Yonsei Medical Journal
Vol. 42, No. 2, pp. 185-193, 2001

Evaluation of a Simultaneous HIV Antigen and Antibody Detection Test in Korean Population

Kwang-Il Park[1], June Myung Kim[2], and Hyon-Suk Kim[1]

Departments of [1]Clinical Pathology and [2]Internal Medicine, Yonsei University College of Medicine, Seoul, Korea.

Current diagnosis of human immunodeficiency virus (HIV) infection relies on the detection of anti-HIV antibodies by enzyme-linked immunosorbent assay (ELISA). Recently, kits detecting both p24 antigenemia and anti-HIV1/anti-HIV2 antibodies have been developed. Thus, it is necessary to compare those kits developed as such. The aim of this study was to evaluate the diagnostic efficiency of a simultaneous detection test of p24 antigen and anti-HIV1/2 antibodies in a low prevalence area.

Eight hundred and four randomly selected sera proven negative for HIV infection and 110 sera from 54 patients diagnosed as HIV infected, obtained between 1999 and 2000, were used for this study. One commercial lot of panels composed of consecutive sera obtained from known HIV-infected patient was included. Anti-HIV1/2 antibodies were detected by two different commercial ELISA kits, one from Korean and the other from German manufacturer. P24 antigen test was performed by ELISA. The simultaneous HIV antigen and antibody detection test was carried out. In the meantime, HIV RNA PCR and anti-HIV1 and anti-HIV2 western blot assays were also performed to confirm the test results in cases the test results didn't agree.

The simultaneous detection kit showed 100% sensitivity and 99.6% specificity. Furthermore, the test displayed the possibility of earlier diagnosis than conventional anti-HIV1/2 ELISA with the results obtained from a group of consecutive panel sera infected with HIV. From these results, we concluded that the simultaneous HIV antigen and antibody detection test can be applied as a substitute clinical screening test in the place of conventional anti-HIV1/2 ELISA, and there is the probable benefit of early diagnosis.

Key Words: Human immunodeficiency virus (HIV), enzyme-linked immunosorbent assay (ELISA), p24 antigen (p24Ag), anti-HIV1/2

Received November 17, 2000
Accepted December 5, 2000

INTRODUCTION

Since the first description of acquired immunodeficiency syndrome (AIDS) in 1981,[1-3] epidemiological researches revealed that it was an infectious disease transmitted by sexual contact, or by the transfusion of blood products. In 1983, human immunodeficiency virus (HIV) was first identified from a patient with lymphadenopathy[4] and was recognized as the causative agent of AIDS.[5]

HIV is one of the retroviruses belonging to the lentivirus subfamily and was previously called lymphadenopathy associated virus (LAV) or human T lymphotropic virus (HTLV) type III or AIDS-associated retrovirus (ARV).[6] The detection of the virus was problematic until the serologic diagnostic method was introduced, because of genetic variants and lack of homogeneity in *env* gene product.[6-9] Following the introduction of serologic testing to detect anti-HIV antibodies, wide spread infection of HIV was revealed ranging from asymptomatic states to acute progressive states throughout the world.[10,11]

Although HIV infection is rare in Korea, the Korean National Institute of Health reported in 1999 that the number of registered patients exceeds 1,000 since the first reported case in 1987.[12] Today's decision on pathologic status mainly relies on clinical symptoms, although various serological and molecular genetic methods have been developed.[13,14] Quantitative CD4+ T lymphocyte count and quantitative HIV RNA analysis are useful adjuncts for gauging a patients immune status or the progress of disease as well as monitoring the therapeutic efficacy.[14]

Until now, the detection of anti-HIV antibody by enzyme-linked immunosorbent assay (ELISA) has been preferentially adopted in most clinical laboratories.[15-17] However, the antibody takes about 4 weeks to appear after original infection of the virus. Thus, a suitable detection method able to identify the virus during this period is critically needed. To circumvent this problem, p24 antigen detection has been considered by researchers because the antigen is present in most HIV-infected patients in the early stage when generally having low antibody titers. During this period, clinical signs may appear, viremia is high and the risk of transmission is at its greatest.[16,17] Hence, detection at this stage would provide many benefits not only for public healthcare and medical economic savings but also for treatment.[18,19] However, the present diagnostic methods used for the detection of p24 antigenemia by HIV RNA are too costly for routine use in clinical laboratories. Moreover, diagnosis of HIV infection can not be made during the period after the amount of viremia is decreased and before the production of antibody as detectable titer.[20]

