Vitros 5600 Syphilis TPA Assay: Evaluation of an Automated Chemiluminescence Assay for Detection of *Treponema pallidum* Antibodies in a High Prevalence Setting

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Abstract: The performance of the Syphilis TPA assay (Ortho-Clinical Diagnostics) on Vitros 5600 Integrated System was evaluated and demonstrated excellent results. Our data support the use of this assay for test confirmation in the traditional algorithm and for screening for syphilis in a routine automated laboratory setting when using the reverse algorithm.

Syphilis is a bacterial disease caused by the spirochete *Treponema pallidum* subspecies *pallidum*. Syphilis has a broad clinical spectrum, rendering laboratory testing an important tool for diagnosis. Serologic tests are still the mainstay for the diagnosis of syphilis and can be divided into nontreponemal and treponemal assays. Nontreponemal assays can be used to differentiate between active disease and past infection, which is not true for treponemal tests because these tests show a lifelong positivity. Because traditional treponemal tests are labor-intensive, newly introduced automated enzymatic immunoassays (EIAs) and chemiluminescence assays (CLIs) offer a solution to the increasing number of samples sent to the laboratory. Advantages and disadvantages of the different assays are summarized in Table 1.

Traditional algorithms for syphilis diagnostics start with a nontreponemal screening test (e.g., rapid plasma regain, or RPR), and when this test shows reactivity, confirmation is established with a treponemal test (e.g., *T. pallidum* particle agglutination, or TPPA). Such algorithms are thought to be cost-effective for small laboratories. Upon the arrival of automated immunoassays, a reverse algorithm was introduced that makes use of an EIA or CLI as the primary screening test followed by a nontreponemal test only when the former is positive. If the nontreponemal assay turns out to be negative in a patient without a history of syphilis treatment, a second treponemal test, like the TPPA, is advised. Problems in interpretation may occur in case of discordant results, notably the combination of positive CLI with negative RPR and TPPA results.

Syphilis has been reemerging in Belgium over the last decade, mainly among men who have sex with men. Currently, at the Institute of Tropical Medicine, 6500 samples are tested per year from patients attending the HIV/sexually transmitted infection clinic (65%) and the tropical medicine ward (35%), with reinfections constituting most of syphilis infections. Traditionally, a combination of RPR and TPPA has been used for screening and follow-up of syphilis patients. In response to increased test volumes, an automated system, the TPA assay (Ortho-Clinical Diagnostics, Rochester, NY) performed on Vitros 5600 Integrated System (Ortho-Clinical Diagnostics) was introduced. The purpose of this study was to evaluate the diagnostic performance of the automated TPA assay.

The Syphilis TPA assay is an immunometric immunoassay with chemiluminescence detection that uses recombinant TP15, TP17, and TP47 *T. pallidum* antigens for detection of syphilis antibodies. Results are expressed as a ratio of the signal obtained at the clinical cutoff. Ratios less than 0.80, from 0.80 to less than 1.20, and at least 1.20 correspond to negative, undetermined, and positive results, respectively. Both RPR and TPPA were tested using Macro-Vue RPR card tests (Becton, Dickinson and Company, Sparks, MD) and SERODIA-TPPA (Fujirebio Inc, Tokyo, Japan), according to the manufacturer’s instructions. According to ITM’s policy, sample leftovers from patients presenting at the ITM polyclinic can be used for research unless the patients explicitly state their objection. All statistics were performed using Analyse-it Software (Leeds, England, UK).

Intrarun precision was evaluated by replicate measurements of patient samples at 3 levels (negative, weak positive, strong positive). Intrarrun variation was determined by analyzing commercial control material (Syphilis TPA Control Level 2; Ortho Clinical Diagnostics) over independent runs (*n* = 16), using 3 different lot numbers. Results are shown in Table 2. No cross-reactions were detected with sera containing antibodies against the spirochetes *Borrelia burgdorferi* (*n* = 5) and *Leptospira* spp. (*n* = 10) and against *Plasmodium* spp. (*n* = 10). Although cross-reactivity with Yaws, Pinta, and others was not evaluated, laboratories should anticipate that this cross-reaction may occur.

Comparison of the TPA assay with RPR and TPPA was performed by testing in a single-run 62 consecutive samples sent to the laboratory for syphilis screening or follow-up. TPA was positive on all 17 TPPA-positive samples (sensitivity 100.0% with 95% confidence interval, 80.5%–100.0%) and negative on 43 of 45 TPPA-negative samples (specificity 95.6% with 95% confidence interval, 84.9%–99.5%). The 2 TPA-positive and TPPA-negative reactions were registered in patients with a history of treated syphilis, suggesting a higher sensitivity of the TPA assay. These results are similar to reports from other CLIs.6–12 The combination of 3 recombinant antigens (TP15,
TP17, and TP47) has been shown to improve diagnostic sensitivity. Cross-reactions with antibodies against TP47 from oral treponemes are, however, possible, and therefore, the specificity of this antigen can be questioned.

