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Commentary

Modern Methods for the Serological Diagnosis of Syphilis

Dona Xiaoge*

Department of Pathology, Central South University, Yuelu District, Changsha, Hunan, China

DESCRIPTION

Syphilis is a Sexually Transmitted Disease (STD) caused by the spirochete bacterium *Treponema pallidum subsp. pallidum*. Over 11 million cases of adult syphilis are reported worldwide each year. In addition, the disease can be transmitted congenitally affecting 1,500,000 or more infants annually. The course of untreated syphilis infection follows a relapsing course which may span many years. The disease exhibits a variety of clinical presentations, which have been classically divided into four stages. The primary stage of syphilis occurs after an incubation period of 9-90 days, and is usually characterized by the appearance of a single painless ulcer at the site of inoculation. If left untreated, this primary lesion resolves spontaneously with the majority of patients subsequently developing secondary syphilis.

The secondary stage of infection is characterized by rash-like skin lesions that can cover part or all of the body including the soles of the feet and palms of the hands. The rash normally appears 1-6 months after the onset of the primary lesion. Thereafter, the rash will usually resolve spontaneously without treatment and the patient has no apparent signs or symptoms of infection. This period of latency can last many years before development of the late, destructive stage of infection, namely tertiary syphilis. Tertiary syphilis is the final phase of untreated infection. It is characterized by widespread lesions of the skin, bones, and internal organs. Ultimately, severe neurologic and/or cardiovascular lesions may cause possible dementia, insanity, blindness, and sudden death from cardiovascular complications.

The mainstay of syphilis screening and diagnosis is serological testing. The immune response to the disease involves the production of two distinct antibodies, namely non-treponemal antibodies which recognize lipoidal material released from damaged host cells and treponemal antibodies which are specific for *T. pallidum* antigens. At the present time, the detection of both non-treponemal and treponemal antibodies require the use of specialized equipment, with testing being performed in a laboratory by trained technicians. In most settings, the test results may not be available on the same day that the specimen has been collected.

Currently-available laboratory based non-treponemal (cardiolipin) tests include the Venereal Disease Research Laboratory (VDRL), Unheated Serum Reagent (USR), Toluidine Red Unheated Serum (TRUST) and the Rapid Plasma Reagent (RPR) card tests. The VDRL and USR tests require the use of a microscope to visualize a flocculation reaction that occurs when reactive sera are mixed with cardiolipin-containing antigen, while the TRUST and RPR are macroscopic flocculation tests. (4-6) the antigen used in the RPR test is prepared from a modified VDRL antigen suspension containing choline chloride, EDTA and fine charcoal particles as the visualizing agent. All these non-treponemal tests are labor intensive and could be considered subjective. The treponemal tests include the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) test, the Treponema Pallidum Hemagglutination Assay (TPHA), the Treponema Pallidum Passive Particle Agglutination Test (TP-PA), Enzyme Immunoassays (EIAs), Chemiluminescence Assays (CIA) and Immunochromatographic Point of Care (IPOC) assays for specific antibodies to *Treponema pallidum*.

Serum samples from suspected patients are often sent to laboratories for analysis where they are initially screened with a non-treponemal test such as the RPR or VDRL. The reactivity of the sera is then confirmed using a treponemal test such as an FTA-ABS test, TPHA, TP-PA or EIA. If both the non-treponemal and treponemal tests show reactivity, then the patient is counselled and treatment provided.

However, since there is often a considerable time lag in obtaining the results, the patient may not return to the clinical site for treatment. In such cases, not only does the patient remain untreated, but there are opportunities for further spread of the disease. Because syphilis is a disease of worldwide distribution and of public health significance, a rapid point-of-care test that can be used to screen and confirm the serological status of an individual attending an STD or antenatal clinic in resource-poor settings, would allow them to be counselled by the

Correspondence to: Dona Xiaoge, Department of Pathology, Central South University, Yuelu District, Changsha, Hunan, China, E-mail: donax@gmail.cn

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physician as to whether they have active infection that would require immediate treatment before leaving the premises.

CONCLUSION

At present, there is no dual point-of-care test available which is capable of screening and confirming the results on the same device. The goal of the studies reported in this thesis has been to develop and optimize two rapid point-of-care tests for syphilis, capable of detecting both non-treponemal and treponemal antibodies, thus serving as both a screening and confirmatory test for the disease. This is a 3 approach long advocated by the World Health Organization (WHO). The tests developed during the course of these studies have proved simple to perform, require no expertise in interpretation of results and are capable of being read within 2 to 15 minutes.

These tests are ideal for use in field epidemiological studies where laboratory facilities are not available and where a rapid serological diagnosis is important. Two formats of the rapid point-of-care test have been designed, namely an immunofiltration (flow-through) and immunochromatographic (lateral flow) device to be used with whole blood or serum/plasma samples. This thesis also describes the development and optimization of two new laboratory-based tests, an Enzyme Linked Immunosorbent Assay (ELISA) and a multiplex chemiluminescence assay which have also been designed for the simultaneous detection of non-treponemal and treponemal antibodies. These tests have the advantage that they can be fully automated and would be ideal for use in high volume laboratories.

At the present time, there are no points of care or ELISA/chemiluminescence tests capable of detecting non-treponemal antibody anywhere in the world. Viable tests such as those described in this thesis are urgently needed to alleviate both the scourge of adult syphilis and to aid the congenital syphilis elimination efforts of the World Health Organization.