Recently a new method capable of detecting p24 antigen as well as anti-HIV1/2 antibodies simultaneously has been developed and has become valued as a useful screening test which is rapid and economic in highly prevalent areas and as a blood donor screening test as well.[21] However, the test suffers from a risk of false reaction when used in a population group where the prevalence of HIV infection is very low. HIV has numerous mutants and regional specificity has still not been determined. It is thus imperative that the efficiency of newly developed and commercialized diagnostic reagents should be precisely evaluated.

Therefore, we evaluated a simultaneous detection test, which was developed for the simultaneous detection of HIV antigen and antibody at once as screening, among the Korean population in a low epidemic area.

MATERIALS AND METHODS

Subjects

The study consisted of 110 randomly selected sera from 54 patients diagnosed as HIV infection as well as 804 randomly selected sera proven negative for HIV infection, obtained from the outpatient clinic at the Yonsei Health Promotion Center and from volunteers who visited Severance Hospital, Yonsei University College of Medicine between 1999 and 2000. The negative samples were twice examined for anti-HIV1/2 antibodies using both Genedia HIV1/2 v3.0 (Green Cross Corp., Seoul, Korea) and Enzygnost Anti-HIV1/2 Plus (Behringwerke, Marburg, Germany) kits to ensure the negative results, and then selected as the study group.

Five milliliter of venous blood was collected into a plain tube to obtain serum to perform the serological test and at the same time another 5 mL of venous blood was collected into an EDTA tube to obtain plasma for the HIV RNA polymerase chain reaction (PCR). Following centrifugation for more than (at least) 15 minutes at 3,500 rpm within 2 hours after sample collection, they were divided into 3 aliquot tubes. One of them was tested immediately and the others were reserved in a deep freezer at -70℃ for supplementary tests. Besides the patients' samples, commercially available seroconverter panel (SERO-HIV panel, NABI, Miami, FL, U.S.A.) was included to evaluate the specificity and sensitivity.

Methods

The anti-HIV1/2 antibody test, p24 antigen test and 'simultaneous screening test of p24 antigen and HIV antibody' were performed on the prepared samples. Samples showing discrepant results between tests were further studied using HIV RNA quantitation by PCR and anti-HIV1 and anti-HIV2 antibody western blot assays and then their results were compared.

Anti-HIV1/2 antibody test

(1) Genedia HIV1/2 v3.0 test (Green Cross Corp., Seoul, Korea)

The required number of strips was placed on the microplate frame. Each well was filled with 100 uL of sample diluent, and then 50 uL of negative control was applied into 3 wells, positive controls into 2 wells and samples into the other wells. The plates were agitated for 10 seconds at

a frequency of 1,000 per minute to mix, and sealed with plate sealer and incubated for 60 minutes at 37±1℃. After washing the wells 5 times with washing solution, the washing solution was removed completely, 100 uL of conjugate solution, reconstituted immediately prior to usage, was added, and the plate frames were gently tapped for mixing and then incubated for 30 minutes at room temperature avoiding direct light exposure. Finally, 100 uL of stopping solution was added to all the wells and the plates were tapped gently to homogenize the chromogen. OD_{450} (optical densities at 450 nm) was measured by adjusting the zero-point with air blank using an empty cuvette by Behring ELISA Processor II Plus (Behringwerke, Marburg, Germany).

(2) Enzygnost Anti-HIV1/2 plus test (Behringwerke, Marburg, Germany)

Strips were taken out of the kit and placed on the microplate frame. Each well was filled with 25 uL of sample diluent, and then 100 uL of negative control was applied into 4 wells, positive control into 2 wells and samples into other corresponding wells. The plates were sealed and incubated for 60 minutes at 37±1℃. After aspiration, the plates were washed 4 times with washing solution which was then removed completely, 100 uL of substrate solution, reconstituted just before use, was added, and the plates were incubated for 30 minutes at room temperature avoiding direct light exposure. After incubation, 100 uL of stopping solution was added to all the wells.

