Algorithm for recall of HIV reactive Indian blood donors by sequential immunoassays enables selective donor referral for counseling

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ABSTRACT

Background: HIV/AIDS pandemic brought into focus the importance of safe blood donor pool.

Aims: To analyze true seroprevalence of HIV infection in our blood donors and devise an algorithm for donor recall avoiding unnecessary referrals to voluntary counseling and testing centre (VCTC).

Materials and Methods: 39,784 blood units were screened for anti-HIV 1/2 using ELISA immunoassay (IA-1). Samples which were repeat reactive on IA-1 were further tested using two different immunoassays (IA-2 and IA-3) and Western blot (WB). Based on results of these sequential IAs and WB, an algorithm for recall of true HIV seroreactive blood donors is suggested for countries like India where nucleic acid testing or p24 antigen assays are not mandatory and given the limited resources may not be feasible.

Results: The anti-HIV seroreactivity by repeat IA-1, IA-2, IA-3 and WB were 0.16%, 0.11%, 0.098% and 0.07% respectively. Of the 44 IA-1 reactive samples, 95.2% (20/21) of the seroreactive samples by both IA-2 and IA-3 were also WB positive and 100% (6/6) of the non-reactive samples by these IAs were WB negative. IA signal/cutoff ratio was significantly low in biological false reactive donors. WB indeterminate results were largely due to non-specific reactivity to gag protein (p55).

Conclusions: HIV seroreactivity by sequential immunoassays (IA-1, IA-2 and IA-3; comparable to WHO Strategy-III) prior to donor recall results in decreased referral to VCTC as compared to single IA (WHO Strategy-II) being followed currently in India. Moreover, this strategy will repose donor confidence in our blood transfusion services and strengthen voluntary blood donation program.

KEY WORDS: Donor Recall, HIV, ELISA, rapid tests, biological false reactive

Introduction

HIV/AIDS pandemic brought into focus the importance of safe blood donor pool. Presently in India WHO Strategy-I is used for screening donors’ blood for HIV, according to which serum/plasma is tested only once by E/R/S (ELISA/Rapid/Simple) assay for HIV antibodies. If negative, the unit is considered free of HIV and if reactive the unit is discarded. The dilemma in screening low-risk populations such as blood donors is that a large proportion of primary immunoassay reactive results represents false positive reactions also known as biological false reactive (BFR).[1,2] Revealing transfusion transmitted disease (TTD) status to blood donors was not permissible earlier in India. From September 2003, according to Objective 4.16 of Indian Action Plan for Blood Safety, the donor is counseled about TTDs prior to donation and is offered the option of knowing his TTD status provided he has given consent for the same.[3] According to this, donors found seroreactive by initial HIV screening assay are directed by blood transfusion services (BTS) to a linked Voluntary Counseling and Testing Centre (VCTC) for counseling and further confirmatory testing without repeating tests in blood banks.

In India, on an average 52% of the donors are voluntary and 48% are replacement donors.[4] None of the licensed blood banks in India collect blood from paid donors as per the Supreme Court ruling in 1996. In India, more than 90% of the donors are males since the prevalence of iron deficiency anemia in females is 35-64%.[5,6] Unless Indian transfusion practice can approach a totally altruistic donation model with anonymity and education of donors about the test results, stigmatization of HIV positivity without confirmatory tests may lead to unnecessary counseling, personal anxiety and donor deferrals. This can have a negative impact on the voluntary blood donation drive.

This study was conducted to analyze true seroprevalence of HIV infection in our blood donors. On primary HIV 1/2 screen reactive donors’ samples, performance of the sequential immunoassays (IA) were compared with that of confirmatory
western blot (WB) test. This helped us to devise an algorithm of recall for initial HIV screen reactive blood donors so as to avoid unnecessary referrals to VCTC.

Materials and Methods

A retrospective study was conducted from December 2003 to January 2005, in the Department of Transfusion Medicine of a tertiary care hospital. 39,784 blood units were screened for HIV 1/2 antibodies using 3rd generation indirect ELISA kit (Microlisa HIV 1/2, J Mitra and Co. Ltd, India) (IA-1), besides other mandatory tests (anti-HCV, HBsAg, VDRL and malaria). All kits used were approved by National AIDS Control Organization (NACO), India. As per the WHO Strategy-I, all HIV screen reactive blood units were discarded. The sera of screen reactive units were aliquoted and stored at -20°C for future testing before donor recall. To avoid inter-lot variation; repeat IA-1 was done using the same assay as above but with a different lot number. Samples which were repeat reactive on IA-1 were further tested independently using two sequential IAs based on different principles (IA-2 and IA-3). A 3rd generation sandwich ELISA (Genscreen HIV 1/2, Bio-Rad, Marnes la Coquette, France) and a rapid dot blot test (Comb AIDS-RS, Span Diagnostics, Gujrat, India) were used for the sequential IAs. All samples which were repeat reactive by IA-1 were also confirmed by the gold standard confirmatory WB test (HIV W. BLOT, J Mitra and Co. Ltd, India).

The results (positive, negative and indeterminate) of WB were analyzed as per WHO criteria.[1] Manufacturer’s instructions were strictly followed while performing each assay. The results of IA-1, IA-2 and IA-3 were compared with that of WB. The significance of S/C ratio (signal/cut off ratio) by repeat IA-1 was compared with WB reactivity pattern. Based on results of these sequential IAs and WB, a working algorithm for donor recall of true HIV seroreactive blood donors is suggested for countries like India where Nucleic Acid Testing (NAT) or p24 antigen assay are not mandatory and given the limited resources are not yet feasible.

Results

Out of 39,784 blood donor units screened during the study period, 64 were found to be HIV screen reactive by IA-1, thus accounting for a seroreactivity of 0.16%. Of the 64 reactive units, 44 (69%) were further tested by repeat IA-1, IA-2, IA-3 and WB tests. Twenty samples had been discarded and thus could not be retested. All forty four samples were repeat reactive by IA-1. Of these 44 samples, 32, 27 and 20 were IA-2, IA-3 and WB positive respectively as against the expected number of 46 (0.11%), 39 (0.098%) and 29 (0.07%) if adjusted for the discarded 20 samples. Thus, based on WB test, the true HIV seroprevalence of 0.07% is low in blood donors of Chandigarh.

According to the Action Plan for Blood Safety, all the 44 HIV 1/2 screen reactive blood donors should be notified of their TTD status.[1] However, for confirmation before donor recall, we tested IA-1 repeat reactive samples independently by two IAs based on different principles (IA-2 and IA-3). Comparison of seroreactivity performance of IA-2 and IA-3 with that of WB results on 44 IA-1 reactive samples is shown in Table 1. Western blot positive, negative (BFR) and indeterminate results were found in 45.5% (n=20), 43.2% (n=19) and 11.3% (n=5) respectively; of the 44 IA-1 repeat reactive samples tested. Out of 44 IA-1 repeat reactive samples, 32 and 27 donors were found seroreactive by IA-2 and IA-3 respectively. Twenty one donors were found seroreactive independently by both IA-2 and IA-3 and 20 of these (95.2%) were WB positive. Seventeen donor samples found seroreactive by any one of these two IAs were either WB negative (n=13) or gave indeterminate (n=4) results.

Of the 44 repeatedly reactive blood donors, 96% (n=42) were males with a mean age (range) of 51.2 (19-51) years. The median S/C ratio was 2.35 with a range of 1.17 to 9.57. Thus, 22 reactive samples with S/C ratio less than 2.35 comprised the low positive group. 89.5% (17/19) of donor samples showing BFR had S/C ratio less than 2.35 as compared to 5% (1/20) for confirmed positives. Our results also suggest that if S/C ratio is high, the probability of it being positive by WB also increases as 86% (19/22) samples in this group were WB positive. Significance of S/C ratio with WB reactivity pattern is shown in Figure 1.

Comparison of seroreactivity by IA-1, IA-2 and IA-3 with WB results revealed that BFR [43.2% (19/44) vs. 25% (8/32) vs. 18.5% (5/27) respectively] and indeterminate results were observed in 11.3% (5/44) vs. 12.5% (4/32) vs. 7.4% (2/27) respectively. The most common cause of indeterminate results was non-specific reactivity to gag protein (p55) alone in all five donors.

Based on the above results the recommended algorithm for donor recall of HIV screen reactive blood donors is summarized in Figure 2. As compared to conventional strategy based on a single IA for donor recall, sequential IAs (IA-1, IA-2 and IA-3) results in selective donor referral for counseling to VCTC (44 vs. 25). Triple seroreactive (IA-1, IA-2 and IA-3) donors (n=21) can be recalled and referred to VCTC for donor counseling without WB test as none of them were BFR. However, all IA-1
reactive samples which are either IA-2 or IA-3 reactive (i.e., double reactive) require confirmation by WB before donor recall. This is because BFR and WB indeterminate results were observed with these double reactive samples [Table 1]. This will also minimize WB usage (44 vs. 17). Thus, selective use of WB along with sequential IAs prevents BFR donors (43.2%) from unnecessary referral to VCTC.

Table 1: Comparison of IA-2 and IA-3 seroreactivity with that of WB reactivity pattern on 44 HIV 1/2 repeat reactive donor samples by IA-1

<table>
<thead>
<tr>
<th>Western blot reactivity</th>
<th>Immunoassay (IA) reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2 R⁺</td>
<td>IA-2</td>
</tr>
<tr>
<td>Positive (n=20)</td>
<td>20</td>
</tr>
<tr>
<td>Negative (BFR⁺) (n=19)</td>
<td>0</td>
</tr>
<tr>
<td>Indeterminate (n=5)</td>
<td>1</td>
</tr>
<tr>
<td>Total (n=44)</td>
<td>21</td>
</tr>
</tbody>
</table>

*B: Biological False Reactive
⁺: Reactive
⁻: Non-Reactive

result the HIV seroprevalence from use of single ELISA test is certainly high, suggesting BFR or indeterminate results.

Low S/C ratio of an ELISA test can also suggest BFR. [18-20] We found S/C < 2.35 in 89.5% of donor samples showing BFR as compared to 5% for confirmed positives. Similar to our results, in another study 95.4% of BFR results had S/C ratio < 3 as compared to 0% for confirmed positive donors. [13]

In India, blood units that are HIV 1/2 reactive on screening IA are discarded (WHO Strategy-1) and the donor is notified about the test result if he/she has given the pre-donation consent for the same. Notification of false positive or indeterminate results has been reported to cause psychological distress and have negative social effects including disruption of family and work. [21,22] This has led to removal of a significant number of safe donations from the blood supply. [23] Each year, approximately 75,000 individuals in US are informed of having HIV-1 antibodies but less than 10% of these donors are truly infected on confirmatory test. [24,25] Several factors (allergy, acute illness, alloimmunization, autoantibodies or vaccination) may be associated with HIV seroreactivity on a screen IA but a negative or indeterminate WB test. [26]

The BFR rate of an IA can vary significantly between different master lots from the same manufacturer. [27] However, we did not find such difference in HIV seropositivity on repeat ELISA from a different lot using the same manufacturer’s kit (repeat IA-1) and thus did not include this in our proposed algorithm.

A sample with a BFR result on one IA may give a negative result when retested with another assay when there is a minimal overlap (~5%) between the BFR populations of different IAs. [28] Therefore BFR results are often assay specific. This second IA being more specific must have sensitivity similar to that of IA-1. Thus, donor samples found seroreactive by three IAs without incorporating a WB test is a highly efficient and cost effective strategy in confirming true HIV positive blood donors who can be notified of their reactive status. Similarly donors who are non-reactive by both IA-2 and IA-3 may not be notified. This will prevent unnecessary notification to donors found false reactive by initial primary screen. Though these donors would not be notified, a strict follow up of their seroreactive behavior during subsequent donations is essential.
Management and notification of donors with indeterminate anti HIV WB becomes a critical situation for blood collection services. The common cause of indeterminate results on WB assays in voluntary blood donors is non-specific reactivity to gag proteins (p24, p55 and p17). In our study we found p55 as the most common pattern seen on WB indeterminate results. Notification and follow up of ELISA reactive but WB indeterminate donors will help in clarifying their HIV status. In the developed countries, discriminatory tests for donor notification are based on highly sensitive and specific NAT assays. Confirmatory test using NAT on HIV screen reactive blood donors may not be feasible currently in under resourced countries. Fourth generation ELISA using combined antigen/antibody (p24/HIV antibody) assays have improved specificity and represent an alternative to separate antigen determination in diagnostic laboratory, blood donor screening and possibly to NAT in (mini)pools. Such studies from India on screen reactive donors may add new information to our proposed algorithm.

HIV infection has a tremendous psychosocial impact in Indian society. The stigma of being informed about HIV positive status creates personal and family stress. Our study suggests that HIV seroreactivity by sequential immunoassays (IA-1, IA-2 and IA-3) prior to donor recall results in decreased referral to VCTC (comparable to WHO Strategy-III) as compared to single IA (WHO Strategy-I) being followed currently in India. Moreover, this strategy will re-pose donor confidence in our blood transfusion services and strengthen voluntary blood donation program.

References


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