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# Analysis and Testing for AIDS in 92 Homosexuals at a Private Party


## Abstract

On January 17, 2004, the local Chungshan Police Station arrested and investigated 92 homosexuals participating in a drug/sex party at a private residence. Blood specimens of the involved were collected for testing by the Taipei Venereal Disease Center and later verified by the Virology Laboratory of the Center for Disease Control. Of the 92 specimens, 28 tested positive for AIDS, yielding a high rate of 30%. 23 of them tested positive for syphilis, at a rate of 25%. Of the 28 AIDS positive cases, 14 had already been reported and registered by the local health authorities for treatment/follow-up, the remaining 14 were new cases. From the subtype analysis of AIDS virus, 89.2% were shown to be of subtype B, and 10.7% were of subtype E. Testing for hepatitis B surface antigen and hepatitis C antibody was also conducted revealing 27 (30.6%) hepatitis B carriers, and 5 (5.4%) anti-HCV IgG positive results.


## Introduction

HIV (human immunodeficiency virus) is the primary virus that causes AIDS (acquired immunodeficiency syndrome). Since the first isolation of HIV in 1983[1,9], AIDS has been rapidly increasing worldwide. According to estimates

of the World Health Organization, there are currently 34-46 million people in the world infected with HIV, and around 20 million have died of AIDS. By taxonomy, HIV is a lentivirus of the retroviridae family. Under the electron microscope, HIV is a round shaped virus pellet of 110 mm in diameter with a glycoprotein envelope. The inner shell contains two sets of single-ply RNA and enzymes such as reverse transcriptase, integrase, viral protease, and other adjustment proteins necessary for virus cloning. The length of a single-ply RNA of HIV is 9.2 kb, carrying 9 genes[(2)]. Two types have been identified: type 1 (HIV-1), and type 2 (HIV-2), originating in eastern Africa and western Africa respectively. They are serologically distinct. HIV-2 is similar to the simian immunodeficiency virus (SIV) of monkeys. HIV-1 is divided into three groups: major group (M), outlier (O), and new (N). The M group can be further divided according to env genes into 10 subtypes A to J, with more than 20% variation between them. The O and N groups have not yet been subtyped. AIDS is transmitted primarily via blood and body fluids.

Syphilis is a chronic infection caused by *Treponema pallidum*, a spirochete. Drs. E. Hoffman and F. Schaudinn of Germany first detected the pathogenic agent in 1905. The route of transmission is through direct sexual contact including kissing, with infected individuals, and also by blood transfusion. A pregnant woman can transmit the pathogenic agent to her fetus via the placenta causing congenital syphilis in her offspring. Hepatitis B and C are two killers in Taiwan, caused by HBV and HCV. They are transmitted primarily via blood and body fluids. Since the initiation of universal immunization of newborns against hepatitis B in 1986, the carrier rate of grade 1 school children has dropped sharply from 10.5% before immunization to 1.7% in 2000.

**Materials and Methods**

**Subtyping Analysis**

Reverse Transcription -Polymerase Chain Reaction (RT-PCR)[3]

Primer: 44F: ACAGTRCARTGYACACATGG

35R: CACTTCTCCAATTGTCCITCA

33F: CTGTTIAATGGCAGICTAGA

48R: RATGGAGGRGYATACA

1.Reverse Transcription

To 10 ul of virus RNA was added 75mM of KCl, 50mM of Tris-HCl, 3 mM of $MgCl_2$, 10mM of DTT, 0.5mM of dNTP mixture, 38U/ul of RNasin, and 50 pmole of antisense primer, maintained at 70℃ for 10 minutes; 100 units of MMLV-reverse transcriptase (Promega, Cat #M1701) was added, and maintained at 37℃ for 90 minutes.

2. PCR

(a) First round PCR

The cDNA obtained from reverse transcription was used for PCR. 50mM of KCl, 10mM of Trais-HCl, 1.5 mM of $MgCl_2$, 0.1% Triton-X 100, 1mM of dNTP mixture and 50 pmole each of 44F/35R was added to the cDNA; 5 units of Taq polymerase (Invitrogen) was then added, and maintained at 94℃ for three minutes, then 1 minute, then at 48℃ for one minute, 72℃ for two minutes, for a total of 35 reactions, and finally at 72℃ for 15 minutes.

