# Evaluation of a Highly Sensitive Method of Measuring Syphilis TP Antibody, and a New Approach to Syphilis Testing

Hiroshi SHIBATA, Hidehiko MORIYAMA, Yuki TANIGUCHI, Chikafumi MATSUDA and Atsushi NAGAI

*Central Clinical Laboratory, Shimane University Hospital*
*89-1 Enya, Izumo, Shimane, 693-8501 Japan.*

## INTRODUCTION

Syphilis is a typical systemic sexually transmitted disease that is caused by infection by *Treponema pallidum* (TP), a pathogenic spirochete. Immunoserological tests with serum samples are generally used for testing TP infection. These are typically TP antibody (TP-Ab) assays wherein a TP bacterial component is used as the antigen, and serological test for syphilis (STS) that uses the phospholipid cardiolipin as the antigen.

In recent years, along with the advancement of testing methods, syphilis TP-Ab testing has been automated and there have been considerable advances in improving sensitivity, speed and labor savings. We shall report here the results of basic investigations on TP-Ab measurements using a recombinant TP antigen (TP-Ag) and the automated immunoassay system HISCL-2000*i* (HISCL-2000*i*; Sysmex Corporation) that uses a chemiluminescent enzyme immunoassay (CLEIA), and describe the procedure of syphilis testing that incorporates this highly sensitive assay.

## SAMPLES

A total of 373 samples, which included patients' serum samples that the clinical laboratory of our hospital had received for syphilis testing and serum samples from hospital staff collected for health checkups, were tested. The measuring system and reagent were HISCL-2000*i* and HISCL Anti-TP (HISCL-TP; Sysmex). This is a chemiluminescent immunoassay where the reagent facilitates reaction between the sample (TP-Ab) and a biotinylated TP-Ag. In the next step, there is binding with streptavidin-coated magnetic particles. Then, after bound/free (B/F) separation, the complex is further bound to ALP-labeled TP-Ag. After another B/F separation, the chemiluminescent substrate CDP-*Star* is added and the intensity of luminescence measured.

We used VIRATROL (Sysmex) for examining the accuracy of the measurement, and an enzyme linked immunosorbent assay using TPauto·FS (KW) (ELSIA-TP; Sysmex) as the control for comparing the test results. Samples with disagreement of findings were investigated through testing by TP hemagglutination assay (TPHA) (Fujirebio Inc.), rapid plasma reagin (RPR) carbon antigen test (Dainippon Sumitomo Pharma), and fluorescent Treponema antibody absorption test (FTA-ABS) (Nihon BCG Supply). To compare the reactivity of each reagent after TP infection, we used serum of TP infected rabbits sampled on different days. Lumipulse® ⅡTP-N (Lumipulse®-TP) (Fujirebio), Mediace® TPLA® (TPLA®) (Kyokuto Seiyaku), TPHA (Fujirebio), RPR and glass plate tests for syphilis (glass plate test) (Dainippon Sumitomo Pharma) were used for comparison. The effect of interfering substances was investigated using Interference Check A Plus (Sysmex). Specificity was examined using serum of children (n = 12), pregnant women (n = 8) and myeloma patients (n = 11), and RF positive serum (n = 9). The names of the persons who were the source of the serums used for testing and other investigations were hidden and the serums were used as per the policies of the Japanese Society of Laboratory Medicine.

Note: This article is reprinted from Kensa to Gijutsu 2009; 37 (13): 1472-1474, with kind permission from Igaku Shoin.

## METHODS AND RESULTS

Within-run reproducibility was calculated by analyzing samples of two concentrations (mean 10.8, cut-off index (C.O.I.) 24.7, n = 20) was 2.14 to 2.50 %. The between-day reproducibility (mean 10.4, C.O.I. 23.9, n = 10) determined by similar measurements was 4.50 to 6.28%. The distribution of negative results among 373 samples was not greater than the cut-off value of 1.0. Linearity of dilution result was confirmed up to C.O.I. 16.1 using diluted samples of TP-Ab positive serums. The overall concordance between results of HISCL-TP and ELSIA-TP was 99.1%, with all cases that were positive in ELSIA-TP being positive in HISCL-TP as well, and two of the samples that were negative in ELSIA-TP being positive in HISCL-TP. These two disagreement samples were confirmed by RPR, FTA-ABS, and TPHA. Both of samples were negative in the RPR but positive in FTA-ABS and TPHA.

