

Review Article

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## Can a Duplex Non-treponemal and Treponemal Serological Test Help in Improving Syphilis Testing?

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### ABSTRACT

Treponemal and non-treponemal serological tests are the most commonly used for syphilis diagnosis. The traditional syphilis testing algorithm recommends the screening of samples with a non-treponemal test. Whereas, the reverse and European Centers for Disease Control (ECDC) testing algorithms advocate the use of a treponemal test for syphilis screening. The popularity of treponemal tests has recently gained interest due to their availability in the automated test format. In contrast to that, non-treponemal tests are not available in such format. Though, their usefulness cannot be underestimated. They are very helpful in determining active syphilis infection, and monitoring the progress of treatment. Hence, combing both different tests on a single platform can be very useful to those laboratories that are engaged with huge number of sample testing. Such test could possibly be designed in a duplex chemiluminescent immunoassay (CLIA) system. It can aid in improving routine syphilis screening with the advantage of fast testing, better result interpretation, and a potential for automation. In addition to that, it can satisfy the requirements of described testing algorithms. However, this duplex test needs to have sensitivity, and specificity equivalent to currently used serological tests for advancing its further use in syphilis diagnosis.

### Keywords

Syphilis,  
Treponemal test,  
Non-treponemal  
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## Introduction

Syphilis is a disease caused by the bacterium *Treponema pallidum* subspecies *pallidum*. It belongs to the *Spirochaetaceae* family and is gram-negative, helical shaped, slender, motile bacteria. This disease is transmitted majorly through sexual route. However, other modes are also possible for its transmission and includes from syphilis infected mother to her fetus while in the uterus or at the time of birth, rarely through blood transfusion, and may through non-sexual contact (Goh, 2005).

Syphilis is known for many centuries. An Italian war of 1495 (the diseases of Naples) was the reported earlier evidence. Many incidences were reported thereafter throughout the world (Frith, 2012). The origin of this disease is mysterious, and few hypotheses were proposed to understand it (Singh *et al.*, 1999). The original Columbian hypothesis described the endemic presence of syphilis in the new world, and later was transferred to the old world through

Columbus crew. The other hypothesis, pre-Columbian, stated central Africa as the place of origin for syphilis, and afterward was introduced to Europe before Columbus voyage. These two are the main theories proposed for syphilis origin. However, the Unitarian theory described that venereal and non-venereal treponematoses were derived from the single disease, and syphilis etiological agent was considered as a variant originated due to the social and environmental condition of the late 15<sup>th</sup> century that favored its extensive transmission through sexual contact (Singh *et al.*, 1999 and Frith, 2012). The origin of the term syphilis has an interesting history too. Earlier it was known by wide variety of names. Most were linked with the name of different countries that believed to be responsible for its spread. Later, Girolamo Fracastoro coined the term syphilis in 1530, and narrated it in his epic poem called, 'syphilis, sive morbus gallicus' (Frith, 2012).

A syphilis infection progresses through multiple stages (LaFond *et al.*, 2006). A primary stage is characterized by the appearance of chancre, and is normally found on the genital organ. However, it can be observed on the other body sites such as oral cavity, anal canal, and rectum too. After bacterial exposure, it takes about 10 to 90 days of incubation time for its appearance (LaFond *et al.*, 2006). During this period, bacteria invade different tissues and give rise to a symptomatic stage called secondary syphilis. The appearance of symptoms like skin rash, muscle pain, sore throat, and malaise are common during this stage. Within three months, the symptoms of secondary syphilis heal spontaneously, and are followed by an asymptomatic latent stage. This stage can be divided into an early or late latent syphilis depending upon the duration of exposure (Singh *et al.*, 1999).

Antibiotic treatment is effective in curing this stage. However, a failure to treatment advances the infection further to the tertiary stage, which is the last stage. Gummatous syphilis, cardiovascular syphilis, and neurosyphilis are the outcome of the untreated infection.

Syphilis is categorized as a curable type of sexually transmitted disease due to the availability of the effective treatment options (Gottlieb *et al.*, 2014). Penicillin is the most efficient in treating all the stages of infection. It is also useful in treating congenital syphilis. However, in case of penicillin allergy, other optional antibiotics such as ceftriaxone, erythromycin, and azithromycin are recommended (Gottlieb *et al.*, 2014; Kingston *et al.*, 2008; Hook *et al.*, 2002; Riedner *et al.*, 2005 and Blandford *et al.*, 2003). Azithromycin is useful in treating syphilis due to its easy oral administration, and this approach is useful in resource-limited settings (Blandford *et al.*, 2003).