(b) Nest-PCR

To 5 ul of the product of the first PCR was taken was added 50mM of KCl, 10mM of Tris-HCl, 1.5mM of $MgCl_2$, 0.1% Triton-X 100, 1mM of dNTP mixture, and 50 pmole each of 33F/48R, a further 5 units of Taq polymerase (Invitrogen) was added, maintained at 94℃ for 3 minutes, at 94℃ for one minute, 52℃ for

one minute, and 72℃ for two minutes, for a total of 35 reactions and finally at 72℃ for 15 minutes.

**Sequencing**

ABI Prism(™) BigDye(™) Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) was used to mark the nucleic acid to be analyzed. Purity of nucleic acid has an impact on sequencing, nucleic acid of high purity ($OD_{260/280}$>1.8) was used as a mold for sequencing. Quantity of nucleic acid was, 200-500 ng for double-ply DNA (such as plasmid), 50-100 ng for single-ply DNA, and 30-90 ng for PCR products. An adequate amount of nucleic acid mold, 3 μl premix (including tris-HCl buffer, pH 9.0, $MgCl_2$, dNTP mix, labeled A-dye terminator, C-dye terminator, G-dye terminator, T-dye terminator, AmpliTag DNA polymerase FS with thermally stable pyrophosphatase), 3.2-5.0 pmole of nucleic acid primer (2-Rabies-F/2-Rabies-R in the present study), were mixed evenly with water to a volume of 10 μl, and covered with paraffin oil. The tubes were placed in a PCR reactor at 94℃, reacting

25 times at 94℃ for 30 seconds, 55℃ for 15 seconds, and 60℃ for four minutes, and finally stopped at 4℃.

**Phylogenetic Tree Analysis**

The RT-PCR sequencing and the sequencing of subtype reference virus strains including TW20, TW71, TW115, TW8637, W112, TW8523, HIVMN, HIVSF2, TW78, THAI-B, TW8602, TW8610, TW31, HIVJY1, IVNDK, W61, TW8616, THAI-E, TW8604, TW8629, TW98, TW8621, IVD747, HIVNOF, BV217, I525A, HIVKENY, HIVZ321, HIVB7944, VI557, HIVMP51, HIVAN70C[3] were analyzed with Molecular Evolutionary Genetics Analysis (MEGA) version 2.1 by the neighbor-joining method.

**Methods for the Testing of HIV Antibodies**

1. Particle agglutination (PA) (Fujirebio Inc.)

75 μl of serum dilute was injected in the first hole of the micro-titration plate; and 25 μl each in the second to fourth holes. 25 μl each of the positive control serum was injected into the second to eighth holes for the dilution of serum. A pipette was used to draw 25 μl of serum specimen into the first hole, and mixed evenly. 25 μl each was drawn and placed into the second and the third holes, and mixed evenly. 25 μ was drawn from the fourth hole and discarded with the pipette to the high-pressure sterilization waste disposal container (positive control serum was discarded until it was diluted to the eighth hole). 25 μl of unsensitized particles was added to the second hole as negative serum control. 25 μl of sensitized particles type 1 was added to the third hole; and 25 μl of sensitized particles type 2 to the fourth hole. For positive control serum, sensitized particles type 1 was added to the third through eighth holes; and sensitized particles type 2 to the third through eighth holes. The micro-titration plate was agitated and maintained evenly at room temperature for two hours (prevented from removal or oscillation) to allow the specific antibody in serum to agglutinate with antigen. The agglutination was observed.

