Original Article

Extensive tests for extermination: Need for incorporation of molecular detection methods of human immunodeficiency virus in screening algorithm in tertiary hospitals in India

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Abstract

Context: A downward trend is being observed in the incidence of human immunodeficiency virus (HIV) infection in India due to strategic activities of National AIDS Control Organisation (NACO) in the last 24 years. Opt-out testing has consistently shown high seroprevalence in our tertiary care center. **Aim:** This study aims to audit opt-out testing and compare various commercial test kits used to detect HIV seroprevalence in patients in our tertiary care institute and suggest new algorithm for HIV testing in tertiary hospitals in India. **Materials and Methods:** Retrospective analysis of 30,021 samples tested in Department of Immunopathology using opt-out testing delinked from the NACO-sponsored testing for Integrated Counselling and Testing Centre (ICTC) was performed. Study population comprised of presurgery and emergency patients which at the time of our reporting were not included in ICTC testing. **Results:** Microlisa was the first test performed on 76% samples. 1.02% cases were reactive only with Microlisa and negative with other rapid kits hence were reported as negative, according to NACO scheme of reporting. Advanced testing algorithm followed by centre for disease control (CDC) showed that 80% of these 4th-generation positive and rapid test-negative patients turned out to be acute HIV infections on molecular testing. **Conclusion:** Patients in tertiary referral center constitute high-risk population and should be screened with 4th-generation enzyme-linked immunosorbent assay which incorporates p24 antigen. Those which are found indeterminate should have molecular testing by nucleic acid amplification test or real-time polymerase chain reaction, as our study has demonstrated that 1.02% of these cases may harbor acute HIV infection.

Key words: Human immunodeficiency virus, molecular test, screening

INTRODUCTION

The incidence of human immunodeficiency virus (HIV) in our tertiary care center in North India has always been higher, using the opt-out strategy to cover presurgery, sick children, and emergency situations; 2.1%[1] versus 0.27%[2] in the general population. The opt-out was performed according to CDC guidelines, using 4th-generation

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enzyme-linked immunosorbent assay (ELISA). Due to delay in purchase of the same, intermittently other approved rapid kits were also used. This gave us an opportunity to compare the performance of all rapid kits with that of 4th-generation ELISA and develop an algorithm for testing for HIV/AIDS in a tertiary care hospital in the context of North India. Our retrospective analysis was also able to demonstrate a large number of patients that may harbor acute HIV infection and thus remain undetected by National AIDS Control Organisation (NACO)-sponsored screening strategies utilizing rapid tests. Thus, in this study, we compare different test kits, elaborate test principles involved, and suggest important alterations in screening algorithms in tertiary hospital settings.

MATERIALS AND METHODS

Ethical justification: This was a retrospective analysis of tests performed on the basis of opt-out screening which does not require any written consent. No additional invasive procedure was performed, and the identity of patients has not been disclosed.

A retrospective study of patients who had undergone HIV testing was done from January 2009 to December 2013 in the Department of Immunopathology at a tertiary care center in North India. During this period, a total of 30,021 serum samples were received from various units of our institute for HIV screening, namely emergency ward, pediatric ward, surgical wards, and gynecology wards. The patients to be tested were decided by the clinicians based on clinical presentation or as a part of preprocedure testing.

The HIV testing was performed independently from NACO, using the various kits: Microlisa (J Mitra and Co. Pvt. Ltd., India), Immunocomb (Inverness medical innovations Orgenics Ltd., Israel; Version: 60432002/EH17/OR), Genedia HIV Ag-Ab ELISA (Green Cross Medical Science Corp., Korea), HIV Tridot (Diagnostic enterprises, India), and SD Bioline HIV 1/2 3.0 test (Bio Standard Diagnostics Pvt. Ltd, India).

The tests were performed with one kit which, if found reactive, was repeated with two other kits. The sample was reported as positive and referred to Integrated Counselling and Testing Centre (ICTC) if all the three kits were reactive. If the test was found to be nonreactive with other two kits, it was reported as negative. In case, the test was reactive with two kits, the patient was advised to get a repeat test after 2–4 weeks. Molecular testing could not be performed in these cases as the same was not available in the department during the study period.

