International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: 10.7439/ijbar
CODEN: IJBABN
Original Research Article

Comparison of analytical sensitivity of HIV diagnostic kits using HIV dilutional panel

G. Bindra*, R. Chhabra, S. Sharma, PSC Anand, B. Bhushan and S. Singh

National Institute of Biologicals, Ministry of Health and Family Welfare, Govt. of India, A-32, Sector 62, Institutional Area, Noida-201307, India

***Correspondence Info:**
G. Bindra
Scientist –III
National Institute of Biologicals (NIB)
(Ministry of Health and Family Welfare)
A-32, Sector 62, Institutional Area, Noida-201307
Phone No.: +91-9868404538, +91-0120-2400022
FAX Number: 0120-2403014
E-mail: gbindra@nib.gov.in

Abstract

An array of tests have been developed over a period of time for screening and diagnosis of HIV infection which include tests for detection of antigen and antibody, viral nucleic acid and the virus itself. Amongst the available tests, serodiagnosis by ELISA is widely being used. It offers a relatively inexpensive procedure with ease of performance and ability to effectively screen a large number of specimens. Our current study is designed to compare the performance of two different HIV diagnostic kits having different coated antigens using HIV dilutional panel. Kit 'X' had synthetic peptides (3rd generation), and the Kit 'Y' had recombinant antigens (2nd generation) coated on the microtitre plates. 10 HIV-1 plasma panel members which were collected from various blood banks were used for comparison of their performance. The plasma samples were diluted in human plasma (base matrix) negative for anti HIV, anti HCV, HBsAg, VDRL and HTLV I/II. Serial dilutions in the range of 10 to10,000 fold for each sample were used for this study. These kits were compared in terms of antibody titre which is defined as the dilution at which the sample OD to cut off (S/Co) value is 1. It was seen that Kit 'X' is more sensitive as compared to Kit 'Y' for all the HIV panel members. This indicates that Kit 'X' had better analytical sensitivity performance as compared to Kit 'Y' since the S/Co $\simeq$ 1.0 of Kit 'X' is at a higher dilution (1:1000) than of Kit 'Y' (1:100). Thus this indicates that dilutional panel can help the manufacturers to design a diagnostic kit which is able to detect the infection at an early stage of disease. It was seen that the S/Co $\simeq$ 1.0 of kit X at a higher dilution

Keywords: recombinant antigens, synthetic peptide, analytical sensitivity

1. Introduction

HIV infection has become a modern day plague as it produces a prolonged, gradually progressive illness that eventually leads to opportunistic infections, malignancy and death. Ever since first AIDS case was reported, the impact of the epidemic on human race has been dramatic.

A variety of tests have been developed over a period of time for screening and diagnosis of HIV infection, these include detection of antigen and antibody, viral nucleic acid and virus detection. Amongst available tests for HIV detection, ELISA is widely being used. ELISA constitutes the most important tool for screening purposes. It offers a relatively inexpensive procedure with ease of performance & ability to effectively screen a large number of specimens.

The period between infection and antibody detection has been progressively shortened with the implementation of more sensitive assay techniques. The degree of sensitivity of ELISA tests for detecting antibodies to HIV has been of concern since long time. The higher the sensitivity of the kit, the higher the probability of detecting true positives. Researchers have used serial dilutions of Western blot confirmed HIV antibody positive sera to compare the sensitivity of various tests and have found some assays to be 10-1000 times more sensitive than others.[1] FDA recommends use of dilutional analysis of known positives for comparing the analytical sensitivity of different HIV antibody assays.[2] Our present work is an expansion of these studies and was designated to compare the performance of two different HIV diagnostic ELISA kits using dilutional panel.

2. Material and methods

2.1 Sample collection and panel preparation

Plasma samples from various blood banks and hospitals were collected and screened for HIV-1. Samples

found to be reactive for HIV-1 but non reactive for other infectious agents like HBsAg, HCV, VDRL and HTLV I/II were used for study. These HIV-1 reactive samples were confirmed by Western Blot and Line Immunoassay. Ten samples were used for this study.

2.2 Plasma dilution preparation

HIV-1 reactive plasma samples were diluted serially 10 fold in human plasma (base matrix) which was negative for anti HIV, anti HCV, HBsAg, VDRL and HTLV I/II. The dilutions were made freshly just before use, under aseptic conditions and ranged from 10 fold to 10,000 fold.

2.3 Kits used for dilutional study

Table 1: Kits used for dilutional study

S. No.	Name of kit	Antigens coated
1.	'X' 3rd generation	Synthetic peptide representing immunodominant epitopes of HIV-1 & HIV-2
2.	'Y' 2nd generation	Genetically engineered HIV-1 env proteins (gp 41 & gp 120), HIV-2 env (gp 36 & gp 105) & HIV-1 gag proteins (p24) coated.