OD_{450} was measured by Behring ELISA Processor III (Behringwerke, Marburg, Germany).

p24 Antigen detection test

The Vironostika HIV-1 antigen Microelisa system (Organon Teknika, Boseind, Netherlands) was used. Each well was filled with 25 uL of sample diluent followed by the addition of 100 uL of negative control, positive control and samples. After mixing, incubation was carried out for 60 minutes at 37±1℃. The wells were washed 4 times with washing solution which was then removed completely, 100 uL of substrate solution (tetramethyl benzidine dihydrochloride, TMB) was added and the plates were gently tapped to homogenize the chromogen and then incubated for 30 minutes at room temperature avoiding direct light exposure. The reaction was stopped by adding 100 uL of stopping solution into all wells. OD_{450} was measured and the absorbances of the negative controls, positive controls, as well as samples were analyzed.

Simultaneous HIV antigen and antibody detection test

VIDAS HIV DUO (bioMerieux, Lyon, France) kit was used to detect HIV antigen and anti-HIV1/2 antibodies. The required number of HIV4 strips and HIV4 SPRs (Solid Phase Receptacles), including the samples, controls and standard to be tested, were placed on the VIDAS Preparation/Loading tray and the appropriate number of assay protocol was entered using the keyboard to create a work list. The positive control was identified by 'C1' and the negative control was identified by 'C2' and the tests were run in duplicate. Two hundred microliter of sample or control was added to the strip sample well, the VIDAS SPRs and strips were inserted into the positions indicated on the screen and after this, all the assay steps were automatically controlled by the instrument.

HIV RNA Polymerase chain reaction (PCR)

AMPLICOR HIV-1 test (Roche Diagnostic Systems, Inc., Branchburg, NJ, U.S.A.) kit was used for HIV RNA quantitation by PCR. To each tube 600 uL of the prepared dissolving reaction solution and 200 uL of samples and controls were added. After incubation for 10 minutes at room temperature, 800 uL of 100% isopropanol was added, the cover closed and the reagents/additives mixed. The reaction mixture was centrifuged at 15,000 rpm at room temperature and the supernatant was removed with RNase free tip with care being taken not to touch the pellet. The pellet was washed with 70% ethanol. To each tube, 400uL of HIV-1 sample diluent was added and mixed for 10 seconds. Then, 50 uL each of positive and negative controls as well as samples were transferred and prepared into MicroAmp reaction tubes and subjected to PCR.

The PCR reaction was performed on the Perkin-Elmer GeneAmp PCR System 9600 (PE Applied Biosystem, Inc., Foster City, CA, U.S.A.). Reverse transcription and PCR were performed as

follows: Two minutes at 50℃, 30 minutes at 60℃, 10 seconds at 95℃, 10 seconds at 55℃, 10 seconds at 72℃ (repeated 4 times), 10 seconds at 90℃, 10 seconds at 60℃, 10 seconds at 72℃ (repeated 26 times), 15 minutes at 72℃. On completion of PCR, the tubes were immediately removed and 100 uL of denaturation solution was added and mixed.

Then 100 uL of hybridization buffer solution was added into each HIV-1 MWP (microwell plate) contained in the kit. Denatured amplicon 25uL, was added into the wells of MWP, on which HIV-specific oligonucleotides were coated. This MWP was incubated for 1 hour at 37C and then washed and dried on absorbent paper towels. Avidin-horseradish peroxidase (AV-HRP) conjugate solution 100uL, was added into each well, incubated for 15 minutes at 37℃ and the MWP washed and dried. Substrate solution 100 uL, was added into each well and incubated for 10 minutes in a dark room. A positive result was determined by color change from colorless to blue. Stop reagent 100 uL was added to each well. Within 10 minutes of adding stop reagent, OD_{450} was measured by ELISA Processor.

