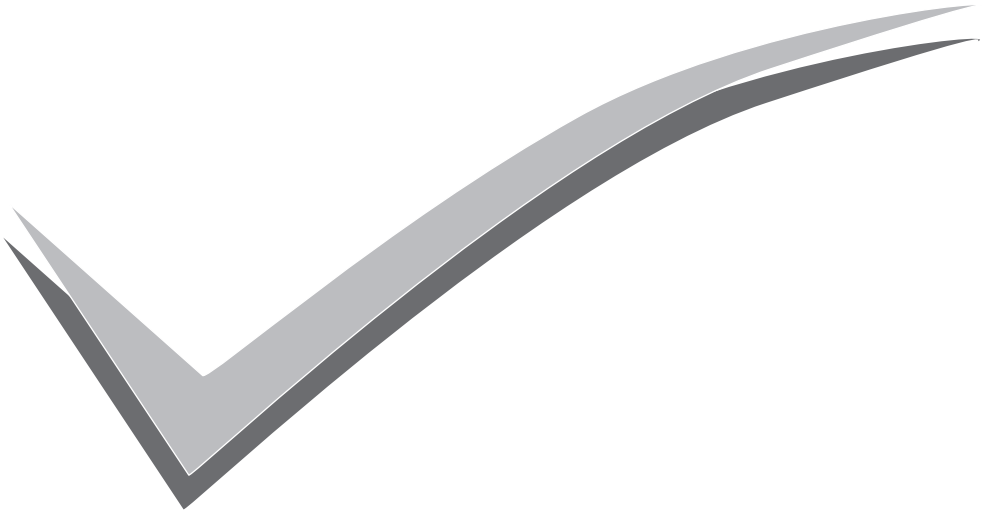
Performance Evaluations



QUALISA® 4.0

4th generation ELISA for HIV 1/2

Tulip Group

Performance Evaluations

INDEX	
S. No.	**Name of the Evaluation Body**
1.	Comparative evaluation with other 4th generation HIV1/2 ELISA at Choithram Hospital, Madhya Pradesh, India

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Dated: 14/05/2010

To,

M/S. Qualpro Diagnostics,

Please find attached the evaluation report of performance of Qualisa HIV 4.0 in comparison with other fourth generation HIV assays.

The following brands were used to conduct the study: Vironostika HIV Uniform Ag/Ab/A508D/7/2010, Microlisa HIV Ag/Ab EIA 03109/09/2010, Eliscan HIV Advance (Lot No.: HHIAA-0709/Oct 2010), Qualisa HIV 4.0 for HIV Ag/Ab Elisa kit (lot No.49006 Expiry May 2010) & Retrolisa HIV 3.0 (Lot: 47030, Exp: 5/2010)

Samples: One hundred and fifty HIV antibody reactive (by third generation EIA assays) sera samples and one hundred and fifty HIV antibody non-reactive sera samples were included for the study.

Also HIV-1 Seroconversion panel "AN" and HIV-1 seroconversion panel "Z" along with secondary standard p24 Antigen was used in the study to evaluate the p24 sensitivity and arrive at sero conversion period for the kits used in the study.

A complete evaluation report is enclosed along with this letter.

(Dr. D. S. Chitnis)

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Choosing the most reliable HIV fourth generation assay- A Challenge!

AIM: To assess the reliability of HIV 4th generation assays by estimating their analytical sensitivity and clinical sensitivity using HIV p24 antigen dilutions and HIV seroconversion panels.

Study design and methods: Studies using seroconversion panels, p24 antigen dilution and antibody sensitivity panels enable the comparison of different HIV assays available in the market[7]. 4th generation HIV Enzyme immunoassays (combined HIV Ag/Ab EIA) from four manufacturers were used for the assessment of their performance. Their analytical sensitivity was determined using dilutions of HIV 1-p24 antigen (traceable to HIV -1 p24 antigen, 90/636, 1st international reference reagent) and their clinical sensitivity was determined using seroconversion Panels "AN" and "Z" (HIV -1 seroconversion panels from Boston Biomedica Inc. U.S.A). One third generation HIV EIA assay was also included in the study to assess the superiority of 4th generation assays over third generation assays. The evaluation was carried out at Choithram Hospital & Research Centre, Indore, under the supervision of Dr. D.S Chitnis (Ph.D), (Professor and Head Microbiology & Immunology).

Results: Overall, Qualisa HIV 4.0 had the best clinical as well as analytical sensitivity in the study. Qualisa HIV 4.0 was reactive from seroconversion Panel AN sera PRB 939-07 (collected 21 days since first bleed) onwards, whereas Vironostika HIV Uni-form II Ag/Ab, Microlisa HIV and Retrolisa HIV were positive from Panel AN sera PRB 939-08 (collected 23 days since first bleed) onwards. Qualisa HIV 4.0 and Vironostika HIV uni-form II Ag/Ab both were positive with panel Z sera PRB-926-03 (7 days since first bleed) onwards whereas Microlisa HIV, Eliscan HIV advance and Retrolisa HIV 3.0 were positive with panel sera PRB926-05 onwards (27 days since first bleed). Qualisa HIV 4.0 and Microlisa HIV were reactive at 26pg/ml HIV p24 Ag concentration. Whereas Vironostika HIV uni-form II Ag/Ab gave positive results at 130 pg/ml p24 Ag ,while Eliscan HIV advance had a detection limit of 650 pg/ml of HIV p24 antigen.

