Dianne Little,[1] *M.B.B.S. and James A. J. Ferris,*[1] *M.D., M.R.C. Path.*

Determination of Human Immunodeficiency Virus Antibody Status in Forensic Autopsy Cases in Vancouver Using a Recombinant Immunoblot Assay

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ABSTRACT: Sera from 207 forensic autopsy cases were tested for the presence of antibody to the human immunodeficiency virus (HIV) using a recombinant immunoblot assay (RIBA) technique developed by Chiron Corporation of Emeryville, California—the Chiron RIBA™-HIV216 test system. Out of these cases, 172 autopsies were of individuals with no known risk factors for HIV infection, and of these, 169 had no detectable antibodies to HIV. In 2 cases, the serum reacted with p24 alone on the RIBA™-HIV216 assay, but these results were not confirmed by further testing and are considered to be false positive reactions. In 1 case, the serum reacted only with gp41 on the RIBA™-HIV216 test but was nonreactive with further testing. This result has been designated equivocal. Of the 35 cases at high risk of HIV infection, 4 had antibodies to HIV detected in postmortem serum samples. The sensitivity (100%) and specificity (98.5%) of the RIBA™-HIV216 test system are high. However, the test appears to be more suitable for routine diagnosis of HIV infection than for rapid screening in the mortuary.

KEYWORDS: pathology and biology, human immunodeficiency virus, postmortem examination

Acquired immunodeficiency syndrome (AIDS) was first described in 1981 in homosexual and bisexual men [*1*] and was later found in intravenous drug users, hemophiliacs, and recipients of blood transfusions. The etiological agent has been identified as a retrovirus [*2–5*], now known as the human immunodeficiency virus (HIV).

To date, the serological diagnosis of HIV infection has involved initial screening with an enzyme-linked immunosorbent assay (ELISA) using purified whole virus lysates. However, a significant number of false positive results occur; therefore, these results must be confirmed by the Western blot, radioimmunoprecipitation, or immunofluorescence assays. Recently, newer tests have been developed using recombinant viral antigens, which appear to produce fewer false positive reactions [*6–11*], particularly reactions attributable to reactivity to the cell substrate used to grow the virus [*12,13*].

A significant number of forensic autopsies are carried out on subjects at high risk for HIV infection. The majority of these come from the intravenous drug user and homosexual/bisexual male groups. The autopsy procedure carries with it certain risks to per-

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[1]Clinical fellow and professor, respectively, Department of Forensic Pathology, University of British Columbia, Vancouver, British Columbia, Canada.

sonnel, particularly of incurring incised wounds and exposure of non-intact skin and mucous membranes to blood. There are now several well-documented cases of HIV infection occurring in health care workers with no other risk factors who had been exposed to HIV-infected blood, although the rate of infection appears to be less than 1%, even with a needle-stick [*14–17*]. It is not practical or economical to take full infectious case precautions [*18,19*] with every autopsy. It would, therefore, be advantageous to know a patient's HIV serological status prior to autopsy.

This study was undertaken with three main purposes: first, to determine the number of forensic autopsy cases in Vancouver, British Columbia, Canada, in which the decedent had antibody to HIV, particularly among those at high risk for HIV infection; second, to formulate guidelines for a policy regarding HIV antibody screening in forensic autopsy cases in Vancouver; and, last, to evaluate whether the test system supplied was suitable for routine screening for antibody to HIV in a mortuary situation.

It should be stressed, however, that, regardless of whether or not screening for the presence of antibody to HIV is undertaken and regardless of the results of such testing, all cases should be treated as potentially infectious, and appropriate precautions should be taken when performing autopsies.

Materials and Methods

Blood was collected at the time of autopsy and immediately centrifuged. The sera were refrigerated (at 2 to 8°C), and samples not tested within seven days of collection were frozen (at −20°C) for later assay.