Retrospective testing of 158 samples from 20 patients allowed us to evaluate the utility of the TPA assay for assessing the response to therapy. Generally, TPA rose parallel to the RPR and TPPA titers in case of reinfection and TPA and TPPA both decreased after treatment. Results from TPA tended to show less fluctuation than those from TPPA. T. pallidum particle agglutination results are subject to the interpretation of the technician, whereas the TPA assay offers an objective ratio that is easier for follow-up.

After routine implementation of the TPA assay, every sample sent to the laboratory for diagnosis of syphilis was analyzed by RPR and the TPA assay. TPA-positive samples were confirmed by TPPA, unless the previous sample from the patient was TPPA positive. In the period from the 21st of June 2013 to the 31st of October 2013, 2240 samples from 2120 patients were analyzed by TPA on Vitros 5600. Of 2240 samples, 324 (14.5%) and 1648 (73.6%) were reported positive and negative by both RPR and TPA, respectively. Negative RPR and positive TPA were found in 258 samples (11.5%), most of them probably reflecting treated infections, whereas 10 RPR-positive samples (0.4%) were TPPA and TPA negative. Upon investigation of the clinical history of the 10 patients, 1 primary syphilis infection (TPA ratio, 0.16), 1 treated syphilis infection (TPA ratio, 0.75), and 8 possibly false-positive RPR reactions (TPA ratio range, 0.01–0.04) were found. TPA ratios were significantly lower in samples with a negative RPR than those from RPR-positive samples (324 vs. 322) were reactive: one with RPR 1/1 was considered to be false positive and the other was known to belong to a patient with a treated infection (RPR 1/4). We consider that the TPA assay is a valuable confirmatory test in the traditional algorithm.

When using the TPA assay instead of the TPPA as a confirmatory test in the traditional algorithm, 2 additional samples (324 vs. 322) were reactive: one with RPR 1/1 was considered to be false positive and the other was known to belong to a patient with a treated infection (RPR 1/4). We conclude that the TPA assay is a valuable confirmation test in the traditional algorithm. In the reverse algorithm, on a total of 582 TPA reactive samples, 31 (5.3%) showed discordant results (CLIA+, RPR −, TPPA −). This is lower than reported in other studies and can again be explained by the high syphilis prevalence in the study population.

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<th>TABLE 1: Advantages and Disadvantages of Nontreponemal and Treponemal Tests (Loeffelholz, Binnicker, Sena)</th>
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<td><strong>Nontreponemal tests (RPR, VDRL)</strong></td>
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<td><strong>Automated treponemal tests (EIA, CLIA)</strong></td>
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<td><strong>Disadvantages</strong></td>
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*A false-negative response caused by a high antibody titer which interferes with antibody-antigen binding.

**VDRL** indicates venereal disease research laboratory; **TPHA**, *T. pallidum* haemagglutination test; **FTA-Abs**, fluorescent treponemal antibody absorption test.
Overall, when using the traditional algorithm 0.36% (8/2240) of the results were considered to be false positive compared with 0.80% (18/2240) when using the reverse algorithm. The choice for a certain algorithm depends on several factors like disease prevalence, pretest probability, and the need for automation and cost-effectiveness. The higher number of reactive samples in the reverse algorithm could lead to more patient follow-ups and overtreatment. Taking into account the patient’s previous results can partially overcome this drawback. The increase in TPA ratio in patients with reinfections suggests the usefulness of the TPA assay as an ancillary diagnostic tool in this condition. However, more studies are required to confirm this finding.

In conclusion, the TPA assay on Vitros 5600 Integrated System demonstrated an excellent analytical performance for the detection of *T. pallidum* antibodies. The TPA assay can be used for confirmation in the traditional algorithm and is eligible to screen specimens for syphilis in a routine automated laboratory setting when using the reverse algorithm or a combination of TPA and RPR as in our setting.

Figure 1. TPA ratio’s on Vitros 5600 (y-axis) compared with RPR titers (x-axis; A) and TPPA titers (x-axis; B).

Figure 2. Traditional algorithm (A) and reverse algorithm (B) applied to the data collected after implementation in our laboratory. Two of 324 samples were nonreactive by TPPA, of which 1 was a treated infection. For 13 samples, no TPPA was obtained (5%) as previous samples from these patients were already TPA and TPPA positive.
REFERENCES


