correctly reporting negative results in the absence of infection). This suggests that the two Siemens assays might be potentially less likely to generate false-positive results than the Biokit assay; however, we recognize that such results will likely vary between studies and populations.

4. Discussion

When new tests become available, their accuracy can best be established by direct comparison of results to a reference standard. Such a standard could be an individual method of proven and accepted accuracy or an established diagnostic algorithm [7]. However, in the case of syphilis testing, no single test or definitive reference standard is available for every stage of the disease [2]. Only dark field microscopy provides visual confirmation of *T. pallidum* infection, but applicability of this method is limited to acute infection in which open lesions can be cultured. Thus, syphilis diagnosis relies predominantly on serological testing, requiring results from both nontreponemal and treponemal methods. Since these two types of tests target different biomarkers, statistical accuracy of new treponemal tests can be evaluated by comparing agreement of results to already-established treponemal methods [7]. Such analysis is also useful to determine if a new test may be used in place of, or alongside of, a test currently in use.

At our institution, we used our own variant of a reverse-algorithm protocol to determine statistical positive, negative, and total agreement between the treponemal Centaur Syphilis assay and each of the 2 other treponemal assays in use by our laboratory: the IMMULITE 2000 Syphilis Screen and the Biokit bioelisa assay. All 3 of these tests are immunoassays employing recombinant *T. pallidum* surface antigens to detect total antibody in serum. Results demonstrated 100% positive, negative, and total agreement between the 2 Siemens assays. However, it should be noted that they are characteristic of performance determined by an independent laboratory and do not represent the manufacturer's claims (results may vary from laboratory to laboratory and can be affected by syphilis prevalence in the population examined). The results also demonstrated 99% positive agreement, 100% negative agreement, and 99.92% total agreement between each of the Siemens assays and the Biokit assay. This high level of agreement may be due in part to the detection of antibodies to Tp17 antigens by all 3 treponemal assays.

Table 2
Comparison of the syphilis assays evaluated in this study.

		IMMULITE	
		Positive	Negative
Centaur	Positive	100	0
	Negative	0	1151
	# Agreed/total	*Agreement*	*95% CI*
Overall agreement	1251/1251	100%	99.7–100%
Positive agreement	100/100	100%	96.4–100%
Negative agreement	1151/1151	100%	99.7–100%
		Biokit bioelisa	
		Positive	Negative
Siemens (*IMMULITE or Centaur*)	Positive	100	0
	Negative	1	1150
	# Agreed/total	*Agreement*	*95% CI*
Overall agreement	1250/1251	99.92%	99.6–100%
Positive agreement	100/101	99%	96.4–100%
Negative agreement	1150/1150	100%	99.7–100%

While the intent of this study was only to establish comparable performance and usability of the ADVIA Centaur assay for our laboratory, an interesting secondary observation indicates results that are inconsistent with two earlier reports by the CDC addressing the accuracy of primary treponemal testing [10,11]. In both CDC reports, when treponemal EIA assays were applied as the initial syphilis test, over 50% of the positive results were found to be negative using RPR. In the 2008 study, 17% of the EIA-positive/RPR-negative results could not be confirmed using a second treponemal method (FTA-ABS or TPPA); similar results were reported by Binnicker et al. in a much smaller study [1]. In the 2011 report, up to 60% of the treponemal-positive/nontreponemal-negative (EIA+/RPR−) samples could not be confirmed using TPPA in low-prevalence populations, and up to 18.6% could not be confirmed in high-prevalence populations. On the basis of these studies, the CDC suggests that treponemal-specific EIAs (or at least the three used in their study) can generate a high rate of false-positive results when used for primary analysis and recommends confirming EIA+/RPR− results using the TPPA method.

In contrast, in this study it appears that none of the treponemal-positive samples/VDRL-negative samples were falsely reactive, and all of these samples represent successfully treated past infections. We believe that we did not see false-positives because the EIA results were confirmed using multiple EIA assays before proceeding to nontreponemal testing. This suggests that the number of false-positive results in the final confirmatory step might be reduced by modifying the current reverse algorithm (i.e., treponemal testing followed by nontreponemal testing, and then confirmation with a second treponemal assay if initial and nontreponemal testing results are discordant) to the proposed algorithm in which two different nontreponemal methods are used initially to confirm treponemal positivity before determining active vs. past infection using a nontreponemal assay. Because treponemal-positive/nontreponemal-positive results would normally not require a second confirmatory treponemal test, our algorithm will likely increase the total cost of testing. However, it would streamline the laboratory testing process by further reducing the number of manual nontreponemal tests performed. At the very least, this study indicates that a second treponemal EIA should provide confirmatory results comparable to what may be achieved using TPPA. In fact, neither the TPPA nor the TPHA methods are used now by most laboratories in the Netherlands (especially large laboratories), as they are manual and thus more time-consuming and prone to error when performed in large volume.

Use of a second EIA rather than TPPA is also potentially supported by a recent study by Binnicker et al. In this study, the commercial treponemal EIA assays evaluated performed comparably to TPPA, suggesting that a second or third treponemal EIA can serve as an acceptable confirmatory tool as long as its sensitivity and specificity are comparable to that of TPPA [12]. A study conducted for the WHO by Ebel et al. determined that the Biokit bioelisa assay has 99.5% sensitivity and 99.4% specificity [6]. Although this test was compared to FTA-ABS rather than TPPA, sensitivity and specificity are comparable to the seven assays evaluated by Binnicker et al. (sensitivity was 98.9% for TPPA and ranged from 96.8% to 100% for the other six assays; specificity was 97.6% for TPPA and ranged from 94.3% to 99% for the other six assays) [12]. In a

more recent study by Cole et al., when the Biokit assay was evaluated alongside TPPA (sensitivity = 99.1, 95% CI: 85.1–100%; specificity = 100%) and several other treponemal methods, it was found to have 98.2% sensitivity (95% CI: 93.8–99.8%) and 100% specificity [13]. The >99% agreement of the Siemens assays with the Biokit assay indicates that sensitivity and specificity are likely comparable. We therefore feel that the use of the three treponemal assays in this study constitutes sufficient evidence for confirmation of reactivity.

5. Limitations of the study

The primary limitation of this study was that we did not include well-characterized samples from individuals with diseases known to potentially cross-react with treponemal assays to yield a false-positive result (such as a variety of viral and autoimmune disorders). We also did not include samples from patients known to have problematic presentations, such as very early syphilis, neurosyphilis, coinfection with HIV, or congenital syphilis [2,3]. We did, however, include a large population of pregnant women. Although nontreponemal testing of pregnant women is associated with false-positive results, Larsen et al. point out that treponemal tests can also yield false-positives, although to a lesser extent [3,14]. Regardless, of the 500 pregnant women tested for syphilis by our laboratory, only three were positive according to the treponemal tests; using nontreponemal and Western blot methods, all of these were confirmed to have been the result of a past infection. Since pregnant women constitute a major portion of Star-MDC's routine syphilis-testing population, it was more important for us to establish the performance of the ADVIA Centaur test during pregnancy than for other potentially interfering disease states.

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