2.4 Assay conditions

All the assays were strictly performed as per manufacturer's instruction. The assay was accepted as per validity criteria for each kit.

3. Results and Observations

The observations are shown in the Fig.1

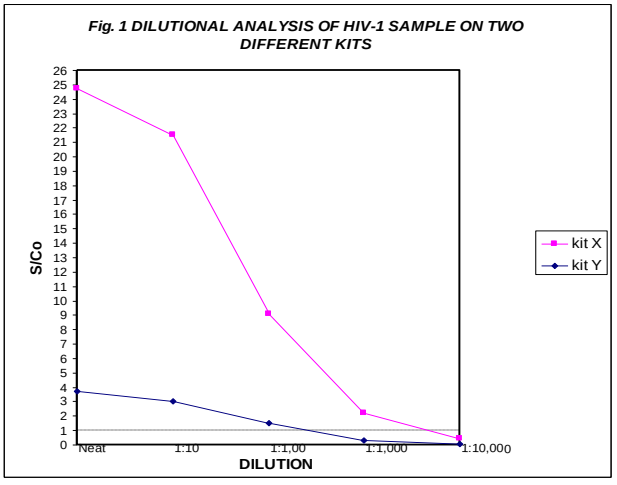
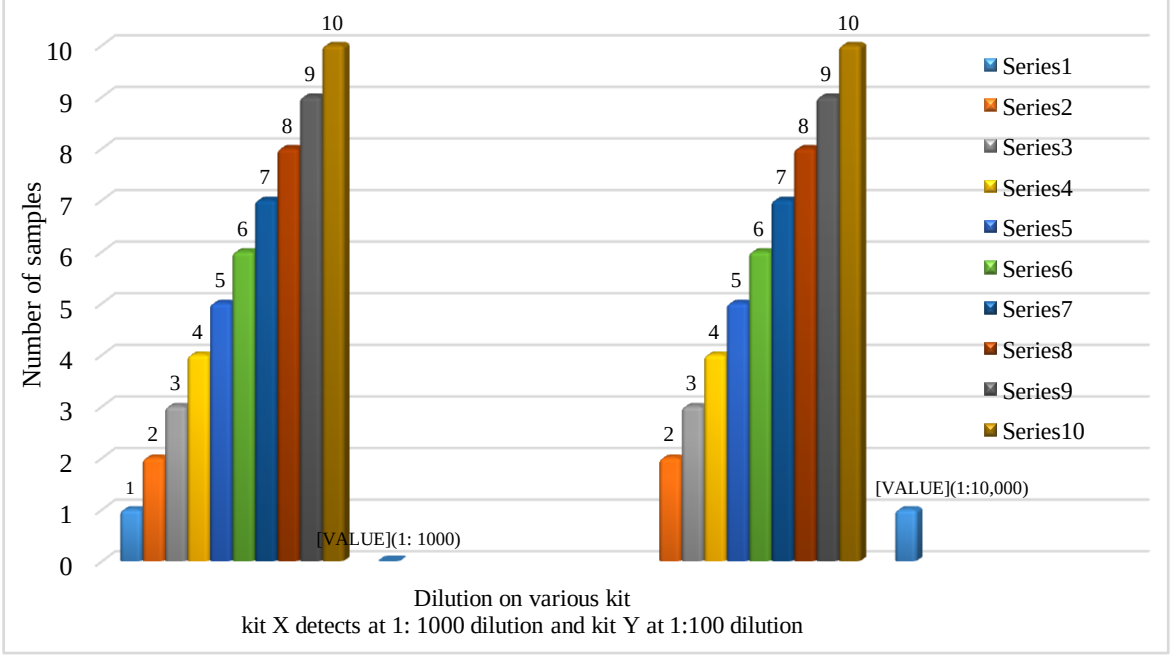


Figure 2: Dilutional analysis of ten HIV -1 reactive samples on two different kits



The diluted reactive samples along with neat reactive samples were tested in duplicate on Kit 'X' and Kit 'Y'. (Fig. 1) The kits were compared in terms of antibody titre which is defined as the dilution at which the sample OD to cut off (S/Co) value is 1. Kit 'X' is more sensitive as compared to Kit 'Y' for all the panel members. This indicates that Kit 'X' has better analytical sensitivity performance as compared to Kit 'Y' since the S/Co ~ 1.0 of it is at a higher dilution (1:1000) than of Kit 'Y' (1:100).

