COMPARISON OF THE PERFORMANCE OF SD BIOLINE SYPHILIS 3.0 ASSAY WITH THE RPR TEST FOR THE SYPHILIS SCREENING IN DAR ES SALAAM, TANZANIA

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Summary

Background: World Health Organization in 2003 launched Sexually Transmitted Diseases Diagnostics Initiative (SDI) with mission to promote the development, evaluation and application of sexually transmitted infection diagnostic tests including syphilis screening appropriate for use in primary health care settings in developing countries.

Objective: To evaluate the performance of SD BIOLINE Syphilis 3.0 test (Standard Diagnostics Inc., South Korea) using routine serum samples from blood donors, antenatal clinic attendees and out patients.

Settings: Mwananyamala and Amana District Hospitals, and Muhimbili University College of Health Sciences, Department of Microbiology and Immunology, Dar es Salaam, Tanzania

Methods: A total of 498 serum samples were initially tested on both SD BIOLINE Syphilis 3.0 and rapid plasma reagin (RPR) tests and were then confirmed on Treponema pallidum hemaagglutination (TPHA) test.

Results: The overall seroprevalence of syphilis was 5.6% (28/498) on TPHA, 8.2% (41/498) on SD BIOLINE Syphilis 3.0 and 9.8% (49/498) on RPR tests. The SD BIOLINE test had higher sensitivity (79% vs. 68%) and specificity (96% vs. 94%) compared to RPR test.

Conclusion: The overall sensitivity (79%) of SD BIOLINE syphilis 3.0 test found is low whereas specificity (96%) found is similar compared to the previous evaluation but higher compared to the sensitivity (68%) and specificity (94%) of the currently used RPR test. SD BIOLINE syphilis 3.0 test offers better sensitivity, specificity and test efficiency than the currently used RPR test.

Recommendation: The SD BIOLINE syphilis 3.0 test offers better sensitivity, specificity, test efficiency and operational characteristics than the currently used RPR test and may be adopted for use in syphilis screening in our settings.

Keywords: Screening, confirmatory assay, syphilis, Dar es Salaam, Tanzania

Introduction

Syphilis is a sexually transmitted infection (STI) caused by the spirochetal bacterium *Treponema pallidum*. Clinical diagnosis of syphilis is based on the detection of antibodies to *T. pallidum* by serology. Among the existing immunological method, the confirmatory treponemal tests are the agglutination format such as the *T. pallidum* hemagglutination assay (TPHA) and the immunostaining analysis by fluorescent treponemal antibody adsorption test (FTA-Abs).[1] The ELISA format[2-4] and immuno-chromatographic format (rapid) to detect antibody of *T. pallidum* are also available. Since even highly purified antigens from inoculated *T. pallidum* may contain a certain amount of contaminating materials such as flagella of *T. pallidum*, native *T. pallidum* antigen may cause a nonspecific reaction in the assay of test serum samples, and this may result in lower sensitivity and poor reproducibility. To circumvent these potential problems in immunoassays, researchers have constructed *T. pallidum* genes for the expression of recombinant antigens in bacterium systems such as *E. coli* and focused on *T. pallidum* membrane protein, which are definitely immunogenic. The major immunoreactive antigens of these membrane proteins have been reported to have a MW 15, 17, 42, and 47 kDa based on western blot analysis.

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The SD BIOLINE Syphilis 3.0 (Multi) test (Standard Diagnostics Inc., Kyonggi-do, South Korea) is a solid phase immunochromatographic assay for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) against *T. pallidum*.[5] This test is intended for professional use as an aid in the screening of syphilis. The aim of the current evaluation was to determine the performance of SD BIOLINE Syphilis 3.0 test compared to the confirmatory assay *T. pallidum* hemagglutination (TPHA) (Plasmatec Laboratory Products Ltd, UK) and the currently existing rapid plasma reagin (RPR) test (Span Diagnostics Ltd, Surat, India) using routine serum samples from blood donors, antenatal clinic attendees and out patients in Dar es Salaam, Tanzania

Materials and Methods

This was a field-based evaluation of BIOLINE Syphilis 3.0 test kit that was conducted from February – August 2005. A total of 498 serum samples was used for evaluation; 248 and 250 from Mwananyamala and Amana district hospitals, respectively. All serum samples were tested on SD BIOLINE Syphilis 3.0 and RPR tests in the district hospitals. Confirmation of all samples (reactive and non-reactive) on TPHA (Plasmatec Laboratory Products Limited) was performed in the Dept. of Microbiology and Immunology, Muhimbili University College of Health Sciences, Dar es Salaam. Testing was performed and results were interpreted according to manufacturer's instructions. All sera with reactive RPR and TPHA were regarded as positive samples for *T. pallidum* infection. Sensitivity, specificity, positive and negative predictive values, and test efficiency were determined based on TPHA as the gold standard method.