Anti-HIV1 and anti-HIV2 Western Blot Assays

NEW LAV BLOT 1 (Sanofi Diagnostics Pasteur, Redmond, WA, U.S.A.) for HIV1 and NEW LAV BLOT 2 (Sanofi Diagnostics Pasteur, Redmond, WA, U.S.A.) for HIV2 were used for western blot assays. The western blot procedures for both HIV1 and HIV2 were the same as follows. The concentrated washing solution/diluent and distilled water were mixed at the ratio of 1:5, and 2 mL of this solution was added to each tray well and gently mixed for 5 minutes. Twenty microliter of positive and negative controls were added to the wells as well as to the samples, incubation proceeded for 2 hours at room temperature with gentle mixing, and then 2 mL of washing solution/diluent was added to each well. After mixing, 2 mL of conjugate solution was added and incubated for 1 hour at room temperature. The contents were removed using an aspirator and dried. The results were interpreted by the criteria established by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) and the Centers of Disease Control and Prevention (CDC).[22]

Precision study on HIV antigen and antibody simultaneous detection test

The degree of precision of the detection test was evaluated using positive and negative controls included in the VIDAS HIV DUO kit. For the total 16 test runs of this study, both control reagents were included, so as to calculate the coefficient of variance (CV) on between-run within-batch. And the CV of within-run within-batch was calculated by testing each control reagent 15 times repeatedly.

Sensitivity and specificity study on HIV antigen and antibody simultaneous detection test

To establish the sensitivity, 110 randomly selected samples obtained from 54 HIV-infected patients were tested. And specificity was established by a screening test performed on 804 randomly selected samples obtained from the Health Promotion Center and from the outpatient clinic. They were examined for anti-HIV1/2 antibodies using two different ELISA kits. The samples producing negative results in both tests and confirmed as negative by clinical history were included and the results were compared with other results.

Comparison of results using seroconverter panel (SERO-HIV, NABI, Miami, FL, U.S.A.)

The results of the HIV antigen and antibody simultaneous detection test were compared with those of the conventional anti-HIV1/2 antibody test and the p24 antigen detection test using commercialized seroconverter panel (SERO-HIV panel, NABI, Miami, FL, U.S.A.). This panel consisted of 10 samples intermittently collected from HIV-infected patients within a certain period of time (Table 1).

RESULTS

Precision of 'HIV antigen and antibody simultaneous detection test'

The between-run within-batch CV for positive and negative controls were 5.1% and 17.8%, respectively, while the within-run within-batch CV were 7.6% and 17.1%, respectively (Table 2).

Table 1. The Characteristics of the HIV-1 Seroconverter Panel (SERO-HIV Quality Assurance Reagent)

Sample Lot No.	Day	Anti-HIV1/2 antibody		HIV-1 antigen	Western blot
		Abbott*	Genetic systems*	Abbott	Ortho/Cambridge
A	1	0.094	0.125	4.169	No Bands
B	3	0.178	0.133	3.289	No Bands
C	8	3.1	0.251	1.093	p24+/-, gp160+/-
D	10	4.809	0.464	0.751	p24, gp160
E	15	5.505	1.004	0.364	p24, gp160 p24, p51+/-
F	21	4.819	1.698	0.324	p55+/-, p66+/-, gp160
G	25	6.628	1.839	0.342	p24, p51, p55+/- p66, gp160
H	28	9.67	2.204	0.271	p17+/-, p24, gp41+/-, p51, p55+/-, p66, gp120+/-, gp160
I	35	12.094	1.884	0.284	p17+/-, p24, gp31+/-, gp41, p51, p55+/-, p66, gp120+/-, gp160
J	39	12.99	2.826	0.262	p17+/-, p24, gp41+/-, p51, p55+/-, p66, gp120+/-, gp160

*Test value (optical density, OD) was obtained by dividing samples value by cut-off value. Samples with a test value higher than or equal to 1 are reported as positive.

Table 2. The Precision of the 'HIV Antigen and Antibody Simultaneous Detection Test'

	Sample	Number of tests	Mean value ± Standard deviation (OD)	Precision (CV, %)
Between-run	Positive control	16	3.47 ± 0.17	5.1
	Negative control	16	0.05 ± 0.008	17.8
Within-run	Positive control	15	3.37 ± 0.26	7.6
	Negative control	15	0.04 ± 0.008	17.1

OD, optical density; CV, coefficient of variation.