Data regarding test kits used and reactive samples were collected from the records, and the sensitivity of each kit was calculated and compared using Chi-square test.

RESULTS

Out of 30,021 samples which were screened for HIV during the study period, 627 (2.1%) were found to be reactive. In the year 2009, 77 samples were reactive out of a total of 4189 samples (1.8%). In 2010, 136 samples were reactive out of 5818 samples (2.3%). In 2010, samples were reactive out of 5818 samples. In 2011, 132 samples were reactive out of 5780 samples (2.3%). In 2012, 139 samples were reactive out of 6814 samples (2.0%). In 2013, 143 samples were reactive out of 7420 samples (1.9%).

Microlisa (fourth-generation) was the most frequently used kit and was used as the first screening test to screen 22,998 samples, out of which 692 (3%) were reactive. Any sample which gave a negative result with this test was reported as nonreactive and was not subjected to further screening. However, if a sample was reactive by Microlisa, then it was subjected to further screening by two other kits using different antigens. Of the 692 reactive samples, 457 samples gave a positive result with two other kits also and were finally reported as reactive. Thus, 235 samples which were positive only by Microlisa and negative by two other kits were finally reported as nonreactive. No molecular testing was performed on these 235 samples.

In 2009–2010, Immunocomb and SD kit were also used in addition to Microlisa to perform HIV screening while in 2011–2013, Genedia and Tridot were used.

Immunocomb was used as the first screening test in 6500 samples, of which 159 (2.4%) were reactive. SD kit showed reactive result in 2 out of 211 (0.95%) samples where it was used as the first screening test. One hundred and ninety samples screened using Genedia as the first screening test showed reactivity in 6 samples (3.2%) whereas Tridot gave reactive result in 3 out of 122 samples (2.5%) using the latter as the first screening test [Table 1].

The rate of detection of HIV infection by Microlisa was more as compared to Immunocomb and was statistically significant with $P = 0.02$. There was no significant difference in the rate of HIV detection among various other kits used.

Table 1: Various test kits and the rate of human immunodeficiency virus detection using these kits

Kit	Principle	Detects	Solid phase	Sensitivity and specificity (%)	Reactive test in present study (%)
Microlisa	Fourth-generation EIA based on "Sandwich ELISA"	Antibodies to HIV-1 and/or HIV-2 and HIV-1 p24 Ag	The microtiter plate is coated with HIV envelope proteins gp41, C terminus of gp120 for HIV-1, and gp36 for HIV-2 and anti-HIV-1 p24 antibodies	100 and 100	692/22,998 (3)
Immunocomb	Indirect solid-Phase EIA	Antibodies to HIV-1 and/or HIV-2	Card with 12 projections (teeth). Each tooth is sensitized at 3 spots. The upper spot contains goat antibodies to human immunoglobulin (internal control); the middle and the lower spots contain HIV-2 and HIV-1 synthetic peptides	100 and 99.4	159/6500 (2.4)
Genedia	Sandwich ELISA	Antibodies to HIV-1 gp41, HIV-1 group O gp41 and HIV-2 gp36 and, HIV-1 p24 antigen	Wells coated with recombinant HIV-1 gp41 Ag, recombinant HIV-1 group O gp41 Ag, recombinant HIV-2 gp36 Ag and monoclonal HIV-1 p24 antibodies	100 and 99.7	6/190 (3.2)
Tridot	Rapid, visual immunoassay test	Antibodies (IgG) to HIV-1 and HIV-2	HIV-1 and 2 antigens (gp41, C terminal of gp120 of HIV-1 and gp36 of HIV-2) immobilized on an immunofiltration membrane	100 and 100	3/122 (2.5)
SD Bioline	Rapid immunochromatographic third generation of one step anti-HIV 1/2 test	Antibodies of all isotypes (IgG, M, A) specific to HIV-1 and HIV-2	Membrane strip precoated with recombinant HIV-1 capture antigen (gp41 and p24) on test band-1 region and with recombinant HIV-2 capture antigen (gp36) on test band-2 region	100 and 99.3	2/211 (0.95)

