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# Syphilis detection using the Siemens ADVIA Centaur Syphilis treponemal assay

Ad Donkers [a], H. Roma Levy [b,*], Annemiek Letens-van Vliet [a]

[a] Star-MDC, Laboratory for Medical Microbiology, Rotterdam, The Netherlands
[b] Siemens Healthcare Diagnostics, Los Angeles, CA,, United States

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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.

* Corresponding author. Tel.: +1 818 274 5689; fax: +1 818 346 0558.
*E-mail address:* helene.levy@siemens.com (H.R. Levy).

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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

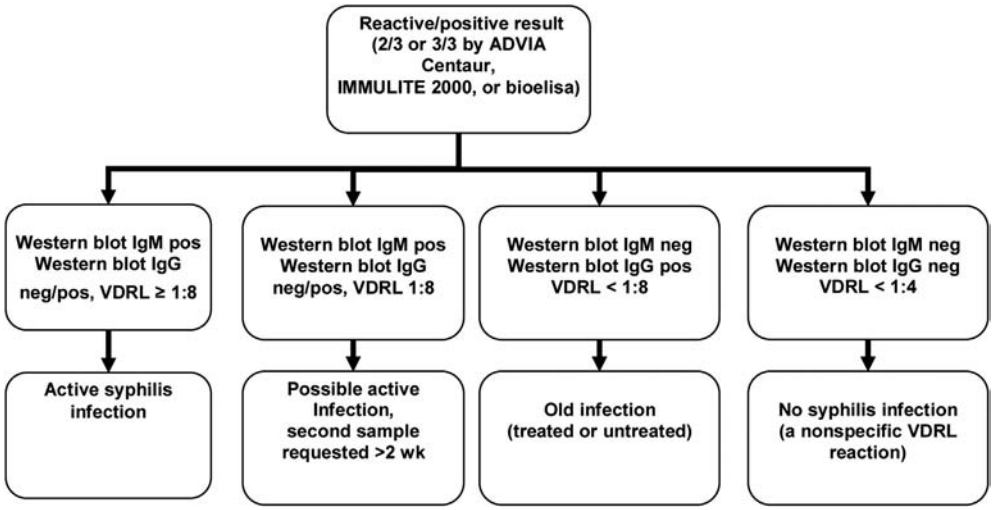


**Fig. 1.** The classification algorithm.