Ling Li,[1] *M.D.; Niel T. Constantine,*[2] *Ph.D.; Xiang Zhang,*[2] *M.D.; and John E. Smialek,*[1] *M.D.*

Determination of Human Immunodeficiency Virus Antibody Status in Forensic Autopsy Cases Using a Rapid and Simple FDA-Licensed Assay

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ABSTRACT: Evaluation of an FDA-licensed rapid assay to detect antibodies to human immunodeficiency virus (HIV) was accomplished by testing 414 serum samples collected sequentially from autopsy cases at the Office of the Chief Medical Examiner, State of Maryland. The time of postmortem collection ranged from 8 to 30 hours. All samples were tested for the presence of antibodies to HIV using a rapid peptide microfiltration assay (SUDS HIV-1 test, Murex Corporation, Norcross, GA), and an enzyme-linked immunosorbent assay (ELISA). Samples yielding repeatedly reactive results were confirmed by Western blot. Of the 414 specimens, 23 (5.6%) produced reactive results by both SUDS and ELISA, and were confirmed by Western blot. One additional sample was repeatedly reactive by ELISA but negative by the SUDS test. This sample produced an indeterminate profile by Western blot (reactivity to only p24) and was negative by several additional retroviral assays. Of the 23 HIV infected cases, 16 had risk factors for HIV infection; 19 were blacks and 18 were male. The SUDS, 10 minute test, exhibited a 100% sensitivity and 100% specificity in comparison to FDA-licensed ELISA and Western blot assays for detecting HIV antibody in autopsy serum specimens. We conclude that this rapid, simply performed assay is accurate and applicable for use in several testing situations, including autopsy rooms.

KEYWORDS: pathology and biology, HIV, assay evaluation, rapid test, autopsy population

Human immunodeficiency virus is the cause of acquired immunodeficiency syndrome (AIDS), and it is estimated that there are at least 1.5 million Americans infected with HIV [*1*]. By the year 1995, the cumulative global number of adults having developed AIDS is expected to increase from more than one million to 4 million; and WHO has recently estimated that 20 million adults and 10 million children will become newly infected with HIV during the 1990s [*1–4*]. By September 1991, 120,000 Americans had died of AIDS; by the end of 1993 at least another 230 000 deaths are anticipated [*5*]. The virus is believed to propagate exclusively through sexual or blood contact [*6*]. Although those who are most likely to contract AIDS from HIV-infected blood are intravenous drug addicts sharing needles and patients who have received inadequately screened

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[1]Research Fellow, Associate Professor, respectively.

[2]Director, Clinical Immunology, Associate Professor; and Research Fellow, respectively, University of Maryland School of Medicine, Department of Pathology, Baltimore, MD.

blood transfusions, the transmission of the virus from individuals to health care workers has received much attention [*6–10*]. It has been well documented that there is significant risk to health care workers of becoming infected with HIV while in the work setting [*11*]. Although there has not been a report of transmission of HIV to a pathologist or autopsy assistant, the chance of such transmission is certainly great enough to cause concern, especially when performing autopsies. Universal precautions should be practical during any procedure requiring the handling of body fluids; however, some medical examiner's offices and other institutions still require that the performance of autopsies on HIV- or hepatitis-infected individuals be conducted in a special "isolation" room [*12*].

Currently, specimens from most of the autopsies performed at the Office of the Chief Medical Examiner, State of Maryland are tested for HIV, but the results are available only after at least one week following the autopsy. Furthermore, the samples need to be sent to a special equipped laboratory for HIV testing. Consequently, the time required to obtain results does not allow for a decision to be made concerning the classification and handling of cases at the time of the autopsy.

A quick and easy-to-perform HIV test for use in an autopsy room could be beneficial in an effort to circumvent the delay in obtaining HIV results, and thus give adequate information to all health care personnel who attend the autopsy procedures. Currently, routine HIV testing using ELISA as a screening method requires at least two hours to perform, and requires somewhat sophisticated equipment and experienced laboratory personnel. Therefore, a routine HIV testing method may not be the method of choice for the purpose that is needed at an autopsy facility.