HIV=Human immunodeficiency virus; EIA=Enzyme immunoassay; ELISA=Enzyme-linked immunosorbent assay

DISCUSSION

HIV infection is a major global health problem. HIV is a nontransforming human retrovirus of lentivirus family. In India, there is prevalence of two genetically different forms of this virus: HIV-1 and HIV-2. The HIV-1 virion consists of an electron-dense, cone-shaped core composed of major capsid protein p24, nucleocapsid protein p7/p9, two copies of genomic RNA, and three viral enzymes (protease, reverse transcriptase, and integrase). The core is surrounded by a matrix protein p17. The outer most lipid envelope is derived from host cell membrane and contains two glycoproteins which are indispensable for HIV infection of cell. p24 antigen is the most easily identified viral antigen, and thus, most of the antibodies used for HIV screening target this antigen.[3]

A total of 34.2 (31.8–35.9) million people were living with HIV in 2011, according to WHO.[4] In 2001, the prevalence of adult HIV in India was 0.41% while in 2006, the prevalence dropped to 0.35%. In 2011, the estimated prevalence further decreased to 0.27%, but still amounting to 20.9 lakh persons.[2] The dynamics of HIV-1 viremia after infection and the sequence of appearance of different laboratory markers have been established after analyzing specimens from seroconversion panels. Figure 1 shows the sequential appearance of laboratory markers for HIV-1 infection.[5-8]

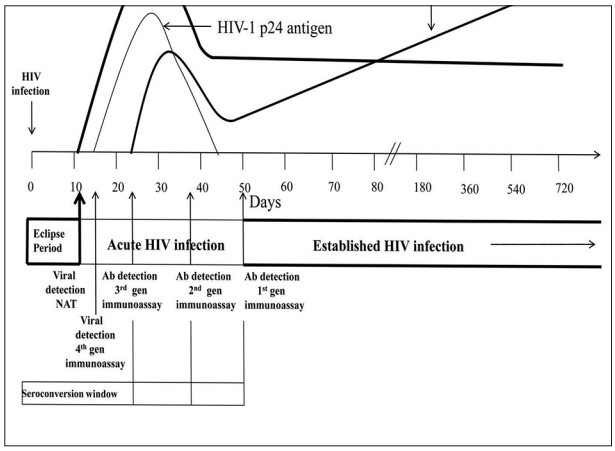

Figure 1: Sequence of appearance of laboratory markers for human immunodeficiency virus-1 infection (Published in Laboratory Testing for the Diagnosis of human immunodeficiency virus infection: Updated Recommendations. Centre for disease control. June 27, 2014 http://stacks.cdc.gov/view/cdc/23447)

NACO was established in India in 1992 to tackle the HIV epidemic, and a comprehensive National AIDS Control Programme (NACP) was started in the following year.[2] NACP I focused on spreading awareness about AIDS and expanded sentinel surveillance. NACP II, introduced in November 1999, focused on behavior change with the establishment of Voluntary Counselling and Testing Centres

and expansion of Prevention of Parent-to-Child Transmission programme (PPTCT). National AIDS Prevention and Control Policy, adopted in 2002, focused on targeted interventions for high-risk groups (HRG) in states with high prevalence, Adoption of National Blood Policy, and launch of National Adolescent Education Programme. It also introduced national Anti-Retroviral Treatment programme, setting up of the National Council on AIDS, chaired by the Prime Minister, and setting up of State AIDS Control Societies in all states.[2] NACP-III, launched in July 2007, integrated care, support, and treatment services with prevention efforts among HRG as well as general population with aim of stopping and reversing the HIV epidemic in India. ICTCs were established with improvement of PPTCT services, strengthening of State AIDS Control Societies and District AIDS Prevention and Control Units, and establishment of Technical Support Units at National and State level to help in the program monitoring and technical areas.[1] At present, NACP IV aims to reduce new infections by 50% (2007 Baseline of NACP III), provide comprehensive care and support to all HIV/AIDS-infected persons, and increase access to treatment services. To achieve these objectives, NACP has proposed some strategies which aim to intensify and consolidate prevention services, focusing on HRG and vulnerable population, expanding information education and communication services for general population and HRG with emphasis on behavior change and demand generation. Other strategies include building capacities at national, state, district, and facility levels and strengthening Strategic Information Management Systems.[2]

