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APPLYING REVERSE ALGORITHM FOR SYPHILIS SCREENING IN RESOURCE POOR SETTINGS

Microbiology

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ABSTRACT

Background and Objective: The diagnosis of this chronic systemic venereal disease with multiple clinical presentations is challenging and is typically made by serologic testing. Traditionally screening is by a non-treponemal antibodies test -VDRL/RPR and confirmed by treponemal antibodies detection. Recently reverse algorithm is advocated, that is to screen first for treponemal antibodies followed by VDRL/RPR. The objective of this study is to apply an economicrapid ICT -Treponemal antibody assay for reverse screening and directly comparing it's performance with traditional algorithm.

Methods: Seven hundred and fifty consecutive sera received from all departments for a period of three months (Jan to March 2018) at Sree Mookambika Institute of Medical Sciences at Kanyakumari district were screened for syphilis using ICT (Tulip Diagnostics) and RPR (Agappee)independently and all reactive sera were tested by TP-HA assay for confirmation (Omega diagnostics)

Results: Out of 750 sera tested, 12 were reactive by ICT Treponemal antibody assay (Reverse screening) and 3 were reactive by RPR Non Treponemal antibody (Traditional). All three reactive by RPR were detected by ICT assay and positive by TP-HA also. Out of the 9 sera reactive by ICT only 4 were positive by TP-HA assay.

Conclusion: In resource poor settings, rapid ICT treponemal antibody assay may be used to apply reverse screening for syphilis. Though reverse screening is prone for higher false reactive rate than traditional algorithm, the higher sensitivity enables to detect more successfully treated past syphilis and late/latent syphilis. A specific third treponemal antibody assay test is important whenever there is discordance between ICT and RPR assays.

KEYWORDS

Resource poor settings, Syphilis Screening, Traditional, Reverse algorithm, TP-HA, ICT, RPR

INTRODUCTION

Syphilis is a curable infection caused by spirochaete, Treponema pallidum. This infection is sexually transmitted and can also be passed on from mother to fetus during pregnancy.Early detection and treatment are also critical in preventing severe long-term complications and onward transmission to sexual partners.[1]

The diagnosis of this chronic systemic venereal disease with multiple clinical presentations is challenging. Though there are direct detection methods like Dark field microscopy, RT PCR, they are not widely available and the main stay of diagnosis is only by serological methods. The serological tests for syphilis are of two types. The Non-treponemal tests e.g. Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) which do not detect antibodies to T.pallidum These tests detect antibodies to reagin which is a combination of cardiolipin, lecithin and cholesterol. The Reagin is not specific for T.pallidum but is generated in response to spirochaete induced damage to cellular membranes and is a useful indicator of the disease activity. T. pallidum specific tests e.g. Fluorescent Treponemal Antibody – Absorption Test (FTA – ABS), T. pallidumhaemagglutination assay (TPHA), enzyme immunoassay (EIA), and CLIA detect antibodies against T.pallidum antigens. [2] Previously, non-Treponemal tests were used to screen serum samples for syphilis and positive samples were confirmed by a treponemal assay[3]. Recently, the WHO, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) released an updated algorithm which suggests that in areas having a low disease prevalence; samples may be screened using a treponemal specific assay and positive samples being analyzed with a non treponemal test to assess disease and treatment status[4 ,5].

When screening positive treponemal test is negative by RPR test it causes diagnostic dilemma. Because it may be due to (A) effectively treated past syphilis (B)early/late/latent syphilis when the sensitivity of RPR is low (C) no syphilis (falsely reactive treponemal test).So CDC recommended a third specific test TP- PA or TP-HA for such samples (Treponema pallidum particle agglutination(TPPA)assay/Treponema pallidum haemagglutination assay). A reactive TP-PAor TP-HA suggest either the (A)past successfully treated or(B) late or latent syphilis and a non reactive TP-PA indicates (C) the screening Treponemal assay is falsely reactive [6].

Many centers have adopted reverse algorithm by initial automated EIA screening for Treponemal antibodies followed by RPR and for discordant samples reflex testing by TP-PA/TP-HA. Many studies were published applying initial automated EIA /CLIA screening for treponemal antibodies. But in resource poor settings automated EIA screening is not widely available. On the other hand, rapid tests like Immunochromatographic tests (ICT) that can use finger prick blood specimens are commercially available for syphilis. They fulfil the WHO ASSURED criteria of being affordable, sensitive, specific, user-friendly requiring minimal trained personnel, rapid and robust (no special storage conditions and can give results in 15 to 20 min), equipment free and deliverable to those who need them.[7] These rapid tests have improved the effectiveness of prevention because of shorter patient wait times, ease of use, lower cost and obviating the need for follow-up and treatment.

This study was designed to evaluate the performance of reverse screening protocol by using a low cost, easy to perform test instead of a costly and technologically more demanding test keeping the limited availability of resources and expertise in many of the places in our setup. Rapid ICT test which detects all classes of Treponemal antibodies(IgM,IgGand IgA) is used as the initial screening test for reverse algorithm and RPR for initial test of traditional screening.TP-HA(omega diagnostics) as the third confirmatory specific Treponemal antibody detection test for all the sera reactive by ICT /RPR tests.