This study was undertaken to assess the applicability and effectiveness of a rapid, simple, and FDA-licensed test for detecting HIV antibody at the time of autopsy. The SUDS test was evaluated for its performance, as well as determining its utility and accuracy when testing samples collected at the time of autopsy. The seroprevalence of HIV infection, the risk factors associated in this population, and the relative threat to autopsy personnel are discussed also.

Materials and Methods

Samples

A total of 414 blood specimens were collected via cardiac puncture at the time of autopsy from cases examined at the Office of the Chief Medical Examiner, State of Maryland between February and April 1992 in a sequential manner. Following centrifugation at 2500 rpm for 10 min at ambient temperature, the serum was recovered and stored frozen at −20°C until testing. About 10% of the samples were hemolyzed, but otherwise seemed adequate for serological testing. The time of postmortem collection ranged from 8 hours to 30 hours. All samples were coded and tested blindly in order that the identity of the individual was unknown. Before performing the autopsies, it was known that eight cases were HIV-1 antibody positive by former medical history.

Data concerning demographics, manner of death, and risk factors were collected for each case. Information on risk factors included a history within the past 10 years of intravenous drug use (IVDU), homosexual or bisexual activity, or if risk factors were unknown, cases were classified as unknown.

Serologic Testing

All samples were tested by the recently FDA-licensed SUDS HIV-1 test (Murex Corporation, Norcross, GA) and an FDA-licensed ELISA (HIV-AB HIV-1/HIV-2, rDNA, Abbott Laboratories, Chicago, IL) according to the procedures recommended by the

manufacturers. Positive and negative controls were included each time the test was performed to monitor the quality of the results. The SUDS test is a manually performed, visually read, 10 min, immunoassay for the qualitative detection of antibodies to HIV-1 in serum or plasma. The SUDS HIV-1 test uses a proprietary microfiltration enzyme immunoassay procedure. The solid phase capture reagent is a mixture of latex particles coated with HIV-1 gag (p24) antigens affinity purified from HIV-1 lysate and a purified synthetic peptide representing a conserved and immunodominant sequence of the HIV-1 transmembrane protein. The SUDS device is a small plastic cartridge designed to filter, concentrate and absorb all liquid reagents added during the test, including the specimen. The absorbent feature is intended to reduce the potential for contamination of the work space and personnel with infectious materials. It is based on a solid phase capture of antigen-coated latex particles, microfiltration, and an antihuman conjugate system with resultant color development. The recommended serum sample volume for the SUDS test is two drops (approximately 30 μL).

The entire test is performed at a recommended room temperature of 20 to 25°C. Human serum or plasma is incubated in a sample cup with reaction diluent and the capture reagent for 3 min. This mixture is transferred, by pouring, into the SUDS test cartridge, where latex particles coated with HIV-1 antigens and (if present) HIV-1 antibodies, are trapped by a glass fiber filter (within 3 min); the liquid is absorbed within the cartridge. Following addition of a wash reagent to remove unbound materials from the latex, a drop of enzyme-labeled antihuman immunoglobulin conjugate is added to detect the presence of bound antibodies. Following a second incubation (3 min), additional wash reagent is then added to remove unbound conjugate. A drop of substrate, a buffered solution of 5-Bromo-4-chloro-3-indolyl phosphate (BCIP), is added to the device. Following a third incubation (2 min), stop solution is added to arrest the reaction. With a reactive specimen, a distinct blue color is observed in the center hole in the bottom of the SUDS cartridge. A portion of the glass fiber filter which does not contain capture reagent is exposed in the two holes adjacent to the center hole. These holes should remain white indicating that the wash steps were completed successfully.

The criteria for a sample to be considered reactive by the SUDS and ELISA were those recommended by the manufactures; reactions by the SUDS test were classified as non-reactive or reactive, and samples exhibiting an optical density reading greater than the calculated cutoff (optical density/cutoff > 1.0) were considered as reactive by ELISA. All specimens reactive by either test were retested in duplicate before confirmation by an HIV-1 Western blot (BioRad, Hercules, CA.). Criteria for positivity by Western blot for HIV-1 were reactivity to any two of p24, gp41, or gp120/160, as recommended by ASTPHLD/CDC.