As stated by NACO, HIV screening/testing can be done for (i) clinical purposes, (ii) research, (iii) seroprevalence studies, (iv) ensuring safety, and (v) voluntarily after counseling for behavior change. The status of HIV infection in an individual is determined by laboratory diagnosis. NACO advocates use of three different kits involving different antigen system and/or different principle of test for detection of HIV infection at ICTCs and PPTCT centers. The first screening test has the highest sensitivity while the second and third tests are with the highest specificity. If the test gives nonreactive result, the sample is considered negative. When the test result is positive, the sample is tested with other two kits and reported positive only if reactive by all the three tests performed. If only two tests are reactive, and the third is nonreactive, it is reported as indeterminate and the patient is called back for repeat testing after 2–4 weeks.[9] NACO uses rapid kits to detect seropositivity. Care has to be taken that at least two kits selected should be able to differentiate between HIV-1 and 2. If the test results are repeatedly indeterminate with these kits or there is difficulty in distinguishing between HIV-1 and 2, Western blot test is advised. NACO has also introduced routine nucleic acid amplification testing (NAT) for HIV screening in all samples received in blood banks to detect cases which may still be in window period.[9] However, the use of Western Blot in HIV has greatly reduced due to availability of good rapid tests and IV generation ELISA.[10]

As per CDC guidelines, HIV screening is recommended for patients between 13 and 64 years of age in all healthcare settings after notifying the patients about the same, unless the patients themselves decline (opt-out screening). HIV testing at least once a year is also recommended for people at high risk for HIV infection. No separate written consent is required for screening which should be included in general consent for medical care. It is further recommended that there is no requirement for prevention counseling with HIV diagnostic testing or screening programs. HIV screening is mandatory in prenatal screening tests, and repeat screening has to be performed in the third trimester in areas with high rates of HIV infection among pregnant women.[11]

Figure 2 depicts the algorithm suggested by CDC for HIV testing. The inference obtained from this algorithm helps in reassuring patients who are uninfected, identifying patients who will likely benefit from treatment, and in reporting evidence of HIV infection to public health authorities.[10] Patients with high-risk behavior and having congruent clinical syndrome should be kept under a high level of suspicion for acute HIV infection. Such patients

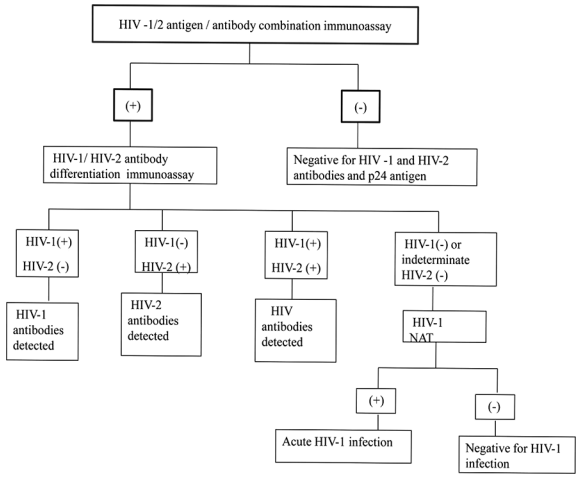

Figure 2: Algorithm suggested by CDC for human immunodeficiency virus testing. (+): Reactive test result, (-): Nonreactive test result, NAT: Nucleic acid test

suspected of acute HIV syndrome should undergo a plasma RNA test in addition to HIV antibody test.[5]

ELISA is a commonly used test to detect HIV antibodies in an individual. The first generation assay used viral lysate as antigen ligand while second and third generation assays use recombinant antigens and synthetic peptides, respectively. The fourth-generation ELISA utilizes recombinant as well as synthetic antigens which detect p24 antigen and antibodies to HIV envelope proteins simultaneously. The first-generation test was sensitive but not very specific. Nowadays, various rapid kits have also been developed to detect HIV infection based on the principle of agglutination, immunochromatography, and immunoconcentration and ELISA. These tests are easy to perform and are less time-consuming. These are equally sensitive and specific as ELISA.[9]

HIV infection in India is exhibiting a downward trend due to strategic activities of the NACO since 1992, which include education, counseling, screening of target population, and treatment of afflicted patients. The testing algorithm was published in 2007. There is a need for revision in the testing algorithm in the light of the present study.