2. Western Blot testing (New Lav Blot I; Bio Rad)

Test paper was picked up with forceps, and the end containing nitric acid was placed in the reactor. In addition to specimens, two more pieces of paper (positive and negative) were also added for the parallel testing of the control group. 2 ml of rinsing buffer was added to each tank and agitated for five minutes to fully moisten the test paper. 20 μl each of serum specimen, and negative and positive control fluids was added into respective tanks, and shaken covered at room temperature for two hours. Fluid in each tank was completely

drawn out with a negative pressure extractor. 2 ml of rinsing buffer was injected, agitated for five minutes, and removed totally. This process was repeated three times. 2 ml of conjugate was injected, covered, and shaken at room temperature for one hour, and washed three times. 2 ml each of chelating agent was injected, and agitated for about five minutes for coloring. Fluid in the reactor was completely drawn out with a negative pressure extractor and washed three times with distilled water to stop the reaction. Results were read by means of a color graph.

**ELISA (Abbott Murex) for Hepatitis B Surface Antigen Testing**

In each hole of the micro-titration plate of hepatitis B surface antigen test reagent, 180 μl of sample diluent was added. 20 μl of specimen and 20 μl of the controls (positive and negative) were put in the holes, mixed evenly, and incubated at 37℃ for culturing for one hour. To each hole was added 50 μl of conjugate, which was then mixed and incubated at 37℃ for culturing for 30 minutes. It was washed with rinsing buffer four times. 100 μl of substrate solution was added to each hole, covered to avoid light, and placed at 37℃ for culturing for 30 minutes. 50 μl of stop solution was added to each hole to measure within 15 minutes the 450 nm light absorption value with a micro-titration spectrometer.

**ELISA (Abbott Murex) for Hepatitis C Antibody Testing**

To each hole of the micro-titration plate of hepatitis C antibody test reagent, 25 μl of sample diluent was added. 75 μl of specimen and 75 μl of the controls (positive and negative) were placed in the holes, mixed evenly, and placed at 37℃ for culturing for one hour. It was rinsed with buffer four times. 100 μl of conjugate was added into each hole, placed at 37℃ for culturing for 30 minutes,

and rinsed with buffer four times. 100 μl of substrate solution was added into each hole, covered to protect from light, and placed at 37℃ for culturing for 30 minutes. To each hole was added 50 μl of stop solution to measure the 450 nm light absorption value within 15 minutes with a micro-titration spectrometer.

**Results**

The 92 specimens sent to the Laboratory were first screened with the particle agglutination method revealing 28 positives. Proceeding according to the operational procedures of AIDS laboratory testing, positive cases screened by the particle agglutination were further confirmed with the Western Blot Test, and determined positive according to the criteria of the World Health Organization (2ENV±GAG±POL) (Figure 3). The 28 specimens were all positive for HIV-1, yielding a high positive rate of 30.4% (28/92). As AIDS is primarily transmitted via blood and body fluids, the 92 specimens were also tested for hepatitis B (HBV) antigens and hepatitis C (HCV) antibodies, as these two viruses are common in Taiwan, and are also transmitted via blood and body fluids. Due to insufficient blood specimens from certain individuals, only 88 were tested for hepatitis B and 91 for hepatitis C. 27 of them were found positive for hepatitis B surface antigens (30.6%, 27/88); and five were positive for hepatitis C antibodies (5.4%, 5/91) (Table 1). Information released by the Taipei Municipal Venereal Disease Control Center revealed that 23 of the 92 male homosexuals had contracted syphilis (25%) (Table 1). Using the HIV-1 positive cases of the private home party as a population for analysis, it was found that 9 of them (36%) tested positive for hepatitis B surface antigens; none of them tested positive for hepatitis C antibodies; and 11 (39.2%) had syphilis infection (Table 2).

Molecular biological techniques were used in the analysis of the AIDS subtypes. Firstly, primers 44F/35R were used with the RT-PCR method for the initial

amplification of HIV C2-V5 section[3]. The RT-PCR products were then used as plate plies for Nest PCR amplification with primers 33F/48R, and by agar electrophoresis and EtBr dyeing, the HIV C2-V3 gene section of 525 bps could be seen (Figure 1). The Nest PCR products were sequenced, and through NCBI gene databank online, the subtypes of HIV were analyzed. It was found that 25 cases (89.2%) were infected with subtype B, and 3 (10.7%) with subtype E. In the phylogenetic tree analysis, gene sections were matched, 345 bps was taken and analyzed for DNA SATR with the neighbor-joining method, and counted bootstrap 1,000 times. It was found that most of the sequences of the present analysis were in the B cluster, while only three were in the E cluster, and the clusters were the reference subtype sequences (A,D,C,F,H,O) (Figure 2).