Conclusion: Though most fourth generation assays detect HIV p24 antigen and HIV antibodies simultaneously, the sensitivity to HIV p24 antigen and HIV Ab between different assays varies significantly in clinical samples and so does the window period. This is possibly because, the sensitivity for HIV p24 antigen is standardized using recombinant HIV p24 antigen standard, where as in clinical samples free as well as conjugated HIV p24 protein may be encountered. Hence even if the analytical sensitivity to HIV p24 Ag is good in some assays, their clinical sensitivity to p24 Ag may be lower depending on how the assay has been optimized optimized. Therefore selecting the most reliable HIV 4th generation assay is a challenging task. A reliable HIV 4th generation assay should; a) have high analytical sensitivity to p24 Ag as well as clinical sensitivity. b) Show linearity in S/CO with increasing p24 Ag concentration. C) Should have shorter window period compared to third generation HIV EIA assays. In this study, overall Qualisa HIV 4.0 demonstrated the highest clinical as well as analytical sensitivity with both the seroconversion panels AN & Z as well as p24 antigen standard dilution simultaneously. Qualisa HIV 4.0 S/CO ratio had the most linear relationship with p24 Ag standard concentration and also correlated well with the characteristics (concentration of Ag and Ab) of both the seroconversion panel samples. Overall Qualisa HIV 4.0 had the shortest window period compared to the other assays used in the evaluation. Though Microlisa HIV had good analytical sensitivity for p24 antigen, its clinical sensitivity was equivalent to a third generation HIV assay with the both seroconversion panels. Secondly, the S/CO of Microlisa HIV showed poor relationship with the p24 Ag standard concentrations. The use of 4th generation HIV assays must be advocated extensively to enable early detection of HIV infection and facilitate safer blood transfusion, prevent the spread of infection, initiate timely treatment and corrective measures. However it must be noted that all fourth generation HIV assays that detect p24 antigen are not always superior to third generation assays. One must therefore be aware about the performance of the available fourth generation assays before putting it on routine use.

Background

Human immunodeficiency virus (HIV) antibody testing was implemented in the eighties in response to the emerging Acquired immunodeficiency syndrome (AIDS) epidemic and widespread concern about the integrity of the national blood supply. HIV testing has now become an integral part of state and national programs aimed at preventing transmission of HIV. With evolving technologies the HIV screening and confirmatory assays have been improving, offering better alternatives to address blood screening, surveillance, diagnosis, and patient management.

Host and viral markers in HIV infection

On exposure to HIV virus, the sequence of chronological appearance of host and viral markers is as follows: Viral RNA, p24 antigen, antibody to HIV antigens. Within 2 weeks after infection (10-14 days), viraemia, as measured by viral RNA, appears to increase exponentially[1] until the humoral and cell mediated immune responses control HIV replication. Viral RNA levels usually reach close to 1 million copies of RNA/ml within a couple of months before gradually decreasing to a fairly constant level known as the set-point[1]. This set-point is important and used to predict the subsequent course of infection and disease. High set-points signal a faster course until development of AIDS and death, while lower set points are associated with longer (slower) disease course. Subsequently, RNA levels gradually increase over time until a point at which viral

replication again increases exponentially at the time of AIDS. In most individuals who are not treated with anti-retroviral therapy, the time to AIDS is about 10 years. The viral protein (p24 antigen) increases parallel to viral RNA as the virus replicates, but its detection is little later than the viral RNA because the amplification methods used for detection of viral RNA are more sensitive than p24 antigen detection methods.

The time interval before the HIV antibody appears known as the serological "window period" is characterized by seronegativity, detectable viraemia (as measured by Viral RNA or p24 antigen), and variable CD4 lymphocyte levels. The detection of specific antibody to HIV signals the end of the window period and labels the individuals as seropositive. Antibody to HIV usually appears at about 3-4 weeks after infection, but depends on the specific antibody method used and variations in the immune response of different individuals. Nevertheless antibody is detected in most persons within 1-2 months regardless of the method used, although there are reports indicating a small percentage of persons may require upto six months for antibody to appear.

The appearance of antibody is a major mechanism in decreasing viraemia, as noted by decrease in viral copies and p24 antigenaemia as antibody levels rise. This is most likely because of p24 antibody binding to viral p24 antigen and limiting viral replication. At some later time, probably because of slow destruction of the immune system, virus replication increases (RNA and p24 antigen increase), anti-p24 levels decrease, and the syndrome of AIDS manifests[1].

Evolution of HIV assays

Host and viral markers that occur during the HIV infection are useful to identify infection, monitor viral replication, disease progression and immune status[1]. The kinetics of their appearance dictates the choice of tests to use depending on the purpose of testing.

Molecular assays for HIV have evolved progressing from strictly research tools to routine methods. Polymerised chain reaction (PCR), Nucleic Acid Sequence Based Amplification (NASBA), and branched DNA methods offer increased sensitivity for early infection. Recently more simplified methods have been developed and marketed that allow for a close estimation of viral copy number (viral load) in blood, thereby eliminating the need for sophisticated methods such as PCR. However these methods are expensive need dedicated equipments and therefore cannot be used in routine laboratories.