In our study, which is from a tertiary care referral center in North India, HIV prevalence was reported to be 2.1%. This observation is similar to findings of previous two studies from our institute, but more than the national prevalence of 0.27%.[2,12,13] This may be attributed to the fact that the study has been conducted in a tertiary care institute where the prevalence is expected to be high, and 22,998 (76%) of samples were screened by a more sensitive 4th-generation ELISA. Also, the cohort of patients tested at the tertiary care center includes patient who are already in the risk category.

In our study, only 2 patients were seropositive for HIV-2, amounting to the incidence of 0.007%. This is even less than the incidence of 0.03% as seen by Tadokar and Kavathekar.[14] We observed a very low prevalence of HIV-2 in our center.

As laboratory tests are the mainstay for diagnosing HIV infection, a variety of international and local diagnostic kits are available for the same. Hence, it is important that sensitivity and specificity of these kits is evaluated before using them in routine practice.[15] An ideal assay should be highly sensitive and specific, easy to perform, reasonably priced, less time-consuming, have long shelf life of reagents, and should not require sophisticated equipments.[16]

Anuradha *et al*. compared Microlisa HIV kit with UBI HIV 1/2 EIA kit and found that the sensitivity and specificity of Microlisa was 100%.[15] Sudha *et al*. compared fourth-generation ELISA with TRIDOT Rapid HIV test for detecting HIV in hospital-based setting, especially in emergency situation. They found discordant result in seven out of 23,609 sera tested. Among these, six cases were found to be reactive only by fourth-generation ELISA while one case was found to be reactive only by Tridot.[17] In our study, 235 out of 22,998 (1.02%) samples were positive only by Microlisa and negative by two other kits, when Microlisa was used as primary screening kit. Two of the rapid tests used in our study (Immunocomb and Tridot) detect only HIV antibodies and not p24 antigen. Thus, when only p24 antigen is present in the blood and antibody levels are undetectable (window period), there is a possibility of missing recent infection. Moreover, Tridot kit detects IgG antibodies only whereas SD Bioline kit detects IgG, A, and M also. This reduces the serological window period by 1 week in HIV-infected individuals.[18] However, in our study, the percentage of cases reactive with SD kit is lesser than that from other kits; this may be explained by less number of samples on which SD kit was used.

Everett *et al*. found higher specificity and only slightly reduced sensitivity of parallel rapid tests as compared to ELISA-based algorithm in field-based settings. As ELISA used in their study also detected p24 antigen, it had greater sensitivity than the rapid tests. The lower specificity of ELISA could be attributed to cross-reactivity with other infections endemic in that region. However, ELISA-based tests are not feasible in field conditions in developing countries because they require longer time, higher cost, specialized equipment, and skilled workforce.[19]

Maity *et al*. compared performance of ELISA and rapid kits for detecting HIV and found that ELISA is a good tool for HIV screening but rapid tests may be added to detect false-positive cases as they have higher specificity.[20]

Waheed *et al*. found that sensitivity and specificity of SD Bioline was 100% and 98.4%, respectively, as compared to 100% sensitivity and specificity for Vironostika ELISA. Anti HIV Capillus had sensitivity of 94.6% and specificity of 100%.[21]

Kannangai *et al*. studied the performance of four rapid kits (SD Bioline, Qualpro's Rapid Immunoconcentration test, Qualpro's rapid test, and CombAids - RS) and observed that all the assays showed 100% sensitivity and specificity in the range of 98.6%–100%.[18]