4. Discussion

All the samples were being picked up at higher dilution i.e. 1000 fold by synthetic peptide based assays (Kit 'X') than recombinant assay (Kit 'Y'), which picks most of them upto 100 fold dilution. Hence the data establishes the fact beyond doubt that synthetic peptide based assay is more sensitive test than recombinant assay as has been quoted [3] that synthetic peptides derived from envelope (env) epitopes have proved to be sensitive & extremely specific diagnostic reagents for HIV infection. Zaaijer *et al* [4] during their

studies with seroconversion panel have concluded that the third generation EIAs detected antibodies earlier than the second generation EIAs. Earlier detection of HIV antibodies by third generation assay compared to second generation EIA used in this study may be because of their ability to detect IgM antibodies, IgG antibodies earlier or to detect antigen-antibody complexes. [4]

Studies indicate that third generation synthetic peptide ELISA reduces the window period from HIV-1 exposure to antibody detection. Hence use of peptide based ELISA test should be considered for use in HIV-1 screening programmes [5] Synthetic peptide based assays in which well defined epitopes of env and gag regions are coated, are by far the simplest format. However, assays based on use of recombinant proteins covering the whole of env/ or gag and additional peptides possibly are more complicated. The quality of the protein, its state of purity, its origin, its size and oligomeric structure may all influence the ability of antibodies to bind.[6]

It is generally agreed that for comparing sensitivity of HIV-1 antibody screening tests, seroconversion panel are the best.[7], but commercially available seroconversion panels are very expensive & the volume is very small for many tests and needs to be used sparingly. [5] Hence a panel consisting of prediluted plasma or serum samples has been used by many researchers. Although serial dilutions may not necessarily define sensitivity in the same manner as clinical studies of sera from patients early in the process of seroconversion[8], dilutional analysis can still be used for comparing the analytical sensitivity of various commercial kits.[9] Dilutional panels do rank the tests differently, compared to seroconversion panel still it has been said that rare seroconversion panel may be extended by use of dilutions of positive members.[10] Even though for comparing sensitivity of various kits seroconversion panels are used it becomes the method of choice but is not cost effective in long run. Thus this indicates that dilutional panel can help the manufacturers to design a diagnostic kit which is able to detect the infection at an early stage of disease, as indicated by our study.

Acknowledgement

The authors are thankful to the blood banks for providing the plasma sample for this study.

References

[1] Stute R: Comparison in sensitivity of 10 HIV antibody detection tests by serial Dilutions of Western blot confirmed samples. *J. Virol. Methods*. 1988; 20: 269-273.

[2] Myers Robert A. Handbook on Performance evaluation of HIV antibody tests, Maryland Department of Health and Mental Hygiene Laboratories, Baltimore, Maryland, USA. 1998

[3] Cheingsong- Popov R, Constantine NT, Weber J, Humoral immune responses and detection during HIV infection, In: HIV- A Practical Approach, Volume 1, Karn J (Ed) IRL Press at Oxford university press, Oxford: 1995: 195-196.

[4] Zaaijer HL, Exel-Oehlers PV, Kraaijeveld T, Altena E, Lelie PN, Early detection of antibodies to HIV-1 by third generation assays. *Lancet* 1992; 340: 770-72.

[5] Tyndall M, Gomez AM, Maitha G, Ndinya- Achola JO, Plummer FA, Rosenthal KL: Early detection of HIV-1 using a synthetic peptide ELISA and a p24 antigen ELISA in men from Kenya. In: IX International Conference on AIDS/ IV STD World Congress, Berlin {abstract PO-C31-3298}, June 1993.

[6] Holmes H, Bowers K & Heath A, NIBSC: WHO working group on reference preparations for testing Hepatitis B, C and HIV diagnostic Kits. Agenda Item 4: Anti HIV -1 reference preparations.

[7] Thorn RM, Braman V, Stella M, Yi A. An assessment of HIV-1 screening test sensitivity using serially diluted positive sera can give misleading results. *Transfusion* 1988; 29 (1): 78-80.

[8] Constatine NT, Guido van der Groen, Belsey EM, Tamashiro H: Sensitivity of HIV-antibody assays determined by seroconversion panels. *AIDS* 1994; 8:1715-1720.

[9] Ujheyi E, Fust G, Mihaleczky G, *et al*: Studies of the sensitivity and reproducibility of commercial kits to detect antibodies to the human immunodeficiency virus. *Transfusion* 1987; 27 (2): 210-212.

[10] Schumacher RT, Garrett PE, Fang CT. Seroconversion panel studies are required for accurate assessment of low end sensitivity in anti- HIV test methods. In: VII International Conference on AIDS. Florence [abstract WC3164], June 1991].