Samples that exhibited discrepant results between either of the screening tests were retested by both methods to ensure that technical error had not occurred and to determine if a result were initially misclassified by either assay. To evaluate the specificity of the SUDS and ELISA, the Western blot results were used as the reference method. The sensitivity of the SUDS test was calculated in reference to the results obtained from ELISA. In addition, any specimens exhibiting discrepant results between the tests were tested for antibodies to HTLV-I/II (Bio-Rad) and HIV-2 (Diagnostic Biotechnology, Singapore) using respective Western blots; discrepant samples were also tested by another synthetic peptide-based rapid assay designed to identify and differentiate antibodies to HIV-1 and HIV-2 (Genie, Genetic Systems, Seattle, WA.).

To further evaluate the sensitivity of the SUDS test in comparison to routine ELISAs, two commercially acquired HIV seroconversion panels (Serologicals Inc., Clarkston, GA) were tested by the SUDS test and results were compared to ELISA results supplied by Serologicals, Inc.

Statistic Analysis

Statistical comparisons of the results were made using the Chi-square test for proportions and the Fisher's exact test. Seroprevalence rates related to age, race, and manner of death were calculated for each category and the ages were grouped in 10-year intervals. The criterion of significant difference was $P < 0.05$. Calculations related to sensitivity and specificity were according to established methods [*13*].

Results

Of the 414 specimens tested, HIV antibodies were detected in 23 (prevalence of 5.6%) by both the SUDS and the ELISA; all were confirmed by the HIV-1 Western blot (Table 1) with reactivity to most viral specific antigens. One additional sample was repeatedly reactive by ELISA (mean optical density/cutoff = 1.5), but negative by the SUDS test. The Western blot profile of this sample exhibited reactivity to only the p24 antigen, and thus was considered as indeterminate. The Genie rapid assay, and HIV-2 and HTLV Western blots all produced negative results for this sample. As indicated in Table 1, the SUDS test exhibited a higher specificity in comparison to the Western blot than did the ELISA, although the true status of the indeterminate sample must be questioned. Results indicated that there was apparently no influence of the hemolyzed samples on the SUDS test result.

Results obtained from the testing of two seroconversion panels are summarized in Table 2. As indicated, both the SUDS and ELISA were able to classify positive samples in both panels earlier than the Western blot. In comparison to the ELISA, the SUDS test detected seroconversion at the same time in panel 2212, and several days earlier when testing panel 2214.

The seroprevalence of HIV-1 and the demographics in our forensic autopsy population are indicated in Table 3. Of the 23 HIV seropositive cases, 18 were male, 19 were black, and 14 were in the IVDU group. There was no significant association of the prevalence of HIV-1 antibody positives in relation to sex. Even though the numbers of blacks and whites were similar, the prevalence of HIV-1 antibody positives was 10.11% (19/188) in blacks compared to 1.80% (4/222) in whites. The prevalence of HIV-1 antibody positives in homicide cases was much higher than in the suicide group, 6.7% (5/75) and 1.8% (1/57), respectively. The total prevalence in this study population was 5.6% (23/414) and there was a very clear clustering, with almost half of the HIV-1 positive cases being drug related.

Table 4 indicates the distribution of HIV-1 seropositive cases related to age and risk factors. The highest prevalence rate was found in the 30–39 age group, followed by the 50 to 59 age group. Of the 23 HIV seropositive cases, 20 (87.0%) were in the 20 to 49

TABLE 1—*Comparison of performances of the SUDS test and ELISA.*

	SUDS Test	ELISA	Western blot
Reactive	23	24	23
Sensitivity[a]	100%	—	
Specificity[b]	100%	99.7%	
Assay time	10 min	2 h	4 h
Cost (US $)	9	1[c]	30

[a]In comparison with the ELISA.
[b]In comparison with the Western blot.
[c]Not including the cost of required equipment.

TABLE 2—*Comparison of HIV-1 antibody test results using two seroconversion panels.*

Sample Date	SUDS test	ELISA (O.D./Cutoff)[a]	WB[a]
Panel 2212			
5–31–90	N	0.29	N
6–04–90	R	3.7	I
6–07–90	R	8.1	P
6–11–90	R	10.0	P
6–14–90	R	5.7	P
Panel 2214			
8–20–90	N	0.38	N
8–23–90	R	0.67	N
8–28–90	R	4.4	I
8–31–90	R	7.2	P
9–04–90	R	9.4	P

N = Negative, R = Reactive, I = indeterminate, P = Positive (ASTPHID/CDC criteria)
[a]ELISA and WB information were provided by Serologicals, Inc.; these data were listed for the ELISA (Abbott) that detected seroconversion at the earliest time.

TABLE 3—*Demographic characteristics of the population.*

	Number (% of population)	Number positive (% of positives)	% Prevalence
Sex			
Male	317 (76.6)	18 (78.3)	5.68
Female	97 (23.4)	5 (21.7)	5.16
Race			
Black	188 (45.4)	19 (82.6)	10.11
White	222 (53.6)	4 (17.4)	1.80
Others	4 (1.0)	0 (0)	0.0
Cause of Death			
Natural	154 (37.2)	7 (30.4)	4.55
Accident	78 (18.8)	0 (0)	0.0
Homicide	75 (18.1)	5 (21.8)	6.67
Suicide	57 (13.8)	1 (4.3)	1.75
Undetermined[a]	50 (12.1)	10 (43.5)	20.00
Total	414	23	5.56

[a]92% (46/50) of cases were drug-related. All of the 10 HIV positive cases died of drug overdose.

TABLE 4—*Distribution of HIV seropositive cases with high-risk factors by age.*

Age (year)	HIV Positive Number (%)	% Prevalence	High-risk factors Number		
			IVDU	Homosexual	Unknown
<19	0 (0)	0	2	0	40
20–29	4 (17.4)	4.65	14	2	70
30–39	12 (52.2)	11.43	29	1	75
40–49	4 (17.4)	4.88	7	0	75
50–59	3 (13.0)	8.11	3	0	34
>60	0 (0)	0	0	0	62
Total	23	5.56	55	3	356

TABLE 5—*Prevalence by high-risk factor.*

Risk factor	Number tested	Number positive	% Prevalence
IVDU	55	14	25.5
Homosexual	3	2	66.7
Unknown	356	7	2.0
Total	414	23	5.6

year old groups. The HIV-1 positive cases in the IVDU group were 25.5% (14/55), and two of the three cases who had a history of homosexual behavior were HIV-1 positive (Table 5).

Discussion

A simple, reliable, and rapid means to detect infection by HIV-1 can be useful for blood screening in situations of casualties, in emergency rooms, autopsy rooms, funeral facilities, and for emergency blood transfusions. Information obtained can be used to determine whether extra vigilance should be practiced, or whether prophylactic treatment should be given during or following occupational or accidental exposure to HIV-1-infected blood/body fluids.

Within the past seven years, more than 130 diagnostic assays from greater than 40 commercial companies have been marketed to detect antibodies to the retroviruses [*13*]. New and improved tests are constantly being produced, some of which are rapid assays and use recombinant genetic technology to produce HIV-1 antigens [*13–15*]. However, all these assays should be thoroughly evaluated, particularly with different populations and in the same location where the test will ultimately be used [*13*].

In this study, the Murex SUDS HIV-1 rapid antibody assay was evaluated using a forensic autopsy population. Our findings suggest that this recently licensed test by the FDA is equivalent to a routine FDA-licensed screening ELISA for detecting antibodies to HIV-1. The SUDS test correctly identified all Western blot confirmed samples and did not produce any false positive reactions. In addition, the test successfully identified seroconversion at the same or earlier time than routine ELISA and Western blot assays. We have previously observed a similar accuracy of the SUDS test when testing fluids other than serum, such as urine and pericardial cavity fluids (unpublished observation). The SUDS test produced no false positives and no false negatives when using urine as samples, suggesting that the test may be applicable in cases where the acquisition of blood may be difficult (such as from children, or persons who do not wish to give blood). In addition, the collection of urine is easier, safer, and more cost effective than the collection of serum.