The SD BIOLINE Syphilis 3.0 test contains a membrane strip, which is pre-coated with recombinant *T. pallidum* antigens (15, 17, 47 KDa) on test band region. The recombinant *T. pallidum* antigens-colloid gold conjugate (15, 17, 47 KDa), patient sample and sample diluent moves along the membrane chromatographically to the test region (T) and forms a visible line as the antigen-antibody-antigen gold particle complex forms. Therefore, the formation of a visible line in the test region (T) indicates a positive result for the detection of *T. pallidum* specific antibodies (IgG, IgA, IgM). When the *T. pallidum* specific antibodies (IgG, IgA, IgM) are absent in the sample, there will be no visible colour band in the test region (T).[5]

The SD BIOLINE Syphilis 3.0 test device has a letter of T and C as "Test Line" and "Control Line", respectively, on the surface of the case. Both the Test Line and Control Line in result window are not visible before applying any samples. The control line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. The SD BIOLINE Syphilis 3.0 test has been

compared with a leading commercial TPHA syphilis test and the overall accuracy of greater or equal to 99%, sensitivity of 99% and specificity of 99.5%.[5]

Results

The overall seroprevalence of syphilis was 5.6% (28/498) on TPHA, 8.2% (41/498) on SD BIOLINE Syphilis 3.0 and 9.8% (49/498) on RPR tests. Seroprevalence of syphilis were 5.6% (14/250) and 5.7% (14/248) on TPHA, 8.4% (21/250) and 8.1% (20/248) on SD BIOLINE Syphilis 3.0 and 8.8% (22/250) and 10.9% (27/248) for Mwananyamala and Amana hospitals, respectively. Sensitivity and specificity were 79% & 68%, and 96% & 94%, whereas positive and negative predictive values, and test efficacy were 54% & 39%; 99% & 94%; and 95% & 92%, for SD BIOLINE Syphilis 3.0 and RPR tests, respectively (Tables 1 and2).

Table 1. Sensitivity, Specificity, Positive and Negative Predictive Values, and Test Efficiency of SD BIOLINE Syphilis 3.0 Test Using Serum Samples from Mwananyamala and Amana District Hospitals (N=498) in Dar es Salaam, Tanzania.

Performance	No.	% (95% CI)
Sensitivity	22/28	79 (59.1 – 91.7)
Specificity	451/470	96 (93.8 – 97.6)
PPV	22/41	54 (37.4 – 69.3)
NPV	451/457	99 (97.2 – 99.5)
TE	22 + 451/498	95 (92.7 – 96.7)

PPV, positive predictive value; NPV, negative predictive value; TE, test efficiency

Table 2. Sensitivity, Specificity, Positive and Negative Predictive Values, and Test Efficiency of RPR Test Using Serum Samples from Mwananyamala and Amana District Hospitals (N=498) in Dar es Salaam, Tanzania.

Performance	No.	% (95% CI)
Sensitivity	19/28	68 (47.7 – 84.1)
Specificity	440/470	94 (91 – 95.7)
PPV	19/49	39 (25.2 – 53.8)
NPV	440/449	94 (96.2 – 99.1)
TE	19 + 440/498	92 (89.5 – 94.4)

PPV, positive predictive value; NPV, negative predictive value; TE, test efficiency

Discussion

World Health Organization (WHO) in 2003 launched Sexually Transmitted Diseases Diagnostics Initiative (SDI) with mission to promote the development, evaluation and application of sexually transmitted infection (STI) diagnostic tests appropriate for use in primary health care settings in developing countries.[6] The current evaluation was aimed at determining the performance of the SD BIOLINE Syphilis 3.0 test kit using routine blood donors, antenatal clinic attendees and out patients' serum samples from Mwananyamala and Amana district hospitals