**Discussion**

Laboratory testing of blood specimens collected from 92 male homosexuals in this particular incident using particle agglutination and Western blot testing confirmed 28 HIV cases. Currently available laboratory testing for AIDS includes ELISA, PA, P24 for HIV antigens, Western blot, and nucleic acid testing (NAT) (including PCR, bDNA, and RT-PCR). In most hospitals and laboratories, ELISA and PA are used for initial screening, and Western blot for confirmation. This laboratory procedure is a standard operation recommended by the WHO and the US CDC. The NAT method, because of its high sensitivity and specificity, is used mainly by central laboratories and research institutes, as it requires trained professionals for operation. This method will eventually be extended to all medical institutions for use. NAT can shorten the open window period from three months to two weeks; it is used by many blood centers of Japan and Europe for the screening of blood and plasma products to improve the quality and efficiency of laboratory testing [5].

Each communicable disease has its own specific route of transmission. By understanding the routes of transmission, most communicable diseases can be effectively prevented. AIDS for instance, is transmitted primarily through three routes: (1) oral, anal or vaginal sexual intercourse with HIV patients, or other exchanges of body fluids; (2) blood; (3) vertical transmission from mother to infant during pregnancy, delivery or breastfeeding. In short, it is transmitted via blood and other body fluids. Other diseases that are similarly transmitted include syphilis, hepatitis B, and hepatitis C [(6)]. Blood specimens of the 92 male homosexuals were, therefore, also tested for hepatitis B surface antigens and hepatitis C antibodies finding 30.6% positive for hepatitis B carriers and 5.4% positive for hepatitis C IgG antibody. The latter result suggests that the individuals had either contracted or been in the process of being infected with hepatitis C. Information concerning syphilis infection as supplied by the Taipei Municipal Venereal Disease Control Center revealed that almost 25% of the cases had contracted syphilis. In the Taiwan area, it is estimated that about 15%-20% of the adult population are hepatitis B carriers; and about 2%-4% are hepatitis C carriers. The carrier rates of hepatitis B and C in the present incident were slightly higher. Individuals with syphilis, hepatitis B and hepatitis C were highly likely to transmit syphilis or other viruses through unsafe sexual behavior, blood and other body fluids. Hepatitis C could also be transmitted through injection.

Male homosexuals, as noted from the present incident, tend to have higher infection rates of diseases such as HIV, HBV and HCV that are transmitted via body fluids. If they practice unprotected sexual behavior, or if they share common needles for drug use, they are likely to become high-risk transmitters of viruses. In homosexuals, in addition to the prevention and testing of AIDS, testing and control of other body-fluid transmitted diseases should also be emphasized. Health authorities, non -governmental groups or organizations

concerned with the prevention and control of AIDS, should also pay attention to the prevention and control of these other communicable diseases.

In the analysis of HIV subtypes, it was found that 89.2% of the HIV cases in the present incident were of subtype B, and 10.7% were of subtype E. Literature shows that in the Taiwan area, HIV subtype B accounts for 68%-72% of the total, and subtype E accounts for 23%-29%[3,7]. In the present incident, the number of subtype B was relatively high, possibly because the cases analyzed were all male homosexuals. It has been shown that different subtypes are prevalent in different ethnic groups; they are also associated with sex and patterns of sexual behaviors. For instance, in Thailand, subtype B is common among intravenous drug addicts; whereas subtype E is transmitted between heterosexuals. Subtype B is more common in Taiwan [7], and is seen more in sub-clusters of male heterosexuals and homosexuals, possibly because they tend to consort together and transmit the infection to each other. Subtype E, however, is more often seen in heterosexuals. Studies of subtypes in Taiwan and abroad suggest that specific patterns of sexual behaviors are associated with the particular type of HIV Virus transmitted, HIV subtype B is likely to be transmitted by anal sex, whereas subtype E is transmitted by vaginal sex. That is why most heterosexual HIV cases are of subtype E [8].

