ORIGINAL ARTICLE

# Comparative analysis of the Traditional, Reverse and European Centre for Disease Prevention and Control algorithms and utilization of WHO NET software for syphilis serodiagnosis in a tertiary care hospital in Chengalpet, South India

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**Abstract**

*Background:* Syphilis is a Sexually Transmitted Disease (STD) caused by *Treponema pallidum* with significant public health importance. Based on the clinical presentation syphilis is classified into primary, secondary, tertiary and latent syphilis. Lab diagnosis of syphilis can be done by serological methods using specific treponemal and non-treponemal tests. Testing strategies like traditional, reverse and European Centre for Disease Prevention and Control (ECDC) are currently used as principal diagnostic algorithms. *Aim and Objectives*: To compare the seropositivity among three diagnostic algorithms for syphilis serodiagnosis and data analysis using WHO NET software. *Material and Methods*: All the samples received for syphilis serology were tested by Rapid Plasma Reagin (RPR) and rapid immunochromatographic card methods. Three diagnostic algorithms were used. In traditional algorithm, the sample was first tested with RPR (non-treponemal test) and if reactive then interpreted as a case of syphilis. In reverse algorithm, a specific treponemal card test was done and if reactive a second non treponemal test like RPR was performed. A second rapid immunochromatographic test was also performed. In the case of the ECDC algorithm two specific card tests were performed and interpreted. WHO NET software was utilized for data entry and analysis. The coefficient of agreement (k value) was analyzed by using QuickCalcs GraphPad software. *Results*: A total of 1292 samples were tested, and 4 (0.3%) samples were reactive by traditional algorithm. By reverse and ECDC algorithm 14 (1.08%) of the samples were considered as reactive. Overall, four samples were identified as cases of definite/current syphilis and ten samples were considered as cases of probable early current / latent or treated syphilis. Reverse algorithm and ECDC algorithm detected more cases. The missed diagnosis percentage was 10 (71.42%) for traditional algorithm. Antenatal patients comprised of 334 (25.85%) samples with seropositivity of 0.29%. Maximum samples were received from the age group of 21 to 30 years 484 (37.46%). Strong degree of agreement was observed between reverse and ECDC algorithm (k value- 0.832). *Conclusion:* Reverse or ECDC algorithm can be implemented in the laboratory routinely to avoid missing cases of early syphilis. The specific treponemal card tests available in immunochromatography formats are easy to perform and interpret. The usage of WHO NET for analysis of data and along with including specific comments individualized for each patient in the lab reports could give valuable insight.

**Keywords:** Syphilis, Seroprevalence, Diagnostic algorithms, Immunochromatograhy, WHO NET

## Introduction

Syphilis is a Sexually Transmitted Disease (STD) caused by *Treponema pallidum* with significant public health importance. Based on the clinical presentation syphilis is classified into primary, secondary, tertiary and latent syphilis [1-2]. Latent syphilis is defined as syphilis characterized by seroreactivity without other evidence of disease. Centre for Disease Control and Prevention (CDC)

recommends to treat latent syphilis especially among antenatal patients. Delayed diagnosis or treatment can lead to serious consequences in pregnancy and congenital malformations [3].

Around six million patients in the age group of 15 to 49 years are newly diagnosed with syphilis every year [4]. Syphilis leads to more than 3 lakh deaths among the fetal and neonatal age group [4-5]. By 2018 to 2030, World Health Organization (WHO) has targeted a 90% reduction in *Treponema pallidum* and *Neisseria gonorrhoea* infections and also less than 50 cases of congenital syphilis in eighty percentage of the nations. Though there was a decline in cases of syphilis due to the preventive measures initiated against HIV infection, cases of syphilis are still reported in certain population group like Men having Sex with Men (MSM) highlighting the need for continuing surveillance [3, 6]. Sethi *et al*. and Kulkarni *et al*., have showed an increase in the prevalence of this infection (0.95% to 1.76% in 6 years) and (0.7% to 1.3%), respectively, indicating the need for continued monitoring of this infection [7-8].