Cases with acute HIV infection form a significant part of HIV epidemic, yet these cases escape detection as there is no antibody production in the initial stages of HIV infection. In addition, there is inherent inability of the routine rapid tests to detect HIV RNA or p24 antigen, and there are logistical and cost issues related to routine use of p24 antigen and HIV RNA assays. Even in our study, molecular testing was not performed as the same was not available in the department during the study period. However, since the stage of acute HIV infection plays a crucial role in transmission of HIV, an acute HIV test which fulfills the "ASSURED" criteria (Affordable, Sensitive, Specific, User-friendly, Robust and rapid, Equipment-free, and Deliverable) and helps in detection of these cases is an essential requirement worldwide.[22,23]

Rapid tests have good reliability for HIV screening. However, cases in which seroconversion has not yet occurred may be missed. In the present study, 1.02% were positive only by Microlisa and hence were reported as negative by the scheme of reporting followed. Molecular testing was not done, which was a limitation of the study. Advanced testing algorithm followed by CDC shows that 80% of these 4th-generation positive and rapid test-negative patients turn out to be acute HIV infections on molecular testing.[10] Hence, molecular tests like NAT or real-time polymerase chain reaction should be used to detect acute HIV cases which escape detection in rapid tests. If RNA testing is not available, a follow-up should be conducted in 2–4 weeks.[24] Furthermore, global funding agencies should focus on empowering tertiary care centers for molecular diagnosis of HIV as cases of acute HIV infection may continue to infect the general population, if left undetected and untreated.

CONCLUSION

Rapid tests provide an accurate and efficient way of screening HIV infection in field-based settings. However, 4th-generation ELISA should be used in hospital-based settings where 1.02% of screened patients have been found to harbor acute HIV infection and remain undetected by rapid methods. Molecular testing should be incorporated into the testing algorithm in these high-risk cases to detect acute HIV infection. Figure 3 shows the proposed testing algorithm for India, in a tertiary care setup.

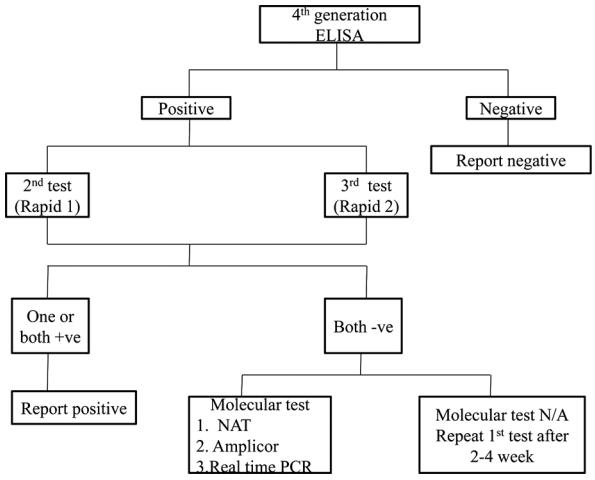

Figure 3: Proposed testing algorithm for tertiary care centers in North India

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. National AIDS Control Organisation. NACO Annual Report 2011-12; 2012. Available from: http://www.nacoonline.org/upload/publication/AnnualReport/NACO_AR_Eng%202011-12.pdf. [Last accessed on 2012 Oct 30].
2. National AIDS Control Programme Phase IV (2012-2017). Strategy Document. Available from: http://www.naco.gov.in/upload/NACP%20-%20IV/NACP-IV%20Strategy%20Document%20.pdf. [Last accessed on 2019 Feb 18].
3. Kumar V, Abbas AK, Aster JC. Diseases of the Immune system. In: Robbins and cotran Pathologic Basis of Disease.9th ed. Elsevier:New Delhi 2014. p. 243-56.
4. World Health Organization. Global Summary of the HIV/AIDS Epidemic. World Health Organization; December, 2011. Available from: http://www.who.int/hiv/data/en/. [Last accessed on 2012 Oct 30].
5. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, *et al.* Dynamics of HIV viremia and antibody seroconversion in plasma donors: Implications for diagnosis and staging of primary HIV infection. AIDS 2003;17:1871-9.
6. Owen SM, Yang C, Spira T, Ou CY, Pau CP, Parekh BS, *et al.* Alternative algorithms for human immunodeficiency virus infection diagnosis using tests that are licensed in the United States. J Clin Microbiol 2008;46:1588-95.
7. Masciotra S, Luo W, Youngpairoj AS, Kennedy MS, Wells S, Ambrose K, *et al.* Performance of the alere determine™ HIV-1/2 ag/Ab combo rapid test with specimens from HIV-1 seroconverters from the US and HIV-2 infected individuals from ivory coast. J Clin Virol 2013;58 Suppl 1:e54-8.
8. Busch MP, Satten GA. Time course of viremia and antibody seroconversion following human immunodeficiency virus exposure. Am J Med 1997;102:117-24.
9. National AIDS Control Organisation. Guidelines for HIV Testing. Available from: http://www.nacoonline.org/upload/Policies%20&%20Guidelines/5-GUIDELINES%20FOR%20HIV%20TESTING.pdf. [Last accessed on 18 Feb 2019].
10. Centers for Disease Control and Prevention and Association of Public Health Laboratories. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations; Published 27 June, 2014. Available from: http://www.stacks.cdc.gov/view/cdc/23447. [Last

