Paul D. Bigbee,[1] M.S.; Prem S. Sarin,[2] Ph.D.; J. C. Humphreys,[3] M.S.; William G. Eubanks,[4] M.S.F.S.; Daisy Sun,[2] M.S.; D. G. Hocken[3]; Arthur Thornton[2]; Dwight E. Adams,[4] Ph.D.; and Michael G. Simic,[3] Ph.D.

Inactivation of Human Immunodeficiency Virus (HIV) by Ionizing Radiation in Body Fluids and Serological Evidence

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ABSTRACT: A method to use ionizing radiation to inactivate HIV (Human Immunodeficiency Virus) in human body fluids was studied in an effort to reduce the risk of accidental infection to forensic science laboratory workers. Experiments conducted indicate that an X-ray absorbed dose of 25 krad was required to completely inactivate HIV. This does not alter forensically important constituents such as enzymes and proteins in body fluids. This method of inactivation of HIV cannot be used on body fluids which will be subjected to deoxyribonucleic acid (DNA) typing.

KEYWORDS: forensic science, Acquired Immunodeficiency Syndrome (AIDS), Human Immunodeficiency Virus (HIV), human T-lymphotropic Virus Type III, ionizing radiation, gamma radiation, X-ray radiation, liquid blood samples, semen, saliva, serology.

Workers in forensic science laboratories examine evidence exhibiting both liquid and dried blood, semen, saliva, urine, vaginal and cervical secretions, human tissue, body parts, and other body fluids on a daily basis. Often this evidence is found in or on sharp objects such as knives, razors, hypodermic needles, and syringes and requires various methods of manipulation for analysis. While working with these body fluids, the possibility of injury, such as needle sticks or cuts, exists and the splashing of liquid blood onto the body, in eyes, mouth, or mucous membranes may occur. Procedures involving centrifugation, vortexing, and maceration potentially create airborne droplets. Quite frequently, and in ever increasing numbers, the body fluids examined in criminal cases are derived

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[1]Special agent, Forensic Science Research and Training Center, FBI Laboratory, FBI Academy, Quantico, VA.

[2]Deputy director, chemist, and biological laboratory technician, respectively, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD.

[3]Physicist, calibration technician, and physicist, respectively, Ionizing Radiation Division, National Institute of Standards and Technology, Gaithersburg, MD.

[4]Special agents, Scientific Analysis Section, FBI Laboratory, Washington, DC.

from individuals infected with disease-causing microoorganisms or those in the "high-risk" populations often infected with the Human Immunodeficiency Virus (HIV), hepatitis B, other sexually transmitted diseases, and tuberculosis. Many procedures for analyzing forensic science evidence differ from clinical or research laboratories and concern about the accidental transmission of the acquired immunodeficiency syndrome (AIDS) virus (HIV), has increased significantly in recent years.

The possibility of HIV being transmitted accidentally to a forensic science laboratory worker is highly remote, as is the possibility of transmission to clinical and research personnel [*1,2*]. However, a few medical and research laboratory workers have been accidentally infected with HIV [*3*].

Previous research has shown that HIV in concentrated amounts can survive in a dried state for several days at room temperature [*1,4*]. However, in concentrations which would normally be found in persons with AIDS, leading experts generally agree that the possibility of the virus surviving beyond a maximum of 72 h in a dried state is highly improbable.[5-8] Resnick et al. [*4*] reported that highly concentrated levels of HIV (20 000 cells per millilitre reverse transcriptase equivalent) can survive for at least 15 days in solutions at room temperature.