Lab diagnosis of syphilis can be done by serological methods using both specific treponemal and non-treponemal tests. Different testing strategies like traditional algorithm and reverse algorithm are put forth by agencies like CDC, WHO and ECDC and are currently used as principal diagnostic algorithms for diagnosing syphilis [9-15].

All these three algorithms have their own advantages and disadvantages. Syphilis can have varied presentation among patients and hence clinicians rely on serological tests. There are only a few studies that have compared the different diagnostic algorithms for diagnosing syphilis [9-10].

WHO NET is a laboratory data management software for clinical microbiology lab introduced by WHO [16]. Though it is primarily utilized for antimicrobial resistance monitoring and surveillance, this software can also be used for configuring serological testing like syphilis testing. It is important that the laboratory chose the appropriate algorithm to avoid missing diagnosis, a methodology that is easy to perform and interpret. This study was undertaken to compare the traditional, reverse and ECDC testing algorithms, and to analyse the degree of correlation among these diagnostic strategies along with the usage of WHO NET software for data entry and analysis.

**Material and Methods**

This cross-sectional study was conducted in the Department of Microbiology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Chengalpet from September 2021 to April 2022.All serum samples received for syphilis serology were tested by the traditional, reverse and ECDC algorithms. The study was approved by the Institutional Ethics Committee.

**Traditional testing algorithm**

In the traditional testing method, the serum samples were tested by Rapid Plasma Reagin (RPR) test, a nonspecific treponemal test. Samples with reactive results were considered as positive for syphilis and non-reactive samples were considered as negative. In case of reactive results, further semiquantitative testing was performed for titres. RPR test is a flocculation test. The nonspecific reagin antibody, if present in the patient's serum, forms floccules on combining with the carbon coated RPR antigen. The test results were interpreted as reactive, weak reactive and non-reactive [9-14].

**Reverse testing algorithm**

In case of reverse testing strategy, all the serum samples were tested with a rapid specific treponemal card test (Modified TPHA) (Syphicheck-WB). In rapid syphicheck-WB test, IgM and IgG class of antibodies against 47 kDa and 17 kDa are detected. All the serum samples showing reactive results (presence of pink to deep purple colored band) were reflexively tested for RPR. The samples showing reactive results from RPR were further tested semi quantitatively for RPR titres. All the samples with non-reactive RPR results were again checked with a second qualitative membrane based immunoassay (Immunopack syphilis card -Reckon diagnostics India). In Immunopack test, recombinant syphilis antigen (17 KDa, 15 KDa, 47 KDa) was used. This cassette test detects treponemal antibodies (IgA, IgM, IgG) against 17 KDa, 15 KDa, 47 KDa treponemal antigens. Presence of pink-purple line in the test region was considered as positive.

Samples showing reactive results with the specific treponemal card test and reactive RPR were considered as definite cases of syphilis. Those samples showing reactive results with only the first treponemal card test and negative RPR were again tested with a second card test. Those samples showing reactive results with both the Syphilis card tests and non-reactive RPR were considered as cases of probable syphilis with one of the following interpretation: Probable early, latent or past treated cases [9-14].

**ECDC Algorithm [13-15]**

As per ECDC guidelines, first a treponemal test (Syphicheck-WB) was performed and if found to be reactive (presence of pink to deep purple color band), it was further confirmed using a second treponemal test: Immunopack syphilis card - Reckon diagnostics India, a qualitative membrane based immunoassay. Samples showing reactive results with both the trepopnemal card tests were considered as likely cases of syphilis. Samples with reactive result with only the first treponemal test were interpreted as cases of probable syphilis with one of the following interpretation: probable early, latent or past treated cases.