accessed on 18 Feb 2019].

11. CDC HIV/AIDS Science Facts: CDC Releases Revised HIV Testing. Recommendations in Healthcare Settings. September, 2006. Available from: http://www.cdc.gov/hiv. [Last accessed on 18 Feb 2019].
12. Minz RW, Singh S, Varma S, Mathuria SN, Aggrawal R, Sehgal S, *et al.* Relevance of opt-out screening for HIV in emergency and pre-surgery patients in a tertiary care center in Northern India: A pilot study. Indian J Pathol Microbiol 2010;53:287-9.
13. Minz RW, Khairwa A, Aggarwal R, Kaur H, Singh S, Sharma A, *et al.* Cost analysis and benefits of opt-out HIV testing at a tertiary care centre in Northern India. Int J STD AIDS 2014;25:341-7.
14. Tadokar VS, Kavathekar MS. Seroprevalence of human immunodeficiency virus type 2 infection from a tertiary care hospital in Pune, Maharashtra: A 2 year study. Indian J Med Microbiol 2013;31:314-5.
15. Anuradha K, Ponamgi SP, Lakshmi V. Evaluation of two commercially available anti human immunodeficiency virus antibody ELISA kits using clinical samples. Indian J Med Microbiol 2002;20:102-4.
16. Raj AA, Kannangai R, Daniel DP, Babu PG, Ramalingam S. Evaluation of commercial microwell ELISA and microparticle EIA for the detection of HIV antibody. Indian J Med Microbiol 2000;18:21-4.
17. Sudha T, Teja VD, Gopal M, Rajesh M, Lakshmi V. Comparative evaluation of TRI-DOT rapid HIV test with fourth-generation ELISA for the detection of human immunodeficiency virus. Clin Microbiol Infect 2005;11:850-2.
18. Kannangai R, Prabu K, Vincent AA, Vijayakumar TS, Sridharan G. Performance evaluation of four different kits available in the Indian market, for the rapid detection of HIV antibody. Indian J Med Microbiol 2003;21:193-5.
19. Everett DB, Baisley K, Changalucha J, Vallely A, Watson-Jones D, Cook C, *et al.* Suitability of simple human immunodeficiency virus rapid tests in clinical trials in community-based clinic settings. J Clin Microbiol 2009;47:1058-62.
20. Maity S, Nandi S, Biswas S, Sadhukhan SK, Saha MK. Performance and diagnostic usefulness of commercially available enzyme linked immunosorbent assay and rapid kits for detection of HIV, HBV and HCV in India. Virol J 2012;9:290.
21. Waheed U, Hayat K, Ahmad B, Waheed Y, Zaheer HA. Evaluation of HIV/AIDS diagnostics kits and formulation of a testing strategy for Pakistan. J Clin Virol 2013;56:367-9.
22. Cohen MC, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 infection: Basic, clinical, and public health perspectives. N Engl J Med 2011;364:1-11.
23. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect 2010;16:1062-9.
24. Centers for Disease Control and Prevention. Detection of acute HIV infection in two evaluations of a new HIV diagnostic testing algorithm – United States, 2011-2013. MMWR Morb Mortal Wkly Rep 2013;62:489-94.