As a result of the increasing concern about handling HIV-infected body fluids, particularly liquid blood samples, the FBI Laboratory began to explore means to render HIV-infected specimens completely safe to handle [*5*]. Traditional means of inactivating the virus, such as use of an autoclave, were ruled out since the proteins of serological importance would be destroyed. In contrast, ionizing radiation, which includes alpha, beta, gamma, X-rays, and high energy electrons, protons, and neutrons, in sufficient quantity, will result in inactivation of living organisms as a result of the radiation-induced damage to DNA. The irradiation of DNA, ribonucleic acid (RNA), or chromosomes may cause breakage either by direct collision with an incoming fast particle or as a result of chemical activity initiated by the radiation. It has been determined experimentally that the energy imparted by the radiation may be used to break chemical bonds and create free radicals, which are always chemically active and which may produce new chemical compounds that may be harmful to the organism [*6*]. This particular aspect of ionizing radiation has been exploited in the sterilization of medical products; polymer modification; prevention of spoilage of packaged meats; radiopasteurization of fruits, seafood, and poultry; and for sterilization of insect pests in stored grain [*7*]. However, proteins and enzymes in biological media are much more resistant to ionizing radiation than DNA. It should also be pointed out that only negligible heat is generated by irradiation [*8*].

Spire et al. [*9*] reported that 250 000 rads of gamma irradiation were required to inactivate HIV in concentrated amounts. However, no previously reported research has examined the effect of high levels of ionizing radiation on the proteins of interest in forensic serology. The FBI Laboratory, working in conjunction with the Ionizing Radiation Division of the National Institute of Standards and Technology (NIST, formerly the National Bureau of Standards) and the Laboratory of Tumor Cell Biology at the National Cancer Institute (NCI), conducted research to address the questions: (1) what

[5]W. Bond, research microbiologist, Centers for Disease Control, U.S. Public Health Service, U.S. Dept. of Health and Human Services, Atlanta, GA, personal communication, 1986.

[6]L. Resnick, Dermatology Department, Mount Sinai Hospital-Medical Center, Miami Beach, FL, personal communication, 1986.

[7]J. Chermann, head of unit, Biology of Retroviruses, Pasteur Institute, Paris, France, personal communication, 1987.

[8]P. Markham, director of virus operations, Bionetics Research Inc., Rockville, MD, personal communication, 1986.

is the absorbed dose of ionizing radiation that is lethal to HIV and (2) is this dose deleterious (for testing purposes) to the biological substances routinely sought in forensic serological analyses?

Methods

The experimentation consisted of three phases. First, liquid and dried blood, semen, and saliva samples from FBI Laboratory donors with appropriate controls were analyzed according to the normal protocol in the Serology Unit, FBI Laboratory (see *Serological Methods*). These samples, along with a bacterial culture of *Serratia marcescens* were then irradiated at the NIST with gamma radiation at 300 000 rads (1 rad = 10^{-2} J·kg^{-1}) and reexamined as before. A second set of samples was then prepared, analyzed, and irradiated along with a second bacterial culture of *Staphylococcus epidermidis* at 1 000 000 rads (1 megarad) of gamma radiation and reexamined.

Since most forensic science laboratories do not have access to a gamma source, the second phase of the project consisted of determining the proper parameters (voltage, current, time, and so forth) of an X-ray machine in the FBI Laboratory to correlate these parameters to absorbed-dose values of X-rays. The NIST calibrated the X-ray machine in the FBI Laboratory and determined the proper voltage, current, distance, and time to relate these factors to absorbed-dose values of X-rays.

The final phase of research was to determine the amount of X-rays required to inactivate HIV completely. This was accomplished by irradiation of concentrated, live HIV (HTLV-IIIB) samples at varying absorbed-dose levels of X-rays, followed by irradiation of serial dilutions of HIV again with varying dose levels of X-rays. When the lowest level of X-ray dose was determined that completely inactivated HIV, prepared blood, semen, and saliva samples, case samples of blood, and bacterial cultures were analyzed, irradiated, and reanalyzed. Prepared standards for blood alcohol determination were also analyzed for ethanol before and after irradiation by X-rays.

Serological Methods

Liquid blood samples were grouped for ABO type by antigen and antibody typing with commercially prepared antisera and indicator cells [*10*]. Lewis groupings were determined by a ficin-capillary tube method [*11*]. Dried bloodstains were subjected to the Kastle-Meyer test (phenolphthalein oxidation) [*12*], the Takayama test (hemochromogen) [*13*], an antihuman test (Ouchterlony method) [*14*], absorption (tube) elution [*15*], and the Lattes Crust ABO typing method [*16*]. Both liquid and dried blood samples were analyzed for eleