Role of *Treponema pallidum* hemagglutination assay for diagnosis of syphilis in low titers of VDRL-reactive sera: A prospective study from a large tertiary care center of East Delhi

Sir,

Since many decades, serological tests remained the keystone for diagnosis of syphilis. Venereal Disease Research Laboratory (VDRL) test is performed solely to screen patients for syphilis, and it still has remained unchallenged due to its simplicity, high sensitivity, and cost-effectiveness. However, serologic tests such as VDRL provide only indirect evidence of syphilis and may be reactive in the absence of clinical evidence of syphilis, also known as biological false positive (BFP). The testing strategy followed in most centers of our country is the first screening by a regain or nontreponemal test such as VDRL followed by a treponemal test, *Treponema pallidum* hemagglutination assay (TPHA) test for confirmation in VDRL-reactive cases, especially in high titers. However, there are several disadvantages with this approach. Screening undiluted specimens with a nontreponemal test alone can yield false-negative reactions in the presence of high titers of antibody (the prozone phenomenon) as seen in secondary syphilis. Nontreponemal tests also lack sensitivity in later stages of infection.\(^1\)\(^2\) To assess the reactivity of TPHA test for diagnosis of syphilis in low (≤1:8) titers of VDRL, a total of 14,319 serum samples were received from antenatal clinic attendees (ANC), sexually transmitted clinics (STD), antiretroviral clinics (ART), and other departments during 1-year period (September 2014–August 2015) in the department of microbiology of University College of Medical Sciences and Guru Teg Bahadur Hospital, New Delhi, which were tested by VDRL followed by TPHA following standard protocol.

All the VDRL-reactive sera were divided into two groups, Group I sera having titer ≤1:8 (low titer) and Group II having titer ≥1:8 (high titer); TPHA was performed in both the groups for detection of anti-treponemal antibodies. Distribution of serum samples received from various departments has been shown in Figure 1. Out of 14,319 sera, 54 samples (0.377%) were found reactive by qualitative VDRL test. Forty samples (0.279%) were reactive in low titers (≤1:8), whereas about 14 samples (0.097%) were reactive in high titers (≥1:8). Table 1 depicts the distribution of VDRL-reactive samples and TPHA-reactive samples in various departments. Out of 40 samples of Group I, 16 samples (40%) were found reactive by TPHA, whereas 24 (60%) were nonreactive by TPHA. Distribution of samples in low and high titers and TPHA reactivity in each group is shown in Table 2. Out of 16 samples having VDRL titer ≤1:8 and TPHA reactive, 10 samples were from STD department, 5 samples from ART, and 1 from ANC [Table 3].

BFP results encountered in routine screening are often difficult to explain and sometimes it may be a cause for anxiety as well as the embarrassment to the patients. Testing strategy in many National AIDS Control Organisation (NACO)-designated STI centers in India is only VDRL testing which is performed at first. TPHA is usually performed in cases having titer ≥1:8, and if found positive, then they are considered syphilitic and are

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**Table 1: Distribution of ANC, STD, and ART patients in different quantitative titers of VDRL**

| Reactive VDRL titer | ANC (%) (n=13,049) | STD (%) (n=462) | ART (%) (n=410) | Total (%) |
|---------------------|---------------------|-----------------|-----------------|-----------|
| U                   | 0                   | 11              | 5               | 16        |
| R: 2                | 2                   | 7               | 3               | 12        |
| R: 4                | 0                   | 3               | 1               | 4         |
| R: 8                | 0                   | 4               | 4               | 8         |
| R: 16               | 1                   | 1               | 0               | 2         |
| R: 32               | 0                   | 2               | 3               | 5         |
| R: 64               | 0                   | 1               | 2               | 3         |
| R: 128              | 0                   | 1               | 3               | 4         |
| R: 256              | 0                   | 0               | 0               | 0         |
| Total               | 3 (0.022)           | 30 (6.49)       | 21 (5.12)       | 54        |

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**Figure 1: Distribution of serum samples received from various departments**
Table 2: Comparison of TPHA in low and high titers of VDRL among ANC, STD, and ART clinic attendees

| Patient groups   | VDRL-reactive sera ≤1:8 titer (Group I, n=40) | VDRL-reactive sera ≥1:8 titer (Group II, n=14) | Total (%) |
|------------------|---------------------------------------------|---------------------------------------------|-----------|
|                  | TPHA + (%) (n=16)                             | TPHA − (%) (n=24)                             |           |
| ANC clinic attendees | 1 (0.007)                                       | 1 (0.007)                                       |           |
| STD clinic attendees | 10 (2.16)                                          | 15 (3.24)                                          |           |
| ART clinic attendees | 5 (1.21%)                                         | 8 (1.95)                                          |           |
| Total             | 16 (0.11)                                          | 24 (0.56%)                                         |           |
|                  | TPHA + (%) (n=11)                             | TPHA − (%) (n=24)                             |           |
| ANC clinic attendees | 1 (0.007)                                       | 0                                              | 3 (0.022) |
| STD clinic attendees | 5 (1.08)                                          | 0                                              | 30 (6.49) |
| ART clinic attendees | 5 (1.21)                                         | 3 (0.73)                                          | 21 (5.12) |
| Total             | 11 (0.07)                                          | 3 (0.02)                                          | 54 (3.73) |

Table 3: Distribution of samples showing TPHA reactive in low titer (≤1:8) of VDRL (n=16)

| Titer of VDRL | ANC | STD | ART | Total |
|---------------|-----|-----|-----|-------|
| U 1           | 0   | 6   | 2   | 8     |
| R: 2          | 1   | 3   | 2   | 6     |
| R: 4          | 0   | 0   | 0   | 0     |
| R: 8          | 0   | 1   | 1   | 2     |
| Total         | 1 (0.007%) | 10 (2.16%) | 05 (1.21%) | 16 |

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Conflicts of interest
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