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Original Research Article

Comparison of syphilis testing by reverse algorithm and conventional method among blood donors in a tertiary care center

Athira Sasidharan[1], Ramesh Bhaskaran[1,*], Nithya M Baiju[1], A.M Rafi[1], Chithra Valsan[2]

[1] *Dept. of Transfusion Medicine, Jubilee Mission Medical college and Research Institute, Thrissur, Kerala, India*
[2] *Dept. of Microbiology, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India*

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ABSTRACT

Introduction: Traditional testing algorithm for syphilis begins with screening non-treponemal test followed by confirmatory treponemal test. Reverse algorithm first screens with a treponemal test; reactive samples are then tested by Rapid plasma reagin test (RPR) which is used to assess disease activity. Discordant syphilis IgG and RPR results are resolved by a second treponemal test, Treponema pallidum hemagglutination assay (TPHA) as recommended by the Centres for Disease Control and Prevention laboratories.

Aim: To evaluate the analytical performance of reverse algorithms in syphilis infection among donors in comparison with traditional methods and the prevalence of syphilis among blood donor populations.

Materials and Methods: This was a cross sectional study done in the department of transfusion medicine from January 2019 to February 2020 which comprised of 8635 blood donors. After donation all the blood samples were screened for syphilis using chemiluminescent immunoassay (Vitros syphilis TPA assay) in Vitros 3600 EQ/ECIQ immunodiagnostic system and RPR (omega RPR test kit) irrespective of each other's result.

Whenever chemiluminescence immunoassay or RPR turns positive, the samples were sent for TPHA (Immutrep TPHA test kit) keeping it as the gold standard method.

Results and Discussion: Among 8635 samples tested, seroreactivity of CLIA (Vitros syphilis TPA assay) was 0.25 % whereas for traditional algorithm 0.13%. Sensitivity, specificity and accuracy of reverse algorithm in comparison with traditional was 100 %, 70.6%, 78.3% and 36.4% ,75% and 65.1% respectively. In comparison with TPHA, Vitros TPA assay in reverse algorithm showed moderate agreement (k=0.56, p=0) which was statistically significant whereas RPR showed slight agreement (k=0.11, p=0.47). Negative predictive value of CLIA was 100% (p=0) which was statistically significant. Positive predictive value of reverse algorithm was 54.5% and 33.3% for traditional algorithm (p=0.04).

1. Introduction

Screening of blood for syphilis is mandatory for issuing safe blood. According to the WHO, blood banks may choose Venereal Disease Research Laboratory, RPReagin

Antibody to non-treponemal antigen is found in active disease and levels subside after successful treatment, while treponemal specific antibodies persist for a long time after the infection has been duly treated. The serological tests most commonly used to screen for the disease are the nontreponemal and treponemal tests. The non-treponemal tests such as RPR or VDRL measures the host's

* *Corresponding author*.
E-mail address: rameshbhaskaran@gmail.com (R. Bhaskaran).

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response to non-treponemal antigen such as cardiolipin and lecithin released from the damaged host cells as well as lipoprotein like material released from the treponema. The treponemal tests such as TPHA, now being replaced by TPPA (T. pallidum particle agglutination assay), and micro hemagglutination assay for treponema pallidum have higher sensitivity for all stages of disease other than exceedingly early primary syphilis.

Recently several enzyme immunoassays, some of which are based on specific T. pallidum recombinant antigen have been developed and evaluated as treponemal test for syphilis

Other technologies in use include the Western blotting test which though not common place are used in some laboratories to resolve questionable results obtained with other treponemal tests. The use of Polymerase Chain Reaction (PCR) though restricted has the advantages of high levels of specificity as compared to serological testing and the ability to detect primary syphilis earlier when serologic tests are non-reactive but lower sensitivity and reduced platelet activity.

In India, according to the Drug and Cosmetics Act & Rules 1945, currently only one screening test for syphilis is mandatory for screening donated blood units. Traditional testing algorithm for syphilis begins with screening non-treponemal test such as RPR, with positive results followed by confirmatory treponemal test such as fluorescent antibody or TPPA.

RPR titers are sensitive, decrease with treatment, and have traditionally been more convenient and less expensive to perform than treponemal tests, rationalizing this algorithm. With increasing automation and decreasing cost, the majority of developed countries have adopted a reverse algorithm which first screens with a treponemal test; reactive samples are then tested by RPR which is used to assess disease activity. Discordant syphilis IgG and RPR results are resolved by a second treponemal test (e.g. TP-PA), as recommended by the Centers for Disease Control (CDC) and Prevention laboratories adopting reverse algorithms.[1] The advantage of Reverse Sequence Syphilis Screening (RSSS) for laboratories is that the number of required manual confirmatory tests will be significantly decreased.

2. Materials and Methods

This was a cross sectional study done in the department of transfusion medicine from January 2019 to February 2020. The study population comprised 8635 blood donors. All the donors who consented for the study were accepted for blood donation. After blood collection all the blood samples in the red tubes were subjected for screening of syphilis. Samples were screened for syphilis using chemiluminescent immunoassay (Vitros syphilis TPA assay) in Vitros 3600 EQ/ECIQ immunodiagnostic system and RPR (omega RPR test kit) irrespective of each other's result. (Figures 1 and 2)

Whenever chemiluminescence immunoassay or RPR turns positive, the samples were sent for TPHA (Immutrep TPHA test kit), TPHA is considered as the gold standard method in the present study and thus, additional samples were taken which were negative in TPHA for comparing the results in both methodologies.

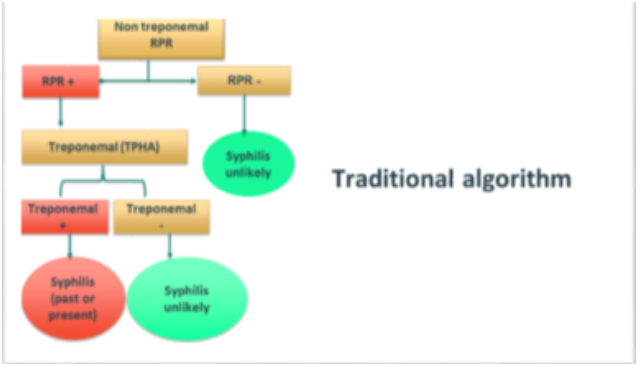

Fig. 1: Traditional algorithm

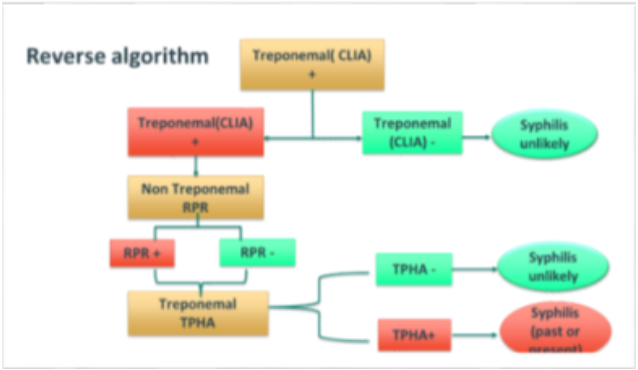

Fig. 2: Reverse algorithm

2.1. Statistical analysis

Categorical and quantitative variables were expressed as frequency (percentage) and mean ± SD respectively. Sensitivity, specificity, positive and negative predictive values, accuracy were used to find the performance analysis of Vitros syphilis TPA assay and RPR when TPHA as the gold standard. Kappa statistics was carried out to assess the agreement of pass between various methods using TPHA as gold standard. Statistical analyses was performed by using a statistical software package SPSS, version 20.0

3. Results

We had a total of 8635 blood donors from January 2019 to February 2020. Voluntary blood donors comprised 3541 (41%) and 5094 (58.9%) were replacement donors. 8309 were males (96.2%) and females comprised of only 326

(3.77%). Average age of study population was 29 yrs

3.1. Performance analysis of reverse algorithm approach

A total of 8635 donor samples were taken for performing reverse algorithm and conventional RPR method. There were 22 samples reactive for syphilis using CLIA out of 8635 donors. Mean age of the reactive sample population was 39.4+/-11.8 yrs. Majority (36.4%) were within 18-30 years of age.

According to reverse algorithm approach, there were 22 (47.8 %) donors who screened positive in Vitros Syphilis TPA Chemiluminescence Immunoassay (CLIA). Only 12 (26.1 %) were confirmed True positive in TPHA, considering TPHA as gold standard. 10 donor samples which were reactive in CLIA was not reactive in TPHA and was classified as false reactive in CLIA. For evaluating performance analysis of reverse algorithm approach additional 24 samples were taken which were confirmed negative in TPHA and performed CLIA and RPR simultaneously and gave negative results.

3.2. Distribution of CLIA reactive RPR reactive

Out of 47.8 % (22) CLIA reactive samples, only 5 were reactive in RPR (10.9%) and 89.1 % was non-reactive with respect to TPHA (Table 1).

Table 1: Syphilis reverse algorithm study analysis

Number of samples screened in CLIA	8635
Number of reactive samples in CLIA	22 (0.25%)
Number of non-reactive samples in CLIA	8613 (99.74%)
Number of concordant samples in RPR	5 (10.86%)
Number of discordant samples with RPR	41
Number of samples subjected to TPHA (gold standard)	46
Number of reactive samples in TPHA	12 (26%)
Number of non-reactive samples in TPHA	34 (73.91%)

On evaluating diagnostic accuracy of CLIA with respect to TPHA there was statistical significance (p=0) with kappa of 0.56. This data clearly states that there is moderate agreement between CLIA and TPHA test (k=0.56).

3.3. Diagnostic accuracy of RPR when TPHA is gold standard in reverse algorithm approach

Out of 22 reactive samples in CLIA, RPR reactive were only 5. All the samples were subjected to TPHA (n= 46) and 4 samples were reactive (CLIA+/RPR+/TPHA+). CLIA+/RPR-/TPHA+ samples were 8. CLIA+/RPR-/TPHA- were 33. There was only one sample which was CLIA+/RPR+/TPHA- which is false positive

3.4. Traditional algorithm analysis

A Total of 8635 samples were performed according to traditional syphilis algorithm. Of those, 12 (0.14%) were reactive in RPR with only 4 (33.3% out of 12) confirming positive with TPHA (RPR+/TPHA+). The other 8 (66.6% out of 12) donors were negative in TPHA (RPR+/TPHA-) showing false positives results in RPR method which could be due to biological false positives

There were 7 samples which were RPR non-reactive but reactive in TPHA (RPR-/ TPHA +) indicating inability of RPR to detect syphilis in latent stages. On analyzing RPR with respect to TPHA, there was no statistical significance (p= 0.469). There was only slight agreement between RPR and TPHA (k =0.11).

Table 2: Overall comparison between reverse algorithm and traditional algorithm approach.

Syphilis screening assay	**Vitros TPA assay in reverse algorithm**	**RPR in traditional**
Period of study	January 2018 – February 2020	
Number of screened samples	8635	8635
Number of reactive samples	22	12
Percentage of reactivity	0.25%	0.13%

Based on CDC reverse algorithm study using Vitros TPA assay as primary screening followed by RPR and confirmation by TPHA test, Reverse algorithm has got significantly higher sensitivity (100% v/s 36.4%) compared to traditional algorithm with p value of 0.00. Reverse algorithm has the higher positive predictive value compared to traditional which is statistically significant (p=0.04).(Table 3)

Negative predictive value in the Reverse algorithm was also significantly high (100%) (p= 0.000) compared to traditional algorithm. Whereas accuracy of reverse algorithm and traditional algorithm was not much statistically significant, (p= 0.171). Reverse algorithm had 78.3% accuracy and the traditional algorithm secured 65.1% accuracy.

Table 3: Comparisonof diagnostic accuracy of reverse algorithm and traditional algorithm considering TPHA as gold standard.

Parameters	**Reverse**	**Traditional**	**P**
Sensitivity	100.0	36.4	0.000
Specificity	70.6	75.0	0.646
Positive Predictive value	54.5	33.3	0.044
Negative Predictive value	100.0	77.4	0.000
Accuracy	78.3	65.1	0.171

4. Discussion

The world health organization estimates that worldwide in 2016, there were 19.9 million prevalent syphilis cases in adolescents and adults between the age group of 15 - 49 years[2] According to 2000-2018 reports the overall rise in cases was attributed to men who have sex with men. In 2018, the rate of syphilis among women were 3 cases per 1,00,000 females[3] according to CDC data. But there is wide decline in transfusion transmitted infection of syphilis because of numerous reasons

1. No direct donor to recipient transfusions.
2. Refrigerated blood components cause inactivation of T pallidum.
3. The decline in rate of syphilis in general population.
4. Self-deferral of blood donors who are ill during infection.
5. Wide use of antibiotics among transfusion recipients.

Even though these are the possible reasons none of these explanations has been qualified or adequately validated hence it's the duty of blood centre to formulate a proper testing methodology for screening syphilis. Transfusion medicine is currently focused on improving blood safety as well as establishing effective, efficient automated diagnostic algorithms for screening blood donors

Seroprevalence of syphilis varies in different methods. In the current study the reverse algorithm prevalence rate is 0.13% (12/8635), whereas in traditional it is 0.05% (4/8635).

In this study we took a sample size of 8635 with 8309 males and 326 females with a mean age group of 29 years. Study by Sonali et al observed higher prevalence of syphilis among males compared to females (9.67% and 2.08%).

According to reports by CDC in 2018,[4,5] the rate of reported primary and secondary cases among men (18.7 /100,000 males) was more compared to women (3 cases. 100,000).

4.1. Comparison of reverse algorithm and traditional algorithm

In the present study total CLIA reactive samples from 8635 were 22 (0.25 %) and 8613(99.74%) were negative and in traditional method 12 (0.13 %) were reactive irrespective of TPHA confirmation. This is slightly comparable with Binnicker et al where 1.5 % samples were reactive in reverse screening and only 0.4% reactive in traditional methods.[6] In Linda et al study 0.52 % were reactive with chemiluminescent immunoassay.

When comparing diagnostic performance, compared to RPR in reverse algorithm, CLIA had sensitivity of 100 % and specificity of 70.6%. This was comparable with various studies. Xiaohui et al study (2010) for the evaluation of chemiluminescence immunoassay and observed sensitivity of 100% and specificity of 99.9% in CLIA compared to TPHA. CLIA showed 99.5% agreement with TPPA. The overall false positive rate of CLIA in the reverse algorithm is 29.4%. This was slightly comparable to a study by V.S Sandel et al, the false positive rate of VDRL and CLIA confirmed cases were 40.5%(n=180) and 37.4% (n=359) respectively. Positive predictive value of 54.5% and accuracy of 78.3 %. CLIA was effective in identification of true negatives which constituted majority of samples in a low prevalence population

With respect to TPHA, CLIA had moderate agreement (p=0) in detecting syphilis with kappa of 0.56. Whereas RPR has only fair agreement (p= 0.004) with kappa of 0.37%.

Sensitivity and specificity of RPR in comparison with TPHA in reverse algorithm is 33.3 % and 97.1 % respectively with false negative rate of 66.7 and false positive rate of 2.9%. The positive predictive value and negative predictive value is the same, 80% and the accuracy is 80.4 %.

RPR reactivity varies with disease status and can be used to follow treatment efficacy and to assess disease recurrence.

From our data on direct comparison of reverse and traditional algorithms, sensitivity and specificity is 100% and 36.4 % which is highly significant (p=0.00). This finding is supported by another study stating overall percent agreement in reverse as 100 % with k=1.00, suggesting that non treponemal assay is nowadays obsolete for diagnosis of syphilis among blood donor populations.

Specificity of reverse algorithm vs traditional algorithm is 70.6% vs 75% which is not statistically significant (p=0.646). Positive predictive value of CLIA is 54.5 % which is statistically significant (p=0.044) when compared with the traditional method (33.3%) which indicates that CLIA predicts whether the person is truly having/ had the disease. Negative predictive value of the reverse algorithm is 100% with p= 0.00 whereas in traditional algorithms 77.4%.

The choice between traditional and reverse algorithms depends on the purpose of screening. For a full-fledged blood bank, implementation of the reverse algorithm is the better option for blood safety. According to the new amendment of the Drug and cosmetic act, syphilis reactive donors are deferred permanently.

In the present study, reverse algorithms led to the discovery of 12 cases of syphilis (26.1%) CLIA+/RPR-/TPHA+ in which diagnosis was either latent syphilis /untreated / unknown treatment of past syphilis. Reverse algorithm is beneficial for the diagnosis of donors with early or late syphilis with nonreactive RPR tests. There are many literatures stating high false positive rates in reverse algorithms which includes Binnicker et al study[6] (28% discordant results) CLIA+/RPR-/TPHA-.

According to CDC if using reverse sequence of screening, a positive specimen on CLIA should be

tested with quantitative nontreponemal test i.e. RPR. If the subsequent non treponemal test is negative, then TPPA/TPHA should be used with positive test indicating syphilis infection.[7]

Since TPHA is considered as gold standard method we should also consider performing TPHA in blood banks, but the limitation would be in terms of its cost. In Thailand TPHA test is used for any general cases which is positive in screening test but not recommended in blood centers.[8] A study on cost utility analysis was done on 2009 and observed cost utility of TPHA higher when compared to RPR test. And cost-effective test in the study was VDRL/RPR.[9]

5. Conclusion

The reverse algorithm is likely to identify more cases of untreated disease in countries such as ours, including early and late/latent syphilis, but with a possibility of detecting donors who had possibly undergone treatment, which could be intentional or un intentional due to the rampant use of various antibiotics for other diseases. However, since the New National Blood Transfusion Guidelines (NBTC) guidelines anyway state permanent deferral for donors testing positive for syphilis, the adoption of the reverse algorithm would ensure enhanced safety of the transfused blood components. This study emphasizes the facts that CLIA is highly sensitive and specific and the indicator results are objective and unequivocal when used for screening for syphilis by adopting the Reverse Sequence Syphilis Screening (RSSS), especially in blood centres with large volumes of samples for testing.

6. Source of Funding

No financial support was received for the work within this manuscript.

7. Conflict of Interest

The authors declare they have no conflict of interest

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Author biography

Athira Sasidharan, Transfusion Medicine

Ramesh Bhaskaran, Associate Professor

Nithya M Baiju, Assistant Professor

A.M Rafi, Fellow in Hemat Oncology and Bone Marrow Transplant Associate Professor

Chithra Valsan, Professor and HOD

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