**Statistical analysis**

All the results were entered in WHO NET software. The positivity percentage of the three algorithms were compared and differences in the detection of reactive results was analysed by WHO NET software through generation of excel sheet [16]. Demographic data like age sex and ward wise data were analysed. The coefficient of agreement 'k' between the testing strategies was analyzed by using QuickCalcs GraphPad software.

**Results**

Out of the 1292 serum samples received for syphilis serology testing, an overall 1.08% (n=14) seropositivity was observed (Table 1). The age, sex and demographic data were analysed using WHO NET software (Table 2). Majority of the samples were from female patients 929 (71.9%). Out of the 1292 samples, 929 (71.9%) were received from obstetrics and gynecology followed by general medicine 460 (35.6%). Antenatal patients comprised 334 (25.85%) of the total samples with majority from the age group of 21 to 30 years 484 (37.46%) (Table 2). By traditional algorithm, 4 (0.3%) of the samples were reactive. By reverse

**Table 1: Seroprevalence of syphilis**

| Total samples tested | Syphilis definite | Probable syphilis (early / current / latent / treated syphilis) | Seroprevalence |
|---|---|---|---|
| 1292 | 4 | 10 | 14 (1.08%) |

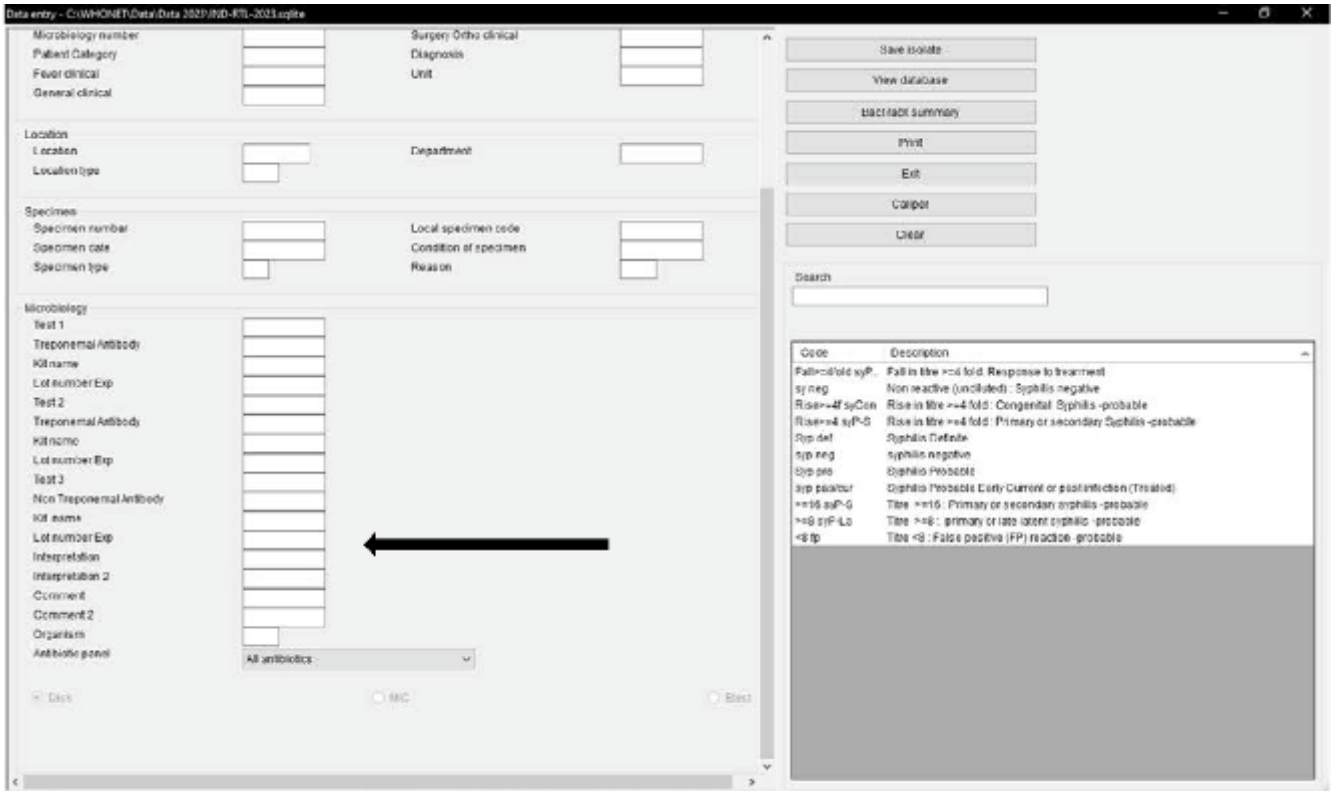


**Figure 1: WHO NET-DATA ENTRY-Patient demographic information with interpretation and comment**

testing algorithm, 14 (1.08%) of the samples were considered as reactive for syphilis.

Out of this fourteen, 4 samples were also RPR reactive with titres > 1:8 and 10 samples were reactive with a second treponemal card test. Four samples that showed reactive with both card and RPR were identified as cases of definite / current syphilis (Table 3). Ten samples non-reactive for RPR and reactive for second card test were considered as cases of probable early current / latent or treated syphilis. By ECDC algorithm, 14 samples were reactive with the first specific treponemal card tests. Out of 14, 10 samples reactive by second treponemal card test were considered as cases probable early current / latent or treated syphilis and 4 samples reactive by RPR were considered as definite cases of syphilis (Table 3).