HIV infection is increasing sharply each year, and the ages of patients are declining. Thus far in Taiwan, there have been 5,839 HIV cases, and the number is increasing. Taiwan is classified by the World AIDS Association as an area of rapid increase in HIV infection, thus more should be done in its prevention and control. In the present incident, almost one-third of the homosexuals were HIV-infected; and a half of those infected had already been registered with the Department of Health for management. It showed that some cases already

registered for management were still having sex with strangers. This probably represented only a tip of the iceberg. More education about safe sex for the general public, homosexuals and already reported, infected individuals should be carried out to help them protect themselves and others.

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**References**

1. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men – New York City and California. MMWR Morb Mortal Wkly Rep 1981; 30: 305-308.
2. Beale KK, Robinson WE, Jr. Combinations of reverse transcriptase, protease, and integrase inhibitors can be synergistic in vitro against drug-sensitive and RT inhibitor-resistant molecular clones of HIV-1. Antiviral Res 2000; 46: 223-232.
3. Yang JY, Lin TL, Luo CC, Chen HY, Twu SJ. Subtyping HIV-1 infections in Taiwan using peptide-enzyme immunoassay, reverse transcription-polymerase chain reaction, and sequencing. J Formos Med Assoc 2001; 100: 89-100.
4. Ohnuma H, Tanaka T, Yoshikawa A, et al. The first large-scale nucleic acid amplification testing (NAT) of donated blood using multiplex reagent for

simultaneous detection of HBV, HCV, and HIV-1 and significance of NAT for HBV. Microbilo Immunol 2001; 45: 667-672.
5. Mine H, Emura H, Miyamote M, et al. High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. J Virol Methods 2003; 112: 145-151.
6. Meng Q, Wong C, Rangachari A, et al. Automated multiplex assay system for simultaneous detection of hepatitis B virus DNA, hepatitis C virus RNA, and human immunodeficiency virus type-1 RNA. J Clin Microbiol 2001; 29: 2937-2945.
7. Chen YM, Lee CM, Lin RY, Chang HJ. Molecular epidemiology and trends of HIV-1 subtypes in Taiwan. J Acquir Immune Defic Syndr Hum Retrovirol 1998; 19: 393-402.
8. Dillner L. HIV subtype may explain sexual transmission. Bmj 1996; 312: 530-531.
9. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983; 220: 868-871.

M.1.2.3.4.5.6.7.8.9.10.11.12.13.14.15.16.17.18.19.20.21.22.23.N

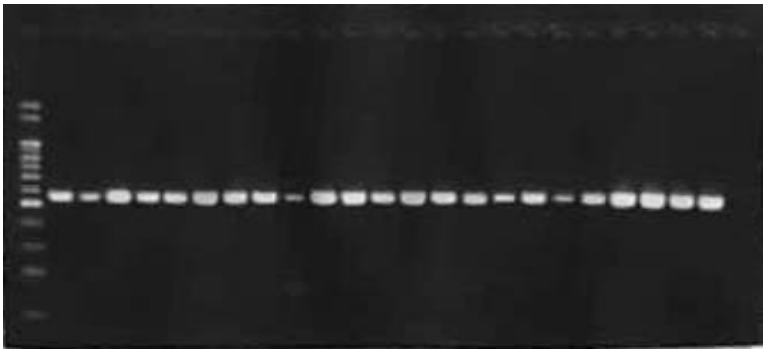

**Figure 1. Nest PCR Electrophoresis**

HIV C2V3 of 525 bps can be found by Nest PCR amplification using 33F/48R primers. M is marker 100 bp ladder; 1-23 are positive cases detected in the present HIV testing; N is the negative control group.

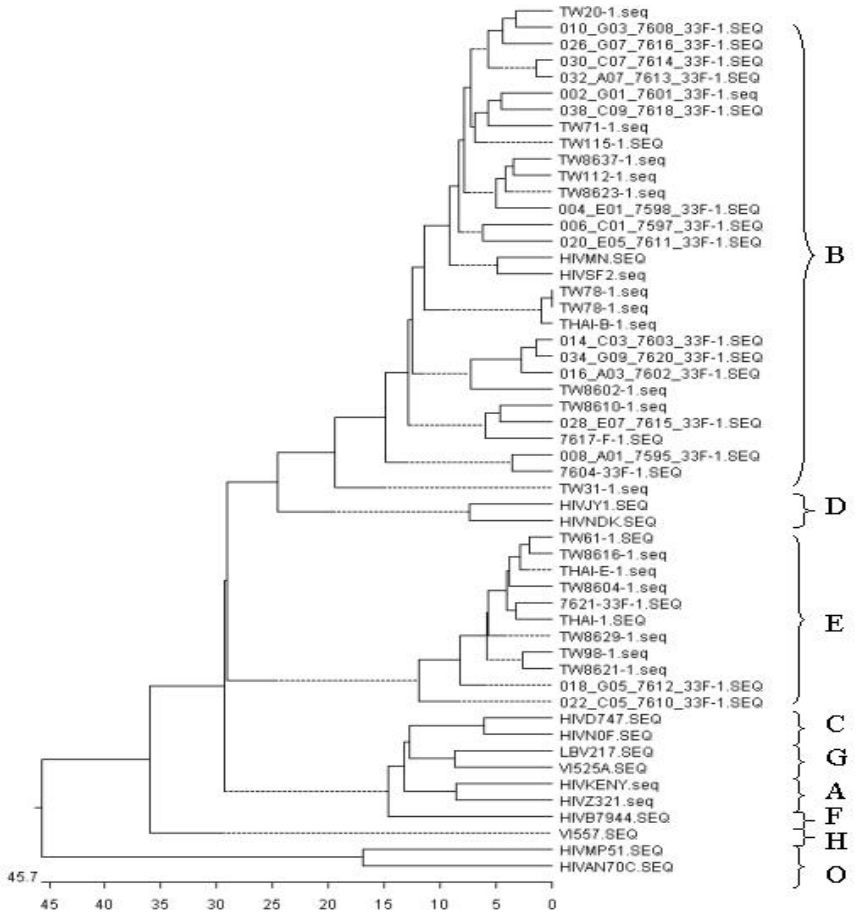


**Figure 2. Phylogenecity Tree Analysis**

Figure shows the phylogenecity tree analysis of HIV subtypes. DNA Star soft is used with the neighbor-joining method. Sequences of HIV reference strains and sequences of the present study are analyzed and compared.

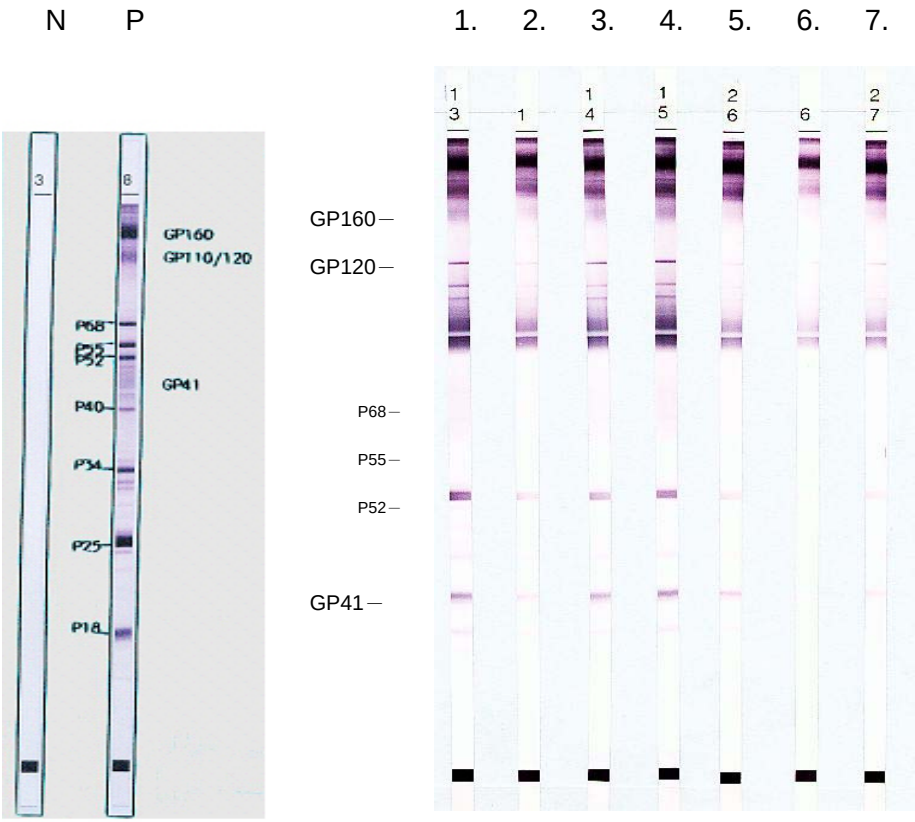


**Figure 3. Analysis by Western Blotting Method**

Figure shows results of the Western blotting testing. Criteria for reading are 2 ENV±GAG±POL. A positive should have at least three antibody reaction lines. N is the negative control group; P is the positive control group; and lanes 1-7 are positive cases detected in the present study.

**Table 1. Testing for HIV-1, HBV and HCV and Positive and Negative Rates**

| | Positive | Negative | Total |
|---|---|---|---|
| HIV-1(PA,WB) | 28 (30.4%) | 64 (69.5%) | 92 |
| Syphilis (RPR,VDRL) | 23 (25.0%) | 69 (75.0%) | 92 |
| HBV antigen | 27(30.6%) | 61(69.3%) | 88 |
| HCV antibody | 5(5.4%) | 86(94.5%) | 91 |

Note: The mount of four specimens was insufficient for HBV antigen testing; that for one specimen was also insufficient for HCV testing.

**Table 2. HIV Subtypes, HBV and HCV Infections in 28 HIV Cases**

| HIV-1 positive | HIV-1 Subtype | Syphilis Testing | | Hepatitis Testing | |
|---|---|---|---|---|---|
| No | Subtype | RPR | VDRL | HBV | HCV |
| 1 | B | Pos | 1:1 | Neg | Neg |
| 2 | B | Pos | 1:1 | Pos | Neg |
| 3 | B | Neg | Neg | Neg | Neg |
| 4 | B | Neg | Neg | Neg | Neg |
| 5 | B | Neg | Neg | Neg | Neg |
| 6 | B | Pos | 1:4 | Neg | Neg |
| 7 | B | Pos | 1:2 | Neg | Neg |
| 8 | B | Pos | 1:4 | Pos | ND |
| 9 | B | Pos | 1:1 | Pos | Neg |
| 10 | B | Neg | Neg | Pos | Neg |
| 11 | B | Neg | Neg | Neg | Neg |
| 12 | B | Pos | 1:2 | ND | Neg |
| 13 | B | Pos | 1:8 | ND | Neg |
| 14 | B | Neg | Neg | Pos | Neg |
| 15 | B | Pos | >1:64 | Neg | Neg |
| 16 | E | Neg | Neg | Pos | Neg |
| 17 | B | Neg | Neg | Neg | Neg |
| 18 | E | Pos | 1:1 | Neg | Neg |
| 19 | B | Neg | Neg | Neg | Neg |
| 20 | B | Neg | Neg | Neg | Neg |
| 21 | B | Neg | Neg | Neg | Neg |
| 22 | B | Neg | Neg | Pos | Neg |
| 23 | B | Neg | Neg | Neg | Neg |
| 24 | B | Neg | Neg | Neg | Neg |
| 25 | B | Neg | Neg | W+ | Neg |
| 26 | B | Neg | Neg | Pos | Neg |
| 27 | E | Neg | Neg | Neg | Neg |
| 28 | B | Pos | 1:1 | ND | Neg |

※Note: Neg is for negative; pos is for positive; W+ is weak positive; and ND is not tested.