As for the effect of interfering substances, no interference was observed up to 19.7 mg/dL of bilirubin F, 21.8 mg/dL of bilirubin C, 487 mg/dL of hemolytic hemoglobin, and turbidity of 1,890 Formazin Turbidity Units. HISCL-TP gave no false positive results in any of the samples of 7-month to 11-year old children's serum, serum of pregnant women 11 to 40 weeks into gestation, serum of patients with M proteinemia (IgG 1,939-4,626 mg/dL, n = 6; IgA 250-3,923 mg/dL, n = 3; and IgM 1,225-3,576 mg/dL, n = 2), and RF (86-1,588 IU/mL) positive serum.

When TP infected rabbit serum was analyzed on different days post infection, the results became positive in RPR, glass plate test, HISCL-TP, and TPLA from day 6 post infection, and the titer gradually elevated from that day onwards. The test result with Lumipulse-TP became positive on day 7 post infection and that with TPHA showed a titer of 1:80 on day 11 (***Table***).

## DISCUSSION

Until now, TP-Ab methods and STS methods have been together used to test for syphilis. The reason for using both methods is that while the STS methods give positive results in the early stage of the infection and reflect the course of the treatment well, the TP-Ab methods show positive results after the lapse of a certain period post infection and continue to give positive results for a longer period, thus showing difference from clinical symptoms. The reason for this is that TPHA, which is a low sensitivity agglutination reaction, is used as the TP-Ab method.

However, these days TP-Ab is measured by high sensitivity methods like latex immunoagglutination, enzyme linked immunoassays, chemiluminescent assays, etc. As shown by comparison of the results using TP infected rabbit serum on different days post TP infection, TPLA and HISCL-TP detected the antibody earlier than the conventional TPHA, and as early as RPR and the glass plate test. This is because these two methods can measure the IgM-TP antibodies as well, which is not possible with TPHA. In the this study there were two cases where the results of HISCL-TP and ELSIA-TP did not agree (HISCL-TP (+) and ELSIA-TP (-)). Both these cases were positive in FTA-ABS and TPHA in spite of being negative in STS. Therefore, their weakly positive result for TP-Ab in HISCL-TP is considered to be due to

***Table***

| Post infection days | TP-Ab methods | | | | | | | STS methods | |
|---|---|---|---|---|---|---|---|---|---|
| | HISCL-TP | | Lumipulse-TP | | TPLA | | TPHA | RPR | Glass plate test |
| | C.O.I. | Int. | C.O.I. | Int. | TU | Int. | Titer | Int. | Int. |
| 1 | 0.0 | - | 0.1 | - | 0.0 | - | 0 | - | - |
| 2 | 0.0 | - | 0.1 | - | 0.0 | - | 0 | - | - |
| 3 | 0.0 | - | 0.1 | - | 0.0 | - | 0 | - | - |
| 4 | 0.1 | - | 0.1 | - | 0.0 | - | 0 | - | - |
| 5 | 0.3 | - | 0.2 | - | 1.1 | - | 0 | - | - |
| 6 | 1.2 | + | 0.9 | - | 25.2 | + | 0 | 1+ | ± |
| 7 | 3.6 | + | 3.2 | + | 80.7 | + | 0 | 2+ | 2+ |
| 8 | 5.6 | + | 5.0 | + | 132.5 | + | 0 | 2+ | 2+ |
| 9 | 9.0 | + | 9.4 | + | 291.3 | + | 40 | 3+ | 3+ |
| 10 | 11.4 | + | 11.5 | + | 345.0 | + | 40 | 3+ | 3+ |
| 11 | 12.7 | + | 14.0 | + | 364.0 | + | 80 | 3+ | 3+ |

anamnestic antibodies. The disagreement in the result obtained in HISCL-TP and ELSIA-TP was caused by the difference in sensitivity.