**Table 2: Demographic data**

| Demographic data | Number (%) |
|---|---|
| **Gender distribution (n=1292)** | |
| **Male** | 363 (28.09%) |
| **Female** | 929 (71.90%) |
| **Age distribution (n=1292)** | |
| **0-20** | 85 (6.57%) |
| **21-30** | 484 (37.46%) |
| **31-40** | 289 (22.36%) |
| **41-50** | 226 (17.49%) |
| **51-60** | 122 (9.44%) |
| **61-70** | 65 (5.03%) |
| **71-86** | 21 (1.62%) |
| **Department / Ward wise data (n=1292)** | |
| **Obstetrics and Gynaecology** | 613 (47.44%) |
| **General medicine including cardiology and pulmonology** | 460 (35.60%) |
| **Emergency ward** | 65 (5.03%) |
| **General surgery including Orthopaedics** | 46 (3.56%) |
| **Intensive care unit** | 32 (2.47%) |
| **Paediatrics** | 29 (2.24%) |
| **Dermatology** | 33 (2.55%) |
| **Others (Otolaryngology, Ophthalmology, Psychiatry)** | 14 (1.08%) |

**Traditional vs reverse algorithm (Table 3)**

The reverse testing algorithm detected 14 samples as reactive where as traditional algorithm identified only 4 cases as reactive. Ten patients were probable early, current /latent or treated syphilis and these cases would have gone undetected if testing were done only by traditional algorithm.

**Traditional vs ECDC algorithm (Table 3)**

In ECDC algorithm, 10 samples were reactive by both treponemal card tests. Reverse algorithm and ECDC algorithm detected more cases of syphilis compared with traditional algorithm. The missed diagnosis percentage was 10 (71.42%) for traditional algorithm when compared with reverse algorithm. ECDC algorithm had a missed diagnosis percentage of 4 (28.57%). Among the 334 antenatal cases, 1 (0.29%) patient in the third trimester was identified as a definite case of syphilis.

**Statistical analysis**

The coefficient of agreement 'k' was 0.468 for traditional vs reverse algorithm indicating moderate agreement between both the testing strategies. Whereas for reverse algorithm and ECDC algorithm the coefficient of agreement was 0.832 indicating strong agreement (Tables 4 and 5).