Sensitivity of 'HIV antigen and antibody simultaneous detection test'

All of the tests performed on the 110 samples obtained from 54 HIV-infected patients showed positive results. Hence, the sensitivity of the 'HIV antigen and antibody simultaneous detection test' was determined to be 100% (Table 3). However, only 17.6% of the samples produced a positive result by the 'p24 antigen detection test'.

Specificity of 'HIV antigen and antibody simultaneous detection test'

All the tests performed on the 804 sera from uninfected individuals were proven to be negative by the 'Anti-HIV1/2 antibody test' but three of them (0.4%) were found to be positive by the HIV antigen and antibody simultaneous detection test (Table 3). On these 3 samples, PCR and western blot assay were all negative (Table 4). The

results above were shown to be reproducible by repeated testing. Among them, patient 1 and patient 2 were outpatients who visited the Health Promotion Center and who had no reported clinical illness. Patient 2 was a 72-year-old woman and no special symptoms or disorders. Patient 3 was an outpatient of orthopedic surgery, and the result was proved to be a false positive. Therefore the specificity of this test was calculated to be 99.6%.

Test results using the seroconverter panel

The results obtained from seroconverter panel using the anti-HIV1/2 antibody detection test, p24 antigen detection test and 'HIV antigen and antibody simultaneous detection test' are given in Table 5. The 'HIV antigen and antibody simultaneous detection test' gave positive results at the early stages of HIV infection, in fact 7 days earlier than the anti-HIV1/2 antibody detection tests.

Table 3. Comparison of the Results of the 'HIV Antigen and Antibody Simultaneous Detection Test' with Anti-Hiv1/2 Test

		'HIV antigen and antibody simultaneous detection test'		Total
		Positive	Negative	
Anti-HIV1/2 test	Positive	110	0	110
	Negative	3	801	804
Total		113	801	914

Table 4. False Positive Results by the 'HIV Antigen and Antibody Simultaneous Detection Test'

	Anit-HIV1 and HIV2 antibody test		P24 antigen test		HIV antigen and antibody simultaneous detection	
	OD	Result	OD	Result	OD	Result
Patient 1	0.059	-	0.038	-	0.36	+
Patient 2	0.036	-	0.026	-	3.54	+
Patient 3	0.042	-	0.031	-	0.4	+
Cut-off value	0.342		0.102		0.35	

*Each OD is a mean value obtained from duplicated testing.

Table 5. Comparison of Each Result Obtained from the HIV-1 Seroconverter Panel (SERO-HIV Quality Assurance Reagent)

Lot No.	Sample Lot No.	Date	Anti-HIV1/2 antibody		P24 antigen test		HIV antigen and antibody simultaneous detection	
			OD*	Result	OD*	Result	OD*	Result
SVO-0231-1	A	1	0.801	-	9.637	+	2.029	+
	B	3	0.853	-	8.284	+	1.514	+
	C	8	1.601	+	2.471	+	1.523	+
	D	10	2.974	+	2.353	+	4.4	+
	E	15	6.436	+	0.559	-	28.343	+
	F	21	10.885	+	0.373	-	30.114	+
	G	25	11.788	+	0.255	-	31.057	+
	H	28	14.128	+	0.265	-	33.086	+
	I	35	12.077	+	0.304	-	35.286	+
	J	39	18.115	+	0.637	-	35.314	+

*Test value (OD) was obtained by dividing the samples value by cut-off value. Samples with a test value higher than or equal to 1 are reported as positive.

DISCUSSION

Cases of HIV infection have been found throughout the world, and thus the necessity for early diagnosis and treatment has increased.[15] The most common method for detecting HIV infection is ELISA using anti-HIV antibodies, with western blot assay being performed for confirmation. ELISA is used for screening due to its high sensitivity, and western blot is used for confirmation due to its high specificity.[22]