In the conventional TPHA, a TP-Ab titer of 80-fold is regarded as the interpretation for positive. The main reason is that with less than 80-fold titer nonspecific agglutination caused by antibodies other than TP-Ab, such as heterophilic antibodies, occurs. Furthermore, TPHA had the shortcoming of not being able to detect IgM antibodies in spite of using the agglutination reaction[1]. On the other hand, the TP-Ag used in modern automated analysis are either highly purified native antigens or recombinant antigens. Therefore, the chance of nonspecific reaction was very much reduced and highly sensitive measurement became possible.

In recent years, latex reagents for automated analysis by the STS methods have also been developed. However, such reagents are known to give biological false positive (BFP) response and required attention to interpretation. Furthermore, there are some reports of false positive results in syphilis tests because of chyle, plasma fibrinogen, and snake venom-derived coagulation promoters[2], and BFP in persons affected by a regional tropical disease caused by a TP subspecies, non-Treponema spirochete infections, autoimmune diseases including antiphospholipid antibody syndrome, febrile diseases like malaria[3], and women with human immunodeficiency virus (HIV) infection[4]. If high sensitivity TP-Ab methods and the STS methods can both detect the disease in the early stage of infection, we suggest that tests that basically use the TP-Ag, which do not cause the BFP reaction, should be used for diagnosing syphilis.

After the advent of penicillin as the therapeutic drug for syphilis, there has been a drastic reduction in syphilis patients and concern about the reduction of even the number of physicians capable of diagnosing syphilis or having the ability to consider the possibility of syphilis in differential diagnosis. Against this background, serological syphilis tests have greater significance[5]. While the conventional manual method is cumbersome and the results can vary from one analyst to another, the new automated immunoassay system has improved accuracy of measurement. Besides this, the automated system has the advantage that positive/negative assessment of the sample can be done objectively from highly reproducible continuous numerical data, which also facilitates easy understanding of small fluctuations in the antibody titer. On the other hand, with conventional TPHA, serial 2-fold dilution is used from a titer of 80 onwards, and positive/negative assessment is done macroscopically in RPR. Another major advantage of HISCL-TP is that it is capable of rapid high throughput analysis with simultaneous output of reports on other infection-related parameters like HBs antigen, HCV antibody, etc.

## CONCLUSION

TP-Ab testing using the automated immunoassay system HISCL-2000*i* was found to have superior reproducibility and sensitivity, with no abnormal reactions attributable to the sample, and thus the assay system showed good performance. The volume of serum required for the assay is only 20μL, and rapid measurement that can be completed in 17 minutes is possible. Thus, the analyzer system and the reagent are useful not only for routine testing but also for emergency testing for infection.

Until now both TP-Ab methods and STS methods were used for syphilis testing. But with the appearance of highly sensitive reagents on the scene, it is natural that the way of looking at the test results would change. With more and more regional governments now permitting National Health Insurance Scheme cover only for one out of the two tests (TP-Ab or STS) for syphilis testing, it would be clinically effective from now on to first screen with a highly sensitive TP-Ab test and then use STS only on TP-Ab positive individuals for diagnosing syphilis and assessing efficacy of treatment.

***References***


1) *Sato T et al. Research of serodiagnosis for Syphilis - Application of hemagglutination test for specific IgM of Treponema pallidum-. Yamaguchi Igaku. 1995; 34: 483-505, (Japanese).*
2) *Noda M et al. Evaluation of reagents for measurement of Anti-Treponema palladium and Anti-Lipoidal antibodies in serum using a latex agglutination immunoassay on an automatic analyzer. Japanese Journal of Medical Technology. 2003; 52(10): 1279-1282, (Japanese).*
3) *Kojima H. Syphilis. Clinical Microbiology. 2005; 32(1): 57-64, (Japanese).*
4) *Augenbraun MH et al. Biological false-positive syphilis test results for women infected with human immunodeficiency virus. Clinical Infection Disease. 1994; 19: 1040-1044*
5) *Nakamura Y et al. Basic evaluation of "Akyurasu-Auto Anti-TP". J. Anal. Bio-Sci. 2006; 29: 349-354, (Japanese).*