**Table 3: Comparative analysis of seropositivity: Traditional vs reverse vs ECDC algorithm**

| Testing method (N=1292) | Samples with reactive results (N=14) | Samples with non-reactive results (N=1279) | Syphilis likely (N=1292) | Syphilis definite / current (N=14) | Probable syphilis early / treated / latent (N=14) | Total positivity (N=1292) |
|---|---|---|---|---|---|---|
| **Traditional algorithm** | | | | | | |
| **First test (method-RPR)** | 4 | 1288 | 4 (0.30%) | 4 (100%) | 0 | 4 (0.3%) |
| **Reverse algorithm** | | | | | | |
| **First test specific treponemal test immune-chromatography (Syphicheck-WB) Modified TPHA** | 14 | 1279 | 14 (1.08%) | 4 (28.5%) | 10 (71.42%) | 14 (1.08%) |
| **Second test RPR** | 4 | - | | | | |
| **Second specific treponemal test immunopack syphilis card test** | 10 | 4 | | | | |
| **ECDC algorithm** | | | | | | |
| **First method specific treponemal test (Syphicheck-WB) Modified TPHA** | 14 | 1279 | 14 (1.08%) | 4 (28.5%) | 10 (71.42%) | 14 (1.08%) |
| **Second specific treponemal card test Immunopack syphilis card test** | 10 | 4 | | | | |
| **RPR test** | 4 | 6 | | | | |

**Table 4: Degree of agreement 'k' value for traditional vs reverse testing algorithm**

| Algorithm | Traditional algorithm | | Total | Kappa value |
|---|---|---|---|---|
| **Reverse algorithm** | **Reactive** | **Non-reactive** | | |
| **Reactive** | 4 | 10 | 14 | 0.442 |
| **Non-reactive** | 0 | 1278 | 1278 | Moderate agreement |
| **Total** | 4 | 1288 | 1292 | |

**Table 5: Degree of agreement 'k' value for traditional vs ECDC testing algorithm**

| Algorithm | ECDC Algorithm | | Total | Agreement Kappa |
|---|---|---|---|---|
| **Reverse testing algorithm** | **Reactive** | **Non-reactive** | | |
| **Reactive** | 10 | 4 | 14 | 0.832 |
| **Non-reactive** | 0 | 1278 | 1278 | Strong agreement |
| **Total** | 10 | 1282 | 1292 | |

**Discussion**

Syphilis is a disease of great public health importance and serodiagnosis is the commonly used mode of laboratory diagnosis. Different diagnostic algorithms have their own advantages and disadvantages [9-14, 17]. Traditional algorithm can miss very early cases and prozone phenomenon can interfere with the accuracy of interpretation of results [14]. In high-risk population, treponemal tests can give high rates of positivity [12]. Reverse algorithm and ECDC algorithm have the advantage of detecting cases earlier. There are only a few studies that compare the positivity rate and degree of agreement between these different strategies.

In a study by Solaimali *et al*., a significant increase in positivity was observed from 0.52 to 2% over 5 years period (2015 to 2020) in India [6-8]. This indicates the necessity for continual monitoring among the high-risk population. In developing countries, with programs in place for HIV prevention and control, the increase in the prevalence of syphilis was ascribed to alterations in the behavior of population [6, 18]. A change in vaginal flora because of contraceptive usage can also lead to genital tract infections [19]. In this study, all the three diagnostic algorithms were compared and immunochromatography formats of card tests with specific treponemal antibodies, which are easy to perform and can be used as a point of care testing, were used. Early diagnosis by choosing the appropriate algorithm and creating awareness are important among the public and high-risk population to prevent transmission.

Our finding of traditional algorithm having missed diagnosing 10 (71.42%) cases was in concordance with the other studies [7-8, 18]. Sethi *et al*., and Kulkarni *et al*., have documented that reverse algorithm was able to identify a greater number of positives by screening like the current study [7-8].