METHOD:

Prospectively collected sera (n 750; one sample per patient) for a period of three months , submitted for routine syphilis testing from all departments of Sree Mookambika Institute of Medical sciences ,Kanyakumari district, are screened by reverse screening using rapid lateral flow ICT kits (Tulip diagnostics).Samples testing reactive by the ICT assay were tested for RPR antibodies.(Agappee); if RPR gave a positive result, the titer of the serum sample was determined to an endpoint. In addition, sera testing reactive by the ICT assay were also analyzed by TP-HA -Qualitative (Omega Diagnostics LTD). In addition to the reverse screening algorithm, each sample was also screened by RPR, with the performing technologist unaware of the results of other testing. The titers of sera that were reactive by RPR were determined to an endpoint and subsequently tested by TP-HA-Qualitative . All testing was performed according to the manufacturers' recommendations.

RESULTS:

Among the 750 samples tested, 12 (1.6%) were reactive by reverse

screening (ICT) compared to 3 (0.4%) by the traditional screening test (RPR) (P = 0.01). All three samples, reactive by RPR were confirmed to be positive by TP-HA; notably, these reactive three samples were also detected by the reverse screening algorithm.

Addition to these three samples, 9 sera (Sl no: 4-12) were reactive by reverse algorithm which were not detected by RPR. Among these, 4 samples were (Sl no :4-7) reactive by TP-HA test, validating the ICT antibody assay. These were interpreted as either past, treated syphilis or latent syphilis. Finally, 5 samples (Sl no:8to12) were reactive by ICT antibody assay but nonreactive by RPR and TP-PA. These results were interpreted as falsely reactive ICT screening results based on negative TP-HA result, (Table 1).

TABLE :1

	Traditional Algorithm		Reverse Algorithm			Interpretation
Sl no	RPR TITRE	TP-HA	ICT	RPR TITRE	TP-HA	
1	+ (128)	+	+	+ (128)	+	Active syphilis
2	+ (64)	+	+	+ (64)	+	Active syphilis
3	+ (1)	+	+	+ (1)	+	Past treated
4	-	NA	+	-	+	Past treated/Latent
5	-	NA	+	-	+	Past treated/Latent
6	-	NA	+	-	+	Past treated/Latent
7	-	NA	+	-	+	Past treated/Latent
8	-	NA	+	-	-	False Positive
9	-	NA	+	-	-	False Positive
10	-	NA	+	-	-	False Positive
11	-	NA	+	-	-	False Positive
12	-	NA	+	-	-	False Positive

DISCUSSION :

This study is the direct comparison of traditional vs reverse screening algorithms for the diagnosis of syphilis in low prevalence population. Rapid ICT kits were used for treponemal antibody assay in reverse screening algorithm. The ICT uses T.pallidum specific antigens to detect antibodies against them in a card or strip format with visual read out.[8] Chemiluminescence based assays are usually used for screening of blood donors in high volume blood banks due to automation facilities, higher testing throughput and objective interpretation of results, however expensive instrumentation is required for them thus limiting their use in resource limited settings.[9] Herring et al,evaluated nine rapid syphilis ICT kits and reported their sensitivity ranging from 84.5 to 97.7% and specificityfrom 93 – 98% when compared against TPHA or T.pallidum particle agglutination (TPPA) as reference method/gold standard.[10]A meta-analysis has shown the sensitivity of different ICTs ranging from 85-100% and specificity in the range of 98 – 100%.[8] ICT is suitable for use in remote and developing regions as they are simple to use, can be transported, stored and performed at room temperature and do not require microscopic or electrical equipment. Moreover, they are cheaper and quicker as compared to other treponemal tests. [10]

Saadia A, observed that the two methods CLIA and ICT had performed equally well and in limited resource settings, the ICT could be used as an alternative for Syphilis screeningand concludedtheir findings demonstrate comparable performance of CLIA and ICT assay for syphilis screening[11]

Our study reveals that reverse screening algorithm using ICT results in higher false positive rate than traditional algorithm. (0.6% versus 0.0%, respectively; P= 0.03). This finding coincides with the findings of previous studies,6/1000[0.6%][12]

and 866/140176[0.6%] reported by CDC [13]

CONCLUSION:

Though false-reactive rate is higher by using ICT for reverse screening algorithm,4 patients with past treated or possible latent syphilis were detected that went undetected by RPR screening. Whether latent, untreated syphilis, or past, successfully treated and resolved syphilis is difficult to determine. So ICT may be used for reverse algorithm in resource poor settings as it is economic, user friendly and doesn't require sophisticated equipments and expertise. This present study supports the higher sensitivity of reverse algorithm to detect early or late/latent syphilis.Our study emphasizes the importance of second treponemal antibody assay when the results of ICT treponemal antibody assay and RPR are discordant.

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