Overall seroprevalence of syphilis was found to be 5.6% on TPHA and relatively higher on both SD BIOLINE syphilis 3.0 (8.2%) and RPR (9.8%) tests. Recent data based on RPR test showed that seroprevalence of syphilis was 5.9% among HIV-1 infected pregnant women in Dar es Salaam, Tanzania.[7] Treponemal antibody tests such as FTA-Abs, TPHA are known for excellent specificity and sensitivity for syphilis antibodies. FTA-Abs assay is the first test to become positive in early syphilis and usually remains positive many years after effective treatment and thus can not be used to judge efficacy of treatment.[1] WHO estimates that there are 12 million new cases of syphilis each year globally, with more than 90% occurring in developing nations.[8] Undetected STIs have serious consequences such as adverse pregnancy outcomes (stillbirth, preterm delivery, congenital syphilis), pelvic inflammatory diseases, tubal infertility and cervical cancer, and most infections have no symptoms. Congenital syphilis remains a leading cause of stillbirths and death among neonates in many developing countries.[8] A recent report shows that syphilis prevalence based on RPR test among antenatal clinic attendees in Dar es Salaam, Tanzania was found to be 5.1%.[9] The difference in seroprevalence could be due to fact that our evaluation included serum samples from blood donors, out patients as well as antenatal clinic attendees in which biologic false positivity to the reagin tests varies.

Our findings also showed that SD BIOLINE syphilis 3.0 test had higher overall sensitivity (79% vs. 68%) and specificity (96% vs. 94%) compared to RPR test. The sensitivity found in our evaluation is relatively lower compared to the findings reported in Mwanza, Tanzania but specificity is similar.[6] Field evaluation that was conducted at Makongoro clinic in Mwanza as one of the SDI laboratory site to evaluate the performance of SD BIOLINE syphilis 3.0 test among 6 rapid syphilis tests showed sensitivity and specificity (using both serum and whole blood) of 91% and 96%, respectively.[6] The low sensitivity found could be attributed to the low sample size used in our evaluation. Fifty TPHA positive samples (40 RPR +, TPHA +; 10 RPR –, TPHA+) and 50 TPHA negative samples (40 RPR –, TPHA –; 10 RPR + TPHA –) were included in the evaluation in Mwanza. Higher positive and negative predictive values (54% vs. 39%; 99% vs. 94%, respectively) were also found with SD BIOLINE syphilis 3.0 test compared to RPR test. The RPR test had relatively higher number of false positive (30 vs. 19) and false negative results (9 vs. 6) compared to SD BIOLINE syphilis 3.0 test. It is known that nontreponemal antigen tests such as RPR or VDRL are subject to biologic false positive results attributable to the occurrence of reagins (a mixture of IgM and IgA antibodies directed against the cardiolipin-cholesterol-lecithin complex) in a variety of human disorders.[1] Prominent among them are infections such as malaria, leprosy, measles and infectious mononucleosis, vaccinations, and collagen vascular diseases (systemic lupus erythromatosus, polyarteritis nodosa, rheumatic disorders).

Regarding the operational characteristics, compared to RPR test, SD BIOLINE syphilis 3.0 test result is available in less than 15 minutes, can be performed in a single or 2 steps,

requires minimal training and no equipment and it is easy to interpret the result. In contrast, RPR test requires electricity for reagent storage, centrifuge machine to separate serum and shaker, humid atmosphere and training. It has been reported that there are barriers to antenatal syphilis screening in Tanzania using RPR test including procurement, cold chain transport & storage constraints, few trained personnel, and inadequate facilities such as fridge, centrifuge machine, shaker and lighting. WHO/SDI recommend that the ideal test should be affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free and deliverable at primary health care setting.[6]

It is concluded that the overall seroprevalence of syphilis was 5.6% on TPHA, 8.2% on SD BIOLINE Syphilis 3.0 and 9.8% on RPR tests. The overall sensitivity (79%) of SD BIOLINE syphilis 3.0 test found is low whereas specificity (96%) found is similar compared to the previous evaluation but higher compared to the sensitivity (68%) and specificity (94%) of the currently used RPR test. The SD BIOLINE syphilis 3.0 test offers better sensitivity, specificity, test efficiency and operational characteristics than the currently used RPR test and may be adopted for use in syphilis screening in our settings.

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