The early HIV EIA assays involved the use of viral lysate, and positive specimens were confirmed by means of western blot. Second generation and third generation EIA's that detected HIV antibodies were developed on the basis of recombinant proteins and synthetic peptides, which increased sensitivity and specificity of the HIV assays and significantly shortened the window period. The availability of standalone HIV p24 EIA's along with HIV antibody detection assays aided in shortening the window period further[1].

In some countries, HIV p24 antigen assays are used in conjunction with third generation assays to improve detection of recent infection. While this may be a good practice there is a constant demand to

Fig 1: Kinetics of HIV infection and host immune response

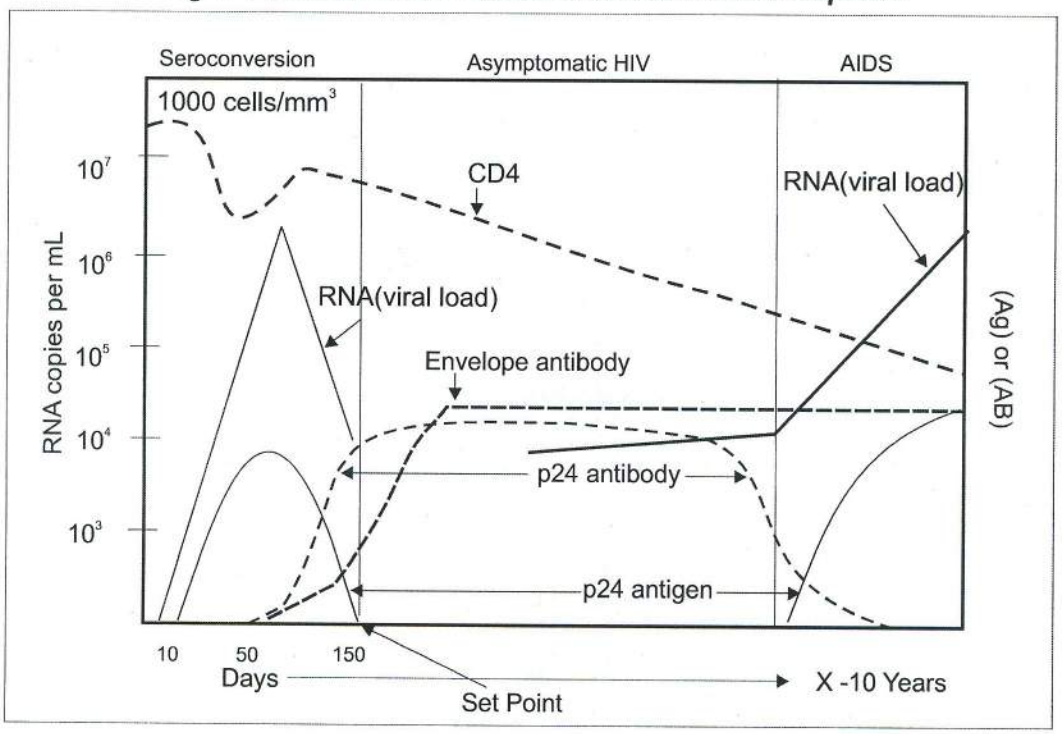
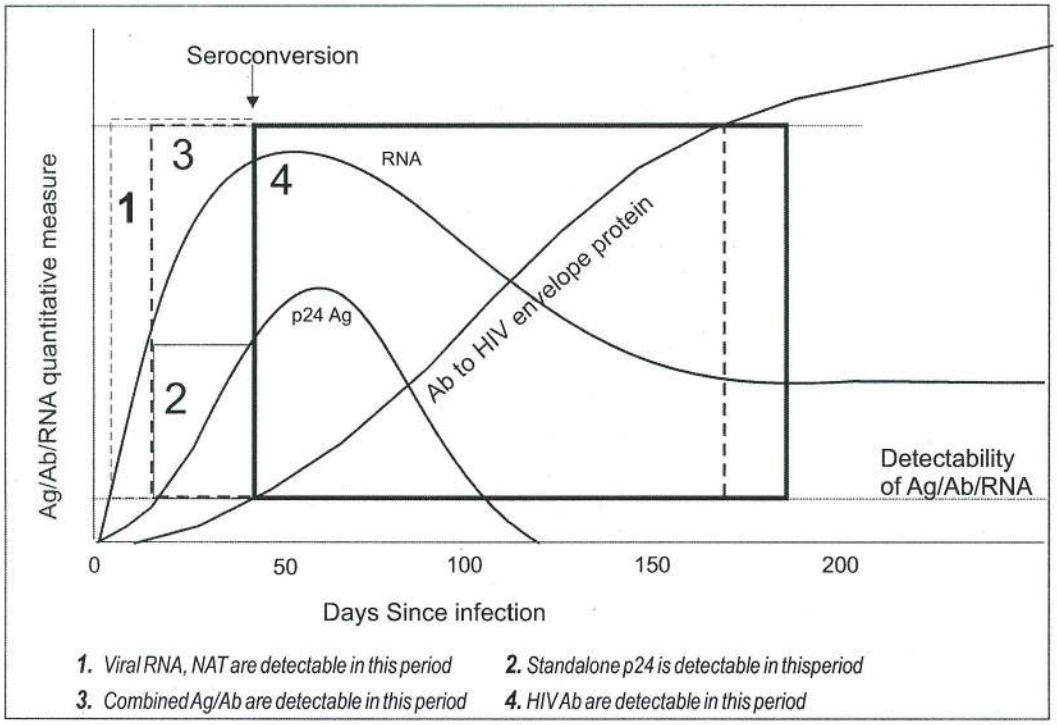


Fig. 2 Detectability of Ag/Ab/RNA by HIV assays in the early phase of HIV infection



reduce the number of test done in the laboratories hence a combined HIV Ag/Ab assay is preferable. The first available HIV combined Ag/Ab assays exhibited a relatively high detection limit for HIV p24 Ag (HIV p24 Ag Limit of Detection (LOD) > 60pg HIV Ag/ml, SFTS standard, French Society of Blood Transfusion)[7]. For this reason, these assays were considered unsuitable to replace Ag only assays which have LOD<20pg HIV Ag/ml (Laperche et al. 2000; Lyet, 2001 a Weber, 2003)[7]. In addition, the overall specificity of these HIV combined Ag/Ab assays for blood donors or patients screening was not as high as HIV Ab only assays. Significant improvement in the performance of the newer HIV combined Ag/Ab assays was observed in assays launched from the year 2001[7]. These assays have demonstrated a high analytical sensitivity obviating the need to perform separate assays.

HIV Fourth generation assay optimization

In India, where epidemic of HIV infections are ongoing, it is extremely important to use tests with shortest possible window period. The use of combined HIV Ag/Ab assays therefore would be beneficial provided, they have sensitivity comparable to that of traditional HIV p24 Ag assays and are better off than third generation HIV antibody assays. Although the fourth generation assays represent a major improvement in terms of sensitivity, optimization of the performance characteristics is required as the fourth generation EIA's combine two different test principles in a single assay format. Though most fourth generation assays claim to detect HIV p24 antigen and HIV antibodies, their sensitivity to HIV p24 antigen and HIV Ab vary significantly in clinical samples and so does the window period. This is possibly because, the sensitivity of the HIV p24 antigen is standardized using recombinant HIV p24 antigen standard, where as in clinical samples free as well as conjugated HIV p24 protein can be encountered. Hence even if the analytical sensitivity to HIV p24 Ag is good for some assays, their clinical sensitivity to p24 Ag may be lower depending on how the assay has been optimized. Secondly the analytical sensitivity to HIV Ab also varies from manufacturer to manufacturer. Therefore selecting the most reliable 4th generation assay is a challenging task. A reliable HIV 4th generation assay should; a) have high analytical sensitivity to p24 Ag as well as clinical sensitivity b) show linearity in S/CO with increasing p24 Ag concentration c) Should have shorter window period compared to third generation HIV EIA assays. This prompted us to investigate the performance of some of the HIV Fourth generation EIA's available in our country.

Study design and methods

Studies using seroconversion panels, p24 antigen dilution and antibody sensitivity panels enable the comparison of different HIV assays available in the market[7]. We compared the reliability of different fourth generation HIV EIA assays available in our country using two HIV seroconversion panels and dilutions of HIV p24 antigen standard. One third generation HIV EIA assay was also included in the study to assess the superiority of 4th generation assays over third generation assays.

Four HIV fourth generation EIA assays ,Qualisa HIV 4.0 (Lot:49006 exp.May 2010),Vironostika HIV Uni-form II Ag/Ab (Lot: A508D/7/2010), Microlisa HIV Ag/Ab (Lot: EIA03109/09/2010), Eliscan HIV Advance (Lot: HHIAA-0709/Oct 2010) and one third generation HIV Ab detection assay "Retrolisa HIV 3.0" (Lot:47030/05/2010)were used for the study. The HIV -1 seroconversion Panel "AN"

(a group of serial bleeds from an individual plasma donor during seroconversion for HIV-1 antibody, p24 antigen, and HIV RNA, Lot No.: Code 90/636) and Panel "Z" (a group of serial bleeds from an individual plasma donor during seroconversion for HIV-1 antibody, p24 antigen, and/or HIV RNA), both manufactured by Boston Biomedica Inc. , USA, were tested with all the assays for assessment of clinical sensitivity. The Panel AN comprised of 9 samples [labeled as PRB939 (E) 01-09] from an individual plasma donor collected between 0 -103 days from time of first bleed. The panel samples were positive by Roche PCR HIV-1 RNA assay (Lot: 88066) from panel sample PRB939-05 onwards and reactive by Abbott HIV antigen test (Lot:20483M101) from Panel sample PRB939-07 onwards. The Panel Z contained 6 samples (labeled as PRB926 01-06) from an individual plasma donor collected between 0 - 32 days from the time of first bleed. The panel samples were positive by Roche PCR HIV RNA assay from panel sample PRB926-02 onwards and reactive by Abbott monoclonal HIV antigen test (Lot: 40265M301) from Panel sample PRB926-03 onwards. The panel samples PRB 926-05 onwards were also positive by 8 U.S. FDA licensed Anti-HIV EIA assays. A secondary standard for HIV-1 p24 Ag, (Lot: OCO 42-28-375-05-Batch 003),traceable to HIV -1 p24 antigen, , 1st international reference reagent) was serially diluted and the dilutions were tested with all the assays used in the evaluation for assessing the P24 Ag sensitivity. 150 HIV antibody reactive sera and 150 HIV antibody non-reactive sera were also tested with all the assays used in the study. The evaluation was carried out at Choithram Hospital & Research Centre, Indore, under the supervision of Dr. D.S Chitnis (Ph.D), (Professor and Head Microbiology & Immunology). All the assay procedures were performed by skilled technicians using the respective manufacturer's instructions. Signal to cut off > 1 was considered as a reactive test result.

Results

Table 1: Comparison of 4th generation HIV assay results with seroconversion panels "AN" and "Z"

Sample/Control/Panel/ Antigen dilution		Vironostika HIV Uni-form II Ag/Ab	Qualisa HIV 4.0	Microlisa HIV	Eliscan HIV Advance	Retrolisa HIV 3.0
Blank		-	-	0.037	-	-
NC		0.069	0.003	0.043	0.015	0.02
NC		0.091	0.001	-	0.016	0.04
NC		0.065	-	-	-	
PC		1.380	2.217	1.692	0.511	1.59
PC		1.235	2.210	1.713	0.71	1.41
PC		1.095	-	1.839	-	-
Cut Off Values		**0.175**	**0.152**	**0.243**	**0.214**	**0.23**
PANEL "AN"						
Panel ID	**Days Since 1st bleed**	**Results (OD in brackets)**				
PRB 939-01	**0**	Neg. (0.051)	Neg.(0.000)	Neg.(0.041)	Neg.(0.15)	Neg.(0.064)
PRB 939-02	**2**	Neg.(0.076)	Neg.(0.000)	Neg.(0.077)	Neg.(0.027)	Neg.(0.042)
PRB-939-03	**7**	Neg. (0.075)	Neg.(0.000)	Neg.(0.126)	Neg.(0.028)	Neg.(0.039)
PRB-939-04	**9**	Neg. (0.134)	Neg.(0.000)	Neg.(0.148)	Neg.(0.032)	Neg.(0.049)
PRB-939-05	**14**	Neg. (0.120)	Neg.(0.044)	Neg.(0.150)	Neg.(0.015)	Neg.(0.043)
PRB-939-06	**16**	Neg. (0.089)	Neg.(0.077)	Neg.(0.178)	Neg.(0.016)	Neg.(0.045)
PRB-939-07	**21**	Neg. (0.085)	Pos(0.547)	Neg.(0.226)	Neg.(0.017)	Neg.(0.091)
PRB-939-08	**23**	Pos(1.40)	Pos(2.423)	Pos(0.245)	Neg.(0.120)	Pos(0.455)
PRB-939-09	**103**	Pos(1.36)	Pos(2.206)	Pos(0.575)	Pos(0.220)	Pos(1.590)
PANEL "Z"						
Panel ID	**Days since 1st bleed**					
PRB 926-01	**0**	Pos(0.25)	Neg.(0.049)	Neg.(0.037)	Neg.(0.020)	Neg.(0.043)
PRB 926-02	**2**	Neg.(0.07)	Neg.(0.042)	Neg.(0.001)	Neg.(0.021)	Neg.(0.043)
PRB 926-03	**7**	Pos(0.55	Pos(0.305)	Neg.(0.009)	Neg.(0.027)	Neg.(0.034)
PRB 926-04	**9**	Pos(0.50)	Pos(0.426)	Neg.(0.226)	Neg.(0.011)	Neg.(0.063)
PRB 926-05	**27**	Pos(1.68)	Pos(1.634)	Pos(1.190)	Pos(0.715)	Pos(0.699)
PRB 926-06	**32**	Pos(1.72)	Pos(1.504)	Pos(1.209)	Pos(0.832)	Pos(0.677)

Neg-Negative, Pos - Positive, PC - Positive control, NC Negative control, OD's above cut off indicate reactive results

Table 2: Comparison of HIV 4th generation assay results with HIV P24 Ag standard dilutions

p 24 Antigen Standard (650 pg/ml)(UD)	Vironostika HIV Uni-form II Ag/Ab	Qualisa HIV 4.0	Microlisa HIV	Eliscan HIV Advance	Retrolisa HIV 3.0
Ag Neat	Pos(0.927)	Pos(1.634)	Pos(0.629)	Pos(0.332)	Ab test kit
Ag 1:5	Pos(0.277)	Pos(1.004)	Pos(0.490)	Neg.(0.115)	Ab test kit
Ag 1:10	Neg.(0.152)	Pos(0.578)	Pos(0.600)	Neg.(0.025)	Ab test kit
Ag 1:20	Neg.(0.102)	Pos(0.332)	Pos(0.422)	Neg.(0.18)	Ab test kit
Ag 1:25	Neg.(0.100)	Pos(0.237)	Pos(0.393)	Neg.(0.14)	Ab test kit