The first generation diagnostic kit for HIV infection using the ELISA method was developed in 1985. It used soluble viral lysate antigen extracted from virus isolated from human T lymphocyte line. However, there were lots of problems, such as recurrent false positive results due to differences in protein structures or relative quantities of expressed protein or discrepancies between results depending on applied methods. In the late 1980s, owing to progress of DNA recombinant technology, it became possible to produce recombinant viral antigen, which made the test more sensitive and specific. Moreover, by the addition of anti-human IgM antibody, second generation diagnostic methods detecting both HIV1 and HIV2-specific antibody were adopted for early diagnosis at the time of seroconversion. However, this test cannot detect HIV1 subtype O found in Europe and Africa because the test uses recombinant protein. In addition, this test suffers from low sensitivity and specificity. Now, an improved method, the so called the third generation technique has been introduced and is currently used in the market. Recombinant HIV-1 *env* (envelope), *gag* (core protein) and HIV2 *env* protein are coated on polystyrene beads so that the bead-antigen-antibody complex could be detected. And specificity and sensitivity results reaching up to 96.9% and 89.9-100%, respectively, have been reported.[22]

However, HIV antibodies can not be detected by these antibody tests between the time of infection and appearance of the first antibodies, a period of 4 to 8 weeks duration.[8] Although viremia is high and the risk of transmission is at its greatest at that time, it is impossible to confirm HIV infection during this period.[19] In addition, current screening tests have given false positive results caused by various conditions, such as autoantibodies (anti-nuclear antibody, anti-mitochondrial antibody, etc.), inactivated sera, frequent freezing and thawing, severe hepatic disease, immunoglobulin therapy, vaccination, kidney graft, Stevens-Jonhson syndrome, alcoholic hepatitis, multiparous women, Epstein-Barr virus infection and various malignant tumors, etc.[23]

To overcome this limitation, the p24 antigen test was developed for early diagnosis of HIV infection and gave a high positive rate when viremia is apparent during primary infection.[21-23] However, after the amount of viremia has diminished and antibody titer has not yet increased, it is difficult to detect the virus. Therefore, this test is not a guaranteed alternative for the current anti-HIV1/2 antibody detection test but could be used as a supplementary test.

Hence, to enhance the efficacy of conventional methods, the HIV antigen and antibody simultaneous detection test has recently been developed and is being applied as a screening test for early diagnosis.[21,23] This test should be comparable to or even more sensitive than the conventional anti-HIV1/2 test. The result obtained from seroconverter panel showed that this test has the ability to detect HIV-infection in primary infections as the p24 antigen detection test does. However, the benefits from the test in terms of its application to acute stage patients, economic savings, and diagnosis for newborns remain to be seen.

It is known that the specificity of this test is almost the same as the current anti-HIV1/2 antibody detection test using ELISA.[17] In our study, the HIV antigen and antibody simultaneous detection test produces a specificity of 99.6%, estimated to be at least equal or superior to the existing method. And the precision of this test was considered acceptable. Cost analysis reveals that it is cost-effective because it is no more expensive than the conventional anti-HIV1/2 ELISA kit and especially because it can cover the core window period of HIV infection. Further studies performed on more clinical trials are needed to assess this method more accurately. Nevertheless, many studies have determined it to be a useful diagnostic test, findings that concur with the results of our study.

In conclusion, this simultaneous HIV antigen

and antibody testing method can be used in diagnosis at the early stages of infection and in screening of high risk groups. And this method may prove to be an improved substitute for the conventional anti-HIV1/2 antibodies and p24 antigen tests, currently used in the U.S.A. and other Western countries, because it costs less without sacrificing sensitivity or specificity. The usefulness of this test was reported to be population-dependent, because where the prevalence of HIV infection is not high such groups have a high risk of false reaction. Thus, we evaluated the test on Korean HIV patients and a control group where the prevalence of HIV infection is low compared to other countries. And we found that the method showed the same sensitivity and comparable specificity to that reported in Western countries. Thus, it is concluded that the method can be adopted regardless of the prevalence rate of HIV infection. However, it is thought that further improvements in specificity may be needed in order to minimize unnecessary retesting in this area.