Latent syphilis is diagnosed when there are serologically reactive results without clinical evidence of syphilis [2, 11]. In the present study, reverse algorithm and ECDC algorithm were able to identify 10 (0.77%) of cases of early /current / latent or treated syphilis. It is very important to follow-up on such cases and perform thorough clinical evaluation as this chronic infection can be easily treated [9]. Reverse algorithm is more sensitive in detecting cases of latent syphilis like the present study [2]. Similar to our findings, studies have reported that with traditional algorithm diagnosis can be missed in certain situations like very early and latent cases [20-21].

In a study by Euheenal *et al*., 0.68% cases were detected as reactive by reverse algorithm vs 0.1% by traditional algorithm [9]. Even though a second treponemal test is performed to resolve discordant results, it is very difficult to differentiate between previously treated and latent syphilis without proper clinical history and documentation of previous treatment [22].

Though in ECDC algorithm, only non-treponemal tests are performed, RPR was done reflexively for all the samples to assess the current active status of the infection. In our study we had used rapid immunochromatographic card test for the serodiagnosis of syphilis with comparable results with both reverse and ECDC algorithm. Strong concordance was observed between reverse algorithm and ECDC algorithm. There are only a few studies that compare the positivity rate and degree of agreement between these different strategies.

All patients with probable early/ current/ latent syphilis were advised to provide detailed history and undergo thorough clinical examination and treatment with a comment entered in the WHONET software.

In traditional algorithm a non treponemal test (RPR) is performed first and if non-reactive no more tests are advised. In the case of early primary syphilis, specific antibodies (IgM) appear first in the blood towards end of week 2, while non-specific antibodies appear late: 2-3 weeks. RPR-based screening may miss early untreated primary syphilis as the window period ranges between 2 to 4 weeks [23]. On the other hand in reverse algorithm, a specific treponemal test is performed first and if positive a non treponemal test (RPR) with titers are performed reflexively to guide patient treatment and follow up.

If the nontreponemal test (RPR) is negative, a second treponemal test (one with a different antigen) is performed in ECDC algorithm. If a second treponemal test is positive, persons with a prior treatment history would not require further management unless sexual history is indicative of re-exposure. A non-reactive specific treponemal test rules out current or past infection. Even though a second treponemal test is performed, it is very difficult to differentiate between previously treated and latent syphilis without proper clinical history and documentation of previous treatment in certain situations. Hence interpretation of the results should always involve thorough history and examination.

Though non treponemal tests have an advantage in cases of monitoring activity by titre values as well as prognosis, if the lab chooses to use only non treponemal tests like RPR (traditional algorithm) as the screening test, the testing should be repeated after 1 to 2 weeks if clinically indicated for non-reactive samples in order to not miss the very early cases of primary syphilis. Such repetition of tests requires follow up with the patients and those samples will give positive results with specific treponemal tests by reverse algorithm.

In case of reverse algorithm /ECDC algorithm: A reactive treponemal test (confirmed by two test format) as per the ECDC guidelines especially in antenatal cases can guide the obstetrician to presumptively diagnose and treat patients to prevent congenital syphilis.

The limitation of our study was that in patients with latent syphilis, no documentation / history was available for exposure or treatment in the past. This could be attributed to the noncompliance on the part of the patient due to the stigma associated with sexually transmitted infection.

**Conclusion**

Reverse or ECDC algorithm testing can be implemented in the laboratory for the serodiagnosis of syphilis routinely to avoid missing cases of early syphilis. The specific treponemal card tests available in immunochromatography formats are easy to perform and comparatively cost effective. Following the reverse or ECDC algorithm would also be of immense value in case of antenatal patients as missing the diagnosis can have serious consequences for the foetus. The lab should choose an algorithm that can detect syphilis cases earlier and use test formats like Immunochromatography that are economical, easy to perform and interpret with lowest missed diagnosis rate. Further, we also found that WHO NET software as user friendly for easy analysis of data and interpretation of the results and to include specific comments individualized for each patient thereby giving valuable insight to the treating physicians.

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