prevalence of syphilis in IDUs on the basis of positive VDRL and TPHA test was 1.3% (2/150). This is lower than the prevalence rates reported in studies carried out in Delhi (6.09%), Germany (3.3%) and Bangladesh (23%). The possible explanation for this could be the difference in the subgroup of population studied and the diagnostic tests employed. In addition, region, gender, ethnic factors and socioeconomic factors which influence the development of sexual behaviour do play a big role in the prevalence of syphilis.

It has been reported that more than 10% of injecting drug users have false positive test results in titres >8. However, we observed false positive reactions in only 2.7% IDUs and that too in titre <8. The present study thus shows that in our financially constrained set-up VDRL test can be reliably employed for screening of IDUs. However, studies on larger scale are required to reach exact conclusion.

References

*N Jindal, A Aggarwal
Department of Microbiology, Govt. Medical College, Amritsar, Punjab -143 001, India

*Corresponding author (email: <neerjarajender@hotmail.com>)
Received: 26-09-07
Accepted: 08-10-07

Experience with a Fourth Generation Human Immunodeficiency Virus Serological Assay at a Tertiary Care Centre in South India

Dear editor,

The human immunodeficiency virus (HIV) epidemic continues to be a burden globally especially in developing countries. Though there is dramatic progress in the diagnostic methodologies, the detection of antibodies continues to be the mainstay of diagnosis in most of these countries. The fourth generation HIV serological assays have been in place in developed countries for a few years. However, they have been introduced in the developing countries only recently. In this study, we have looked at the performance of a fourth generation HIV assay in real-time.

A total of 11, 583 samples received for HIV screening during 2006 September through December were included in this study. The samples were received from patients who were seen in the out patient facility for any procedures/surgery, antenatal screening or with suspicion of HIV infection. The HIV testing was not anonymous or unlinked because counselling services were offered to those in need. In our hospital, a general consent is obtained for all investigations, including blood tests. The HIV antibody testing was done with the sole purpose of better management of HIV infected individuals; the required medical or surgical treatment was never withheld from any patient. The hospital policy is to refer HIV positive individuals to the infectious disease clinic, where counselling services are offered and further course of action determined. This has been the approach followed at our hospital in accordance with the revised guidelines for HIV counselling, testing and referral by CDC (Centre for Disease Control) as it recommends routine HIV testing of all clients from area where the prevalence is >1%.

All the samples were first screened by the Abbott AxSYM HIV Ag/Ab combo test (Abbot, Wiesbaden, Germany). All negative samples were declared negative. All the HIV reactive samples were tested by another fourth generation assay, Vironostika HIV Uni-Form II Ag/Ab (Biomerieux, Boxtel, Netherlands). If the results of the above two assays were concordant those samples were further tested by a second generation assay, HIV TRIDOT (J Mitra and Co. Pvt. Ltd, New Delhi, India). Samples reactive in all the three assays were reported as positive for HIV antibody. Samples which showed discrepant results in the first two assays were further tested in duplicate by Abbott AxSYM (Abbot, Wiesbaden, Germany) and subsequently by two more serological assays Genscreen HIV Ag/Ab, (BIORAD, Marnes LA Coquette, France), Retrocheck (Qualpro Diagnostics, Goa, India) or Genscreen HIV-1/2 (BIO
A total of 171 samples were found reactive by the Abbott AxSYM during the screening. Among these, 112 samples (Group A) were reactive by the second (Vironostika HIV Uni-Form II Ag/Ab) and third (HIV TRIDOT) assay as well and then declared as positive for HIV antibody. The remaining 59 samples showed discordant results in the first two assays. Among these 59 samples (Group B) one sample was negative by repeat testing (in duplicate) in Abbott AxSYM and declared negative and three more samples were declared negative by testing with two more third generation assays, Retrocheck (Qualpro Diagnostics, Goa, India) and Genscreen HIV-1/2 (BIO-RAD, Marnes LA Coquette, France).

When 32 available samples out of remaining 55 were tested for HIV-1 and HIV-2 RNA by real-time PCR, all except one were found negative. These 31 samples were declared negative for HIV. One plasma sample was found negative by Abbott AxSYM and hence not included for HIV RNA testing. For the remaining 23 individuals fresh plasma samples were not available. However original serum samples were available for 18 individuals and those samples were tested by one more fourth generation assay (Genscreen HIV Ag/Ab, BIORAD, Marnes LA Coquette, France) and a third generation assay (Retrocheck Qualpro Diagnostics, Goa, India). All these 18 were found negative by three assays and declared negative. The number of samples tested by each test and their results are also shown in the figure.

The serological window period in HIV is considered to be four to six weeks. The assays that can detect p24 antigen along with antibodies can further reduce this time period. It is reported that the fourth generation HIV serological assays, compared to third generation assay, will reduce the diagnostic window period by further two to five days.[3]

Currently, more and more laboratories in India have started using fourth generation assays routinely for HIV screening. Hence, it is important to know the in-use performance of these assays.

As these assays are highly sensitive there is higher chance of false positive results. In our study it is found that the mean ± SD of the sample rate / cutoff rate of the group A (n = 112) samples was 39.44 ± 12.68 (range 7.1-71.82). All the samples except for two (98.2%) had an S/CO of > 10.00. The mean ± SD of the S/CO of the group B (n = 58) samples was 1.91 ± 1.77 (range 0.9-11.97). All the 58 samples except one (98.3%) had an S/CO of less than 10, while for one of the samples it was 11.97. Calculating an arbitrary cut off of mean S/CO ± 3 SD of the 58 negative samples, the value would be 7.2. All the 58 samples except one (98.3%) had an S/CO of less than 7.2. Among the 170 repeatedly reactive samples by Abbot AxSYM system only 112 (67.5%) were found to be truly reactive for HIV. However, while taking the arbitrary cutoff of the S/CO value 7, among 113 samples 112 (99.1%) were positive for HIV. In this study we could not calculate the accuracy indices of the assays as the negative samples were not tested by any other gold standard assays like HIV-1 and 2 RNA due to financial constraints.

Several HIV positive cases during the seroconversion period have been reported to be detected only by fourth generation assays or HIV RNA detection and these samples turn out to be negative by a variety of third generation assays.[4] Conversely, there are reports of third generation assays.

**Figure:** Algorithm used and number of samples tested in the study

<table>
<thead>
<tr>
<th>Sample</th>
<th>AxSYM testing (112)</th>
<th>REPORT NEGATIVE FOR HIV ANTIBODY</th>
</tr>
</thead>
<tbody>
<tr>
<td>REACTIVE (112)</td>
<td>REACTIVE (112)</td>
<td>REPORT POSITIVE FOR HIV ANTIBODY</td>
</tr>
<tr>
<td>REACTIVE (112)</td>
<td>REACTIVE (112)</td>
<td>REPORT POSITIVE FOR HIV</td>
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<tr>
<td>REACTIVE (112)</td>
<td>REACTIVE (112)</td>
<td>REPORT NEGATIVE FOR HIV</td>
</tr>
</tbody>
</table>

*The number in parenthesis denotes number of samples tested/reactive/negative; ‡Three samples declared negative by testing with another 4th generation and two other 3rd generation assays; †1 sample not tested by PCR as the plasma sample was negative by Abbot AxSYM.*

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EIA showing similar sensitivity to fourth generation EIA (100%) and higher specificity (97.5 versus 95.1%). In our study we were unable to identify any individual who was truly positive only by a fourth generation assay. Based on our findings we believe that any sample which shows an S/CO of <7 may be a false positive result by the Abbot AxSYM system and can be declared negative by testing with two other fourth/third generation assays. However, any sample with >7 S/CO should be considered as positive and if there is any discrepancy with other fourth generation assays it should be sent for molecular assay for the detection of HIV RNA.

References


*R Kannangai, M Moorthy, AJ Kandathil, J Sachithanandham, V Thirupavai, G Nithyanandam, G Sridharan
Department of Clinical Virology, Christian Medical College, Vellore - 632 004 Tamil Nadu, India

An Explanation in Nanostructure Level Based on the View of Energy Change for G333d Mutation Relating to Drug Resistance in HIV-1 Reverse Transcriptase

Dear editor,

Human immunodeficiency virus (HIV) infection is an important infection affecting a million of world population. The best present way in coping with the patients with HIV is controlling of disease with antiretroviral drugs. However, a new emerging problem of antiretroviral drug usage is the problem of drug resistance. Standardised surveillance of transmitted and treatment-associated HIV drug resistance is critical to the success of antiretroviral therapy expansion in developing countries. DNA polymerase and RNAse H activities of HIV reverse transcriptase (RT) have been recognized as potential targets for antiretroviral therapy for many years. The development of medicines targeting the DNA polymerase activity has been highly successful, with currently more than ten drugs approved for the treatment of HIV infection and more candidates in preclinical and clinical development. However, the drug resistance due to the mutation of HIV-RT becomes a new interesting problem in HIV medicine. Mutations at G333D mutation within the reverse transcriptase (RT) gene cause resistance to both zidovudine (AZT) and lamivudine (3TC) in a background of mutations associated with loss of sensitivity to both drugs. HIV strains containing both reverse transcriptase (RT) mutations are resistant to all of the approved NNRRTI drugs. In this work, the author simulated for the required energy corresponding to the G333D mutation of HIV-RH. This study can give an explanation in nanostructure level based on the view of energy change.

This is a calculation-based study. The quantum chemical analysis for overall reaction was performed according to the classical bonding theory. Basically, each chemical reaction possesses its specific reaction energy. The primary assumption in this study is that the required reaction energy for the pharmacological reaction between antiretroviral drug and HIV-RT is equal to “A” kCal/mol for one mole of resultant complex. This parameter is a constant parameter. The net energy requirement in kCal for a reaction depends on the amount of two substrates, one mole of antiretroviral drug and one mole of HIV-RT. Here, another primary assumption is the amount of the two substrates is equal to “B” g and “C” g for antiretroviral drug and HIV-RT in general reaction with wild type of HIV-RT. However, the significant change in the mutants of HIV-RT is the amino acid, which disturbs the amount of the substrate HIV-RT. In this work, the theoretical simulation for the two mutants was performed. Calculation for the amount of HIV-RT in G333D mutation was done. Further calculation for the net energy requirement for a reaction was performed. For the G333D mutation, the main change is Gly (weight = 75.07 g/mol) to Asp (weight = 133.10 g/mol). The change in molecular weight due to this mutant and its corresponding net energy requirement are calculated and presented in the Table. The net energy requirements of G333D mutation is less than wild type.