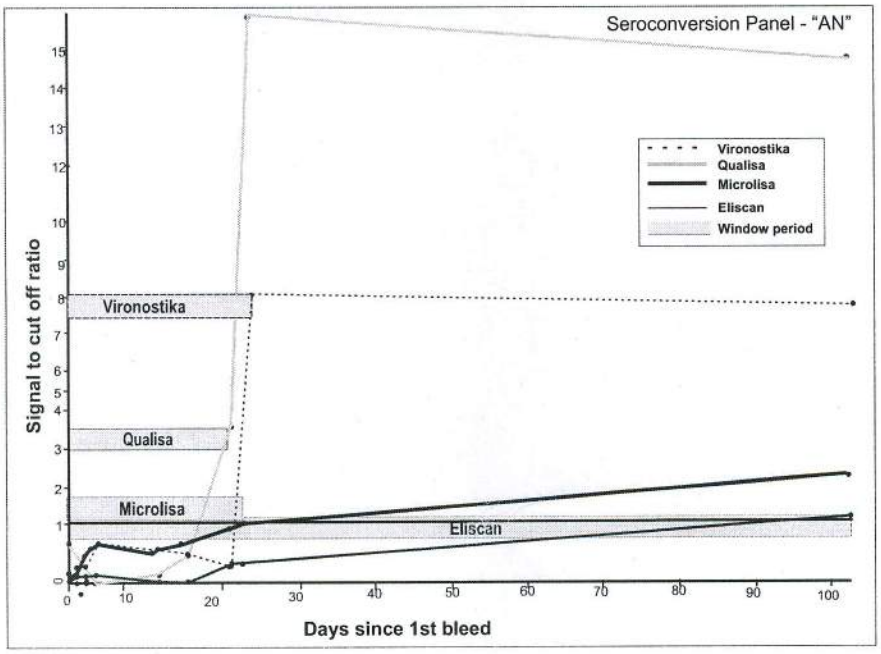
Ag - Antigen, UD - OD's above cut off indicate reactive results

Table 3: comparison of window period of HIV assays with seroconversion panel "AN".

Seroconversion Panel AN		Results (S/CO)					
Panel ID	Days since 1st bleed	RT PCR (Roche HIV RNA)	Qualisa HIV 4.0	Vironostika HIV Uni-form II Ag/Ab	Microlisa HIV	Eliscan HIV advance	Retrolisa HIV 3.0
PRB 939-01	0	BLD	0	0.29	0.16	0.70	0.27
PRB 939-02	2	BLD	0	0.43	0.31	0.12	0.18
PRB-939-03	7	BLD	0	0.42	0.51	0.13	0.17
PRB-939-04	9	BLD	0	0.76	0.60	0.14	0.21
PRB-939-05	14	2×10^4	0.28	0.68	0.61	0.07	0.18
PRB-939-06	16	9×10^4	0.50	0.50	0.73	0.074	0.19
PRB-939-07	21	$>8 \times 10^5$	3.59	0.48	0.93	0.079	0.39
PRB-939-08	23	$>8 \times 10^5$	15.9	8	1.00	0.560	1.97
PRB-939-09	103	2×10^4	14.513	7.77	2.36	1.02	6.91
Window period		14 days	21 days	23 days	23 days	103 days	23 days

*BLD – below limit of detection Note: Samples having S/CO value greater than 1.0 are considered to be reactive.

Fig. 3 Comparison of window period of 4th generation HIV assays with seroconversion Panel "AN"



Note: Samples having S/CO value greater than 1.0 are considered to be reactive.

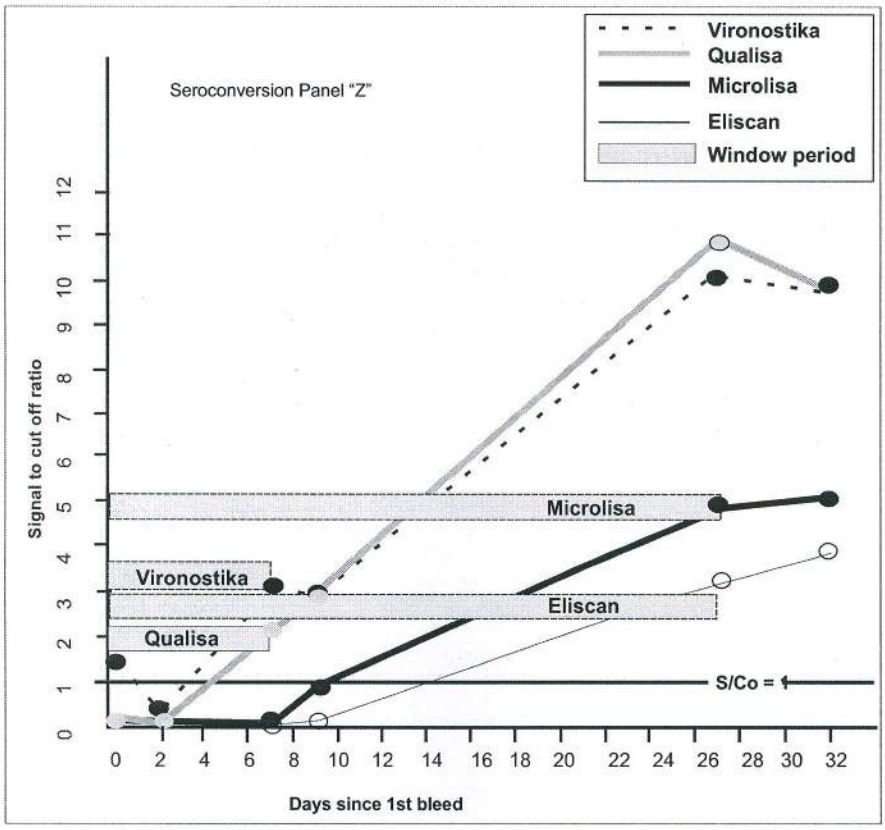
Qualisa HIV 4.0 demonstrated the shortest window period of 21 days (refer table 3.) amongst the HIV 4th generation assays used in the study. It detected HIV infection 2 days before Vironostika HIV Uni-form II Ag/Ab and Microlisa HIV, whereas Eliscan HIV advance showed the longest window period of 103 days. S/CO obtained with Qualisa HIV 4.0 and Microlisa HIV corresponded well with the viral load as detected by RT PCR, however the magnitude of S/CO for Qualisa HIV 4.0 is much higher.