Hyon-Suk Kim, M.D., Ph.D.
Department of Clinical Pathology,
Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea.
Tel: 82-2-361-5863, Fax: 82-2-364-1583,
E-mail: kimhs54@yumc.yonsei.ac.kr

REFERENCES

1. Centers for Disease Control and Prevention. Pneumocystis pneumonia - Los Angeles. Morbidity and Mortality Weekly Report 1981;30:250-2.
2. Centers for Disease Control and Prevention. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men - New York City and California. Morbidity and Mortality Weekly Report 1981;30:305-8.
3. Masur H, Michelis MA, Greene JB, Onorato I, Stouwe RA, Holzman RS, et al. An outbreak of community-acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. N Engl J Med 1981;305:1431-8.
4. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983;220:868-71.
5. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984;224:497-500.
6. Clavel F, Guyader M, Guetard D, Salle M, Montagnier L, Alizon M. Molecular cloning and polymorphism of the human immune deficiency virus type 2. Nature 1986;324:691-5.
7. Smith RS, Naso RB, Rosen J, Whalley A, Hom YL, Hoey K, et al. Antibody to a synthetic oligopeptide in subjects at risk for human immunodeficiency virus infection. J Clin Microbiol 1987;25:1498-504.
8. von Sydow M, Gaines H, Sonnerborg A, Forsgren M, Pehrson PO, Strannegard O. Antigen detection in primary HIV infection. Br J Med 1988;296:238-40.
9. Leys DR, Vanderborght B, Haesevelde MV, Heyndrickx L, van Geel A, Wauters C, et al. Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of West-Central African origin. J Virol 1990;64:1207-16.
10. Tindall B, Cooper DA. Primary HIV infection. Host responses and intervention strategies. AIDS 1991;5:1-14.
11. Levy JA. Pathogenesis of human immunodeficiency virus infection. Microbiol Rev 1993;57:183-289.
12. The Whosaengshinbo. HIV Infection in Korea. 1999; 4846:16.
13. Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of HIV infection. N Engl J Med 1993;328:327-35.
14. Wei X, Ghosh SK, Taylor EM, Johnson VA, Emini EA, Deutsch P, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 1995;373:117-22.
15. Fauci AS, Lane HC. Human Immunodeficiency Virus(HIV) Disease: AIDS and Related Disorders. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, et al., editors. Harrison's principles of Internal Medicine. 14th ed. New York: McGraw-Hill Companies, Inc.; 1998. p.1791-856.
16. Goudsmit J, de Wolf F, Paul DA, Epstein LG, Lange JM, Krone WJ, et al. Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. Lancet 1986;2:177-80.
17. Kelen GD, Shahan JB, Quinn TC. Emergency department-based HIV screening and counseling: experience with rapid and standard serologic testing. Ann Emerg Med 1999;33:147-55.
18. Allain JP, Laurian Y, Paul DA, Verroust F, Leuther M, Gazengel C, et al. Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. N Engl J Med 1987;317:1114-21.
19. Allain JP, Paul DA, Laurian Y, Senn D. Serological markers in early stages of human immunodeficiency virus infection in haemophiliacs. Lancet 1986;2:1233-6.
20. Lane HC. Human immunodeficiency virus. In: Rose NR, de Macarlo EC, Folds JD, Lane HC, Nakamura RM, editors. Manual of Clinical Laboratory Immunology. 5th ed. Washington, DC: ASM Press; 1997. p.763.
21. Hashida S, Hashinaka K, Nishikata I, Oka S, Shimada

K, Saito A, et al. Shortening of the window period in diagnosis of HIV-1 infection by simultaneous detection of p24 antigen and antibody IgG to p17 and reverse transcriptase in serum with ultrasensitive enzyme immunoassay. J Virol Methods 1996;62:43-53.
22. Vasudevachari MB, Richard T, Davey JR, Metcalf JA, Lane HC. Principles and procedures of human immunodeficiency virus serodiagnosis. In: Rose NR, de Macarlo EC, Folds JD, Lane HC, Nakamura RM, editors. Manual of Clinical Laboratory Immunology. 5th ed. Washington, DC: ASM Press; 1997. p.788-99.
23. Hashida S, Hashinaka K, Ishikawa S, Ishikawa E. More reliable diagnosis of infection with human immunodeficiency virus type 1 (HIV-1) by detection of antibody IgGs to pol and gag proteins of HIV-1 and p24 antigen of HIV-1 in urine, saliva, and/or serum with highly sensitive and specific enzyme immunoassay (immune complex transfer enzyme immunoassay): a review. J Clin Lab Anal 1997;11:267-86.