The samples were assayed by a recombinant immunoblot assay (RIBA) technique developed by the Chiron Corporation of Emeryville, California—the Chiron RIBA™-HIV216 test system. Specimens were also tested using conventional methods—the ELISA, Western blot, and immunofluorescence assays.

Principles of the RIBA™-HIV216 Assay

The test system employs nitrocellulose strips, each coated with four recombinant HIV antigens, representative of each of the major structural genes of HIV: two env (envelope)-gene-derived products from the glycoprotein (gp) 120 and gp41 coding regions, p31 from the pol (polymerase) gene, and p24 from the gag (core) gene. Also present on each nitrocellulose strip are two internal controls—one a strong positive sample and one a weak positive sample.

Viral-specific antibodies bind to the individual recombinant HIV antigens on the nitrocellulose strips. Nonspecific antibodies are removed by aspiration and washing. A conjugate of goat anti-human immunoglobulin and horseradish peroxidase (HRP) is added and binds to the strips where antibodies from the test serum have bound. After incubation and subsequent removal of excess conjugate, a substrate of HRP (4-chloro-1-naphthol solution containing hydrogen peroxide) is added. During incubation, a blue-black color develops in proportion to the amount of antibody bound to each of the recombinant HIV antigen bands on the strips. The reaction is terminated by decantation and washing

Assay Procedure

The assay procedure used was the following:

1. Sera were added to the nitrocellulose strips present in the individual assay tubes, and the contents were mixed at room temperature for 2 h on a rocker.
2. The strips were washed in distilled, deionized water and pooled in a beaker.

3. Conjugate was added to the strips and the beaker contents were mixed at room temperature for 30 min on a rotary shaker.
4. The strips were washed with distilled, deionized water, and the enzyme substrate was added and mixed at room temperature for 15 min on a rotary shaker.
5. The strips were washed in distilled, deionized water.
6. The results were read visually by comparing the intensity of the color produced to those of the internal positive controls. The presence of no bands or of bands lighter than the weak positive control constituted a negative test. Samples with antigen responses of an intensity at least equal to that of the weak positive control were considered reactive.

Results and Discussion

During the period December 1987 to June 1988, 207 sera from forensic autopsy cases at Vancouver General Hospital were tested for antibodies to HIV. The study group encompassed all types of forensic autopsy cases. Of these 207 cases, 172 (83%) had no known risk factors for HIV infection, 24 were intravenous drug users, 8 were homosexual males (1 was also an intravenous drug user), and 3 were female prostitutes (2 of whom also used intravenous drugs).

Of those with no known risk factors for HIV infection, the cause of death was divided approximately equally between natural and unnatural causes. Among those at high risk for HIV infection, there was a disproportionately large number of deaths due to unnatural causes (Table 1), which were commonly either acute drug poisoning or homicide (Table 2).

TABLE 1—*Cause of Death.*

Classification	Cases with No Known HIV Risk Factors	Cases at High Risk for HIV Infection
Natural	80	6
Unnatural	90	24
Undetermined	2	0
Total number of cases	172	30

TABLE 2—*Cause of death in cases at high risk for HIV infection.*

Cause	Intravenous Drug User	Homosexual Male	Prostitute (Female)
Overdose, heroin	9	0	0
Overdose, cocaine	6	0	0
Overdose, other drug(s)	1	1	0
Homicide	5	1	3
Motor vehicle accident	1	1	0
Suicide (gunshot wound)	1	0	0
Myocardial infarction	0	2	0
Cancer	1	1	0
AIDS-associated infection	0	2	0
Total number of cases	24	8	3

The RIBA™-HIV216 technique yielded seven positive results; however, only four of these were confirmed by the ELISA, Western blot, and immunofluorescence assays. All four showed strong reactivity with multiple antigen bands on the recombinant immunoblot assay (Table 3) and are considered to be true positive reactions. All of these cases were from groups at high risk for HIV infection. Two cases, both homosexual males, were previously known to have antibody to HIV. One died from complications of AIDS and the other suffered a fatal myocardial infarction. The remaining two cases were previously undiagnosed as having HIV antibodies. One was an intravenous drug user who died from acute cocaine poisoning, and the other was an intravenous-drug-using female prostitute who was beaten and strangled.