Table 4 : : Comparison of window period of HIV assays with seroconversion Panel "Z".

Seroconversion Panel "Z"		Results (S/CO)					
Panel ID	Days since 1st bleed	RT PCR (Roche	Qualisa HIV 4.0	Vironostika HIV Uni-form II Ag/Ab	Microlisa HIV	Eliscan HIV advance	Retrolisa HIV 3.0
PRB 926-01	0	NEG	0.32	1.43 *	0.15	0.09	0.19
PRB 926-02	2	POS	0.28	0.40	0.00	0.10	0.19
PRB 926-03	7	POS	2.01	3.14	0.04	0.13	0.15
PRB 926-04	9	POS	2.80	2.86	0.93	0.05	0.27
PRB 926-05	27	POS	10.75	9.60	4.90	3.34	3.04
PRB 926-06	32	POS	9.89	9.83	4.98	3.89	2.94
Window period		2 days	7 days	7 days	27 days	27 days	27days

BLD – below limit of detection

Fig 4. Comparison of window period of 4th generation HIV assays with seroconversion Panel "Z"



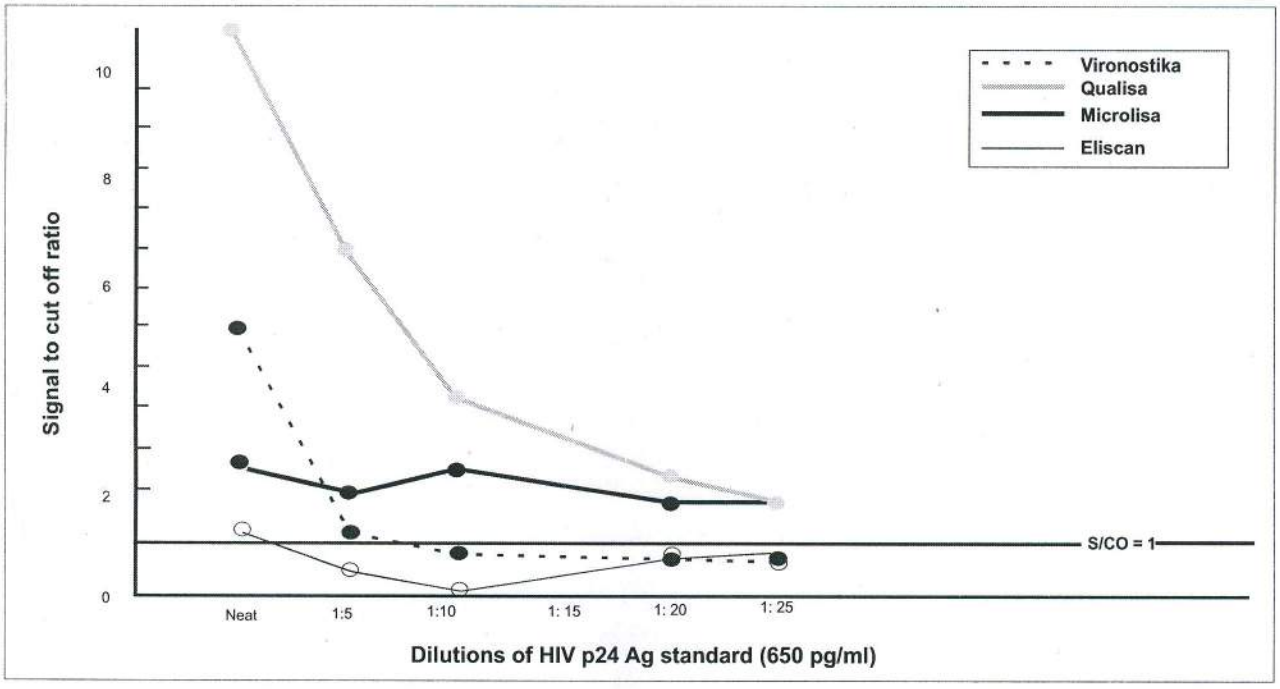
Vironostika HIV Uni-form II Ag/Ab was positive in sample PRB926-01 that is negative with RTPCR (refer table 4). At the same time Vironostika HIV Uni-form II Ag/Ab gave negative result in the next panel sample PRB926-02 collected two days later that is positive with RT PCR (refer Table. 4). Based on the above facts the result of Vironostika HIV Uni-form II Ag/Ab in sample PRB926-01 was considered as doubtful. Therefore both Vironostika HIV Uni-form II Ag/Ab and Qualisa HIV 4.0 were considered to provide positive results from PRB926-03 onwards (PRB926-03 onwards was positive with the HIV Ag test used in the seroconversion panel) indicating a similar window period of 7 days with seroconversion Panel Z. Both Microlisa HIV and Eliscan HIV advance were positive from PRB926-05 onwards with a window period of 27 days. These panel seras (PRB926-05-06) were positive with the 8 anti-HIV assays (3rd generation H*IV assays)* used in the evaluation of panel seras. The S/CO of Qualisa HIV 4.0 corresponded well with the viral load as detected by RT PCR and the magnitude of S/CO of Qualisa HIV 4.0 is the highest amongst all the assays.