Little et al. [*16*], using an HIV immunoblot assay to investigate 207 forensic autopsy cases in Canada in 1988, suggest the potential use for rapid HIV screening tests in the mortuary. They indicated that the disadvantages of the immunoblot included: equipment required, the need for experienced personnel, and that the assay was very time consuming (at least 4 h). Therefore, the authors concluded that the method was not suitable for urgent autopsy cases.

Compared with the immunoblot assay and ELISA, the SUDS synthetic peptide-based rapid test is easy to perform, results are generated in 10 min, and no equipment is required, especially when using urine or other body fluids as samples. For testing blood, the only equipment required is a centrifuge. Furthermore, the ease of performance suggests that personnel without formal training can perform the test easily and accurately.

Data revealing the prevalence of HIV-1 infection in forensic autopsy populations are limited. A study reported previously by our office showed that the prevalence rate of

HIV-1 infection in autopsy cases was about 2.5% in 1988 [*17*]. Similar prevalence rates were also reported by two other studies during the same period [*18,19*]. The 5.6% prevalence rate in the present study is higher than that of previous studies, most likely paralleling the increase in the HIV seroprevalence in the general population.

A comparison of the demographic data from the study reported by the Philadelphia Medical Examiner's Office [*18*] and in the present study reveals some interesting notes. The HIV-1 seroprevalence rate was much higher in blacks than that of whites ($P < 0.001$), even though the numbers in these two groups in this study were similar. This phenomenon was also found in urban emergency populations [*20*]. The HIV-1 seropositive rate in the IVDU group was 25.5% in our study, as compared to only 4.9% in Philadelphia. It is not clear why Philadelphia has not experienced the demographic shift in the HIV-1 positive population to IVDU that is noted in the neighboring city of Baltimore. Our data were more similar to the prevalence reported in San Francisco in which the seroprevalence in IVDU was 6 to 26% [*21*]. A comparison of age related HIV-1 seroprevalence in the autopsy population is difficult, because limited literature is available. The highest prevalence in age groups was between 30 to 39 years of age in this study, which was also similar to a previously reported urban emergency room population [*20*].

Recently, much attention has been devoted to discussions concerning potential risks to doctors and nurses from needlestick injury. Studies have shown that needlestick injury is fairly common, occurring in at least 5% of operations [*22*]. One report investigated pathologists and technicians when performing autopsies to determine the potential for punctures of gloves. The results showed that greater than 8.3% of gloves were punctured and 31.8% of these punctures went unnoticed [*23*]. Therefore, postmortem examination is a potential source of danger to both pathologists and their assistants. Because pathologists are not required to protect patients from infection in the same way as surgeons are, they may be neglect in practicing the necessary precautions to protect themselves against exposure during postmortem examinations. Although universal precautions should be practiced continually in all situations, it is evident from inquiry that this is not always the case. In addition, it is also important to note that during early infection, or the so called "window period," antibody may not be present at all. Although this threat is small, universal vigilance should be practiced in all cases, including during autopsies.

Numerous rapid assays now exist to test for HIV-1 antibodies, but only two of them have been licensed by the FDA [*13*]. One is the Cambridge latex agglutination assay and the other is SUDS from Murex Corporation. The ideal assay should be rapid, inexpensive, highly sensitive and specific, easy to perform and interpret, and require little or no equipment. The SUDS test meets all these requirements, and offers an alternative to routine, more cumbersome techniques. However, verification of these results on larger numbers of both high and low risk populations should be performed.

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Address requests for reprints or additional information to
Niel T. Constantine, Ph.D.
University of Maryland School of Medicine
Department of Pathology
Baltimore, MD 21201