Two of the three cases producing positive results on the RIBA™-HIV216 assay only, showed reactions with p24 alone (Table 3). Previous reports have suggested that a reaction with only p24 may represent antibodies that are cross-reactive to other related viruses [*20–23*] or may be a blot artifact [*24*], and that specimens with such reactions should be considered inconclusive [*25*] and should be further evaluated by other methods [*23*]. Thus, the results for these two cases have been designated false positives. Both cases were from the group with no known risk factors for HIV infection—a 46-year-old woman who died from a head injury following a fall, and an 83-year-old man who died from hemoptysis associated with bronchiectasis.

The third case showing a positive result only with the RIBA™-HIV216 assay displayed reactivity with gp41 alone. The significance of this result is uncertain. It has been suggested [*6,8,9,26*] that currently utilized Western blot techniques are relatively insensitive for detection of envelope antibodies to HIV and that radioimmunoprecipitation is more sensitive in detecting such antibodies [*6*]. It has been stated that good evidence for infection for HIV consists of the presence of antibodies to gp41 and at least one other antigen [*6*] or of the presence of antibodies to at least one core and one envelope antigen [*27*]. However, other studies [*25*] have shown cases with Western blot reactivity to only gp41 which are also reactive by radioimmunoprecipitation. It is possible that such cases may represent HIV infection and that the RIBA™-HIV216 is more sensitive in detecting gp41 antibodies than the conventional assays used, as occurred in the study by O'Shaughnessy [*28*], which also used a recombinant gp41 antigen. Since it is not possible to obtain further serum from the patient for assay by alternate methods, such as radioimmunoprecipitation and HIV antigen testing, to confirm a diagnosis of HIV infection, the case has been designated equivocal. This case was a 44-year-old alcoholic woman who died from pneumonia.

There were no cases of false negative results with the RIBA™-HIV216 technique.

These results indicate a specificity of 98.5% and a sensitivity of 100% for the recombinant immunoblot technique in comparison with conventional assay methods.

TABLE 3—*Antigen band reactivity with the recombinant immunoblot assay.*

Case	Antigens
True positive results	
1	gp120, gp41, p31, p24
2	gp120, gp41, p31, p24
3	gp120, gp41, p31
4	gp120, p31
False positive results	
5	p24
6	p24
Equivocal	
7	gp41

Conclusions

In a population such as that of Vancouver, it appears to be unnecessary and not economically feasible to screen all forensic autopsy cases for antibodies to HIV, since no definite seropositive cases were detected in any of our study group who had no known risk factors for HIV infection.

Screening may be worthwhile in cases at high risk of HIV infection where the HIV antibody status is not known at the time of autopsy. Out of 35 high-risk subjects in our study group, 2 previously unknown seropositive cases were detected. As the total number of such cases is small in a population such as ours, screening for HIV antibody would be economically feasible.

The recombinant immunoblot assay used in this study showed high sensitivity (100%) and specificity (98.5%). However, in evaluating its potential use for rapid screenings for HIV antibody in the mortuary, it exhibited several disadvantages. The assay system necessitates several large pieces of equipment not generally found in a mortuary (specifically, a centrifuge, a rotary mixer, and a rocker) and requires an experienced laboratory technician to perform the testing. In addition, the test is time-consuming, taking approximately 4 h to perform. Thus, it is not suitable for urgent autopsy cases, particularly those occurring outside of routine hours, when a laboratory technician is unavailable. It is, therefore, our opinion that the recombinant immunoblot assay used in this study is more suitable for routine diagnosis of HIV infection than for rapid screening in the mortuary.

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Address requests for reprints or additional information to
James A. J. Ferris, M.D.
Vancouver General Hospital
855 W. 12th Ave.
Vancouver, British Columbia
V5Z 1M9 Canada