Table 5: Sensitivity of the HIV 4th generation assays to the HIV p24 antigen

p-24 Antigen standard dilutions 650pg/ml(UD)	Results (S/CO)			
	Qualisa HIV 4.0	Vironostika HIV Uni-form II Ag/Ab	Microlisa HIV	Eliscan HIV advance
Ag Neat	10.75	5.29	2.58	1.44
Ag 1:5	6.6	1.29	2.01	0.5
Ag 1:10	3.8	0.86	2.46	0.108
Ag 1:20	2.18	0.58	1.73	0.782
Ag 1:25	1.55	0.57	1.61	0.608

Note: Samples having S/CO value greater than 1.0 are considered to be reactive.

Fig. 5. Comparison of sensitivity of 4th generation HIV assays for p24 antigen



Qualisa HIV 4.0 and Microlisa HIV were reactive (S/CO> 1.0) at 1:25 dilution (concentration of 26pg/ml p24 Ag concentration). Whereas Vironostika HIV Uni-form II Ag/Ab gave positive results upto 1:5 dilution (concentration of 130 pg /ml p24 Ag concentration) while Eliscan HIV Advance detected minimum 650 pg/ml of HIV p24 antigen. Qualisa HIV 4.0 had the most linear relationship between S/CO and p24 Ag concentration (refer fig.5).

Table 6: Comparison of Sensitivity and Specificity using known HIV Ab reactive and HIV Ab non-reactive seras.

4th generation HIV EIA assays	HIV Ab reactive sera n=150	HIV Ab non-reactive sera n=150	Sensitivity (%)	Specificity (%)
Vironostika HIV Uni-form II Ag/Ab	150	150	100	100
Qualisa HIV 4.0	150	150	100	100
Microlisa HIV	150	150	100	100
Eliscan HIVadvance	150	150	100	100
Retrolisa HIV 3.0	150	150	100	100

All the HIV assays used in the evaluation demonstrated 100% sensitivity and specificity with 150 known HIV antibody reactive (with third generation assays) sera and 150 known HIV antibody non reactive sera.

Discussion

The availability of fourth generation HIV assays has developed increasing interest in screening of blood bags for safe transfusion and clinical diagnosis. The study by *Weber et al.* demonstrated that fourth generation assays permit an earlier diagnosis of HIV infection than third generation double antigen sandwich assays, by detecting p24 Ag which may be present in samples from individuals with recent HIV infection prior to sero conversion. In these cases the diagnostic window may be reduced by an average of 9 days[2]. In regions where the incidence of HIV infection is high but nucleic acid (NAT) screening of blood donors is unaffordable, HIV combined Ag/Ab assays can enhance the safety of blood donation at reasonable costs[2].

In this study Qualisa HIV 4.0 demonstrated earlier detection of HIV infection both with seroconversion Panels "AN" & "Z" as well as p-24 Ag detection simultaneously. Qualisa HIV 4.0 demonstrated higher clinical sensitivity compared to Microlisa HIV, Eliscan HIV advance and Retrolisa HIV 3.0 with seroconversion Panel "Z". Qualisa HIV 4.0 demonstrated increasing S/CO with increasing days since first bleed except in the last sample of both seroconversion panels, which had lower viral load compared to the previous bleed samples, and were positive with 3rd generation HIV assay (Retrolisa HIV 3.0) indicating lowering of p24 Ag concentrations due to the arrival of anti-p24. The S/CO obtained with Qualisa HIV 4.0 therefore correlate well with the characteristics of seroconversion panels indicating more reliable results compared to all the other assays. Eliscan HIV advance demonstrated lowest sensitivity amongst all the 4th generation assays. Although being a third generation assay Retrolisa HIV 3.0 demonstrated better sensitivity than Eliscan HIV advance with seroconversion Panel "AN".

The clinical sensitivity of HIV assay is measured by the reduction of window period[4]. Qualisa HIV 4.0 proved to have the shortest window period with seroconversion Panel "AN" compared to the other fourth generation HIV assays. Both Vironostika HIV Uni-form II Ag/Ab and Qualisa HIV 4.0 had shorter window periods with Panel "Z" compared to other fourth generation assays. Overall Qualisa HIV 4.0 had the most reliable performance with both the seroconversion panels.

In conclusion, fourth generation HIV assays can contribute to the enhanced safety of blood donation and clinical diagnosis at reasonable costs provided they really shorten the window period. Selecting the most reliable 4th generation assay is the key to early detection of HIV infection. In this study Qualisa HIV 4.0 appears to be appropriately optimized with respect to p24 Ag sensitivity and clinical sensitivity (with seroconversion panels). Qualisa HIV 4.0 had the best correlation between S/CO and analyte concentration (Ag/Ab) with both the seroconversion panels and p24 Ag dilutions, and is therefore the most reliable amongst all the fourth generation HIV assays used in the evaluation While studies using seroconversion panels, p24 antigen dilution series enable the comparison of different assays, case studies provide an insight into the real clinical benefits of the fourth generation assays.However the use of 4th generation HIV assays such as Qualisa HIV 4.0 can be advocated to enable early detection of HIV infection and facilitate safer blood transfusion, prevent the spread of infection, initiate timely treatment and corrective measures.

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The data in the above report was part of evaluation done in our laboratory.

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