### **Diagnosis of Syphilis**

Different techniques are used for the syphilis diagnosis. Direct microscopy from lesion, serological tests, and direct antigen detection test are the major types (Larsen *et al.*, 1995). Among all, serological tests are the most frequently used (Ratnam, 2005). They can be grouped into non-treponemal and treponemal types (Table-1). A non-treponemal group of test deals with the detection of the non-treponemal antibody. It is produced in response to the lipid component (cardiolipin) that is thought to be derived from the damaged host cells, and from bacteria (Ratnam, 2005). It is believed that the cardiolipin is included in the outer membrane of bacteria from the host during infection (Fraser *et al.*, 1998). The Venereal Disease Research Laboratory (VDRL) slide test, Unheated Serum Reagin (USR) test,

Rapid Plasma Reagin (RPR) test, and Tolidine Red Unheated Serum test (TRUST) are the examples of non-treponemal tests. They all in common use an antigen suspension made up of cardiolipin, lecithin and cholesterol, and can detect IgM and IgG classes of non-treponemal antibodies. The presence of a non-treponemal antibody in a suspected syphilis case is an indicator of an active infection, and can aid in diagnosis (French *et al.*, 2009). A semi-quantitative non-treponemal test can be used to determine the end-point titer of a syphilis reactive sample. A recent infection can raise the non-treponemal antibody titer, and a relapse or re-infection can give the persistent titer. A decline in antibody titer indicates recovery from the infection, and it might be due to the effectiveness of treatment (Ratnam, 2005). However, non-treponemal tests have some limitations too. A reactive sample containing large number of non-treponemal antibody can show prozone reaction. It may give a false negative result when tested undiluted (Jurado *et al.*, 1993). Some diseases like malaria, hepatitis, viral pneumonia, chicken pox, systemic lupus erythematosus, and others can give false positive result with non-treponemal tests (Ratnam, 2005). Hence, finding obtained with a non-treponemal test needs further confirmation with a treponemal specific test (CDC, 2008). This group of test includes *T. pallidum* Hemagglutination Assay (TPHA), *T. pallidum* Particle Agglutination Assay (TP-PA), Fluorescent Treponemal Antibody Absorption assay (FTA-ABS), and treponemal antibody specific Enzyme Immunoassay (EIA) tests. These treponemal-specific tests are very sensitive and demonstrated their usefulness in diagnosing early, late or latent stage of syphilis infection (Loeffelholz *et al.*, 2011 and Mishra *et al.*, 2011). However, screening by a treponemal test can give false

positive results in successfully treated cases (Ratnam, 2005). It might be due to the long presence of treponemal specific antibody. Hence, a result obtained with the treponemal specific test needs further confirmation with a non-treponemal test (Seña *et al.*, 2010).

### **Dilemma on Syphilis Testing**

Serodiagnosis of syphilis is dependent on the acceptance of testing algorithm. The traditional algorithm recommends screening by a non-treponemal test. If the sample shows a positive result, then it is confirmed with a treponemal test (CDC, 2008). This practice has been in effect since many years and seems to be very useful so far. The current reverse algorithm is the opposite of this practice. It prefers a treponemal test for screening, and a non-treponemal test is used later for confirmation (Seña *et al.*, 2010). This practice is gaining importance due to the high sensitivity, and test availability in the automated format of the treponemal tests. Another type of screening practice reported recently called the ECDC algorithm (Tong *et al.*, 2014). It uses a treponemal test for screening, and obtained finding is confirmed with another different treponemal test. A non-treponemal test can be used later for monitoring the effectiveness of treatment. All algorithms with their preference for screening and confirmatory test selection are summarized in Table-2.

Out of three testing algorithm described above, which one is better is subject of concern. To answer that, Tong et al. did a study with 24,124 serum samples with all described syphilis testing algorithms. Their study concluded the use of ECDC algorithm as a suitable practice, which begins with a treponemal test for syphilis screening. Contrary to that, Binnicker et al. showed concern on the use of treponemal test (reverse practice) for syphilis screening as it

gave false positive results in low-syphilis prevalence populations in their study (Binnicker *et al.*, 2011). Hence, selection of a screening test is a subject of debate. It may have the impact on syphilis diagnosis. This underscores the need for improving syphilis testing with a duplex test that can fulfill the requirements of different testing algorithms.

### **Obstacles in Developing Automated Non-Treponemal Tests**

EIA and CLIA formats are the most suitable test model that can be automated easily. However, insolubility of non-treponemal cardiolipin antigen in aqueous solvent restricts its further use with such test system. Few studies were reported with the non-treponemal EIA test development. Pedersen *et al.*, (1987) reported an EIA development study with VDRL antigen. An ethanolic solution containing 0.03% (Wt/Vol) cardiolipin, 0.09% (Wt/Vol) cholesterol, and 0.021% (Wt/Vol) lecithin was evaporated on microplate surface. This antigen coated microplate was later used in test system. Described test was able to detect IgM and IgG classes of antibody to the coated VDRL antigen. Their report claimed an equal sensitivity, and specificity of VDRL EIA to the traditional non-treponemal tests. A second report was described based on urease enzyme based non-treponemal VDRL EIA. It was described to be capable of detecting IgG class of antibody (White *et al.*, 1989).

A recent study described a new method in which cardiolipin was oxidized and subsequently conjugated to different molecules like biotin, BSA (Bovine serum albumin), KLH (Keyhole limpet hemocyanin), and others (Castro *et al.*, 2011). Such prepared cardiolipin conjugates were described to be useful in lateral flow test development. A report by Shukla *et al.*, (2015) showed a method for the covalent attachment of cardiolipin to the microplate

directly and was studied in EIA system. The same antigen coating method can also be employed with CLIA platform. Hence, described studies open the gate for the non-treponemal test development with the possibility of automation.

Non-treponemal RPR and VDRL tests are in use for syphilis screening since long. They are easy to perform, cheap, available from different commercial sources, and useful in monitoring syphilis treatment effectiveness (Ratnam, 2005). However, they are not available in the automated format, and obtained results are read visually which can be subjective (Binnicker *et al.*, 2011). A digital flocculation reader was reported that was described to be useful in reading RPR result (Castro *et al.*, 2012). However, still a manual method is followed for sample testing. In contrast to that, EIA test format eliminates the test result subjectivity as a machine reads the result (Pedersen *et al.*, 1987). The testing protocol of an EIA can be followed easily on the automated testing system like DS2 (Dynex Technologies, USA), and can be handy in controlling inter-site testing variability (Gorton *et al.*, 2015). Hence, described obstacles of traditional non-treponemal tests can be overcome with EIA or CLIA test format.

### **Role of Automated Duplex Test in Improving Syphilis Testing**

The screening of syphilis can be made fast by using an automated test format. This approach is the most suitable for those laboratories with a huge workload. Treponemal tests are available in the automated format, and had already proved their high efficiency for syphilis screening (Donkers *et al.*, 2014). Similarly, the availability of a sensitive and specific automated non-treponemal test can help in improving syphilis testing. Both groups of tests are valuable in syphilis diagnosis.

**Table.1** Serological tests for syphilis

Test types	Tests*
Non-treponemal type	VDRL, USR, RPR, TRUST
Treponemal type	TP-HA, TP-PA, FTA-ABS, EIA

\*Refer to text for the abbreviations used in table.

**Table.2** Syphilis screening algorithms

Algorithm	Screening test	Confirmatory test
Traditional	Non-treponemal	Treponemal
Reverse	Treponemal	Non-treponemal
*ECDC	Treponemal	Treponemal

\*Refer to text for the abbreviations used in table.

**Table.3** Possible interpretation scheme for automated duplex syphilis CLIA test

Duplex syphilis CLIA test		Interpretation
Treponemal spot	Non-treponemal spot	
Positive	Positive	True syphilis case
Negative	Negative	True Negative case
Positive	Negative	Need further testing with a different treponemal test (CDC, 2011)
Negative	Positive	Biologically false positive case

Therefore, the availability of an automated duplex treponemal and non-treponemal serological test can aid in improving syphilis screening practice. A test can be optimized in the CLIA test format at where microwell can be coated with non-treponemal and treponemal antigen as a separate spot. A report is available on the use of the multiplex EIA platform for the multiple analyte study (Liew *et al.*, 2007). A similar platform can be utilized for the syphilis duplex test development. This test system can have several advantages. It can be sensitive, economical, reduce time of testing, and workload. Additionally, such test is capable of detecting both non-treponemal and treponemal antibody together. Hence, its availability in future can be very useful and may avoid dilemma on the testing algorithm selection. Such proposed test can screen, and confirm syphilis cases simultaneously. In case of

discrepant test result, a different treponemal test such as TP-PA can be used for further verification (CDC, 2011). The possible scheme of result interpretation with the automated duplex syphilis CLIA test is shown in Table-3.

In India, RPR or VDRL tests is used for syphilis screening (Bala *et al.*, 2013). Whereas, in the United Kingdom and Europe treponemal tests are recommended for the same (Seña *et al.*, 2010 and Naidu *et al.*, 2012). This indicates that syphilis testing practice is not uniform globally. Factors like test cost, availability, and test sensitivity (Zhang *et al.*, 2012) should also be taken into account while advocating the test use. Non-treponemal tests are very economical but lack the high sensitivity of treponemal tests. On the other hand, treponemal tests have the risk of getting false positive result in a population with low-prevalence of

syphilis (Binnicker *et al.*, 2011). The verification of the screening test result with another test is a time consuming and costly approach. Hence, for fast, economical, and efficient syphilis testing, a duplex non-treponemal and treponemal serological test could be an ideal solution. Such suggested format is possible with CLIA system, and it has potential for automation. However, more studies are required to make the proposed duplex test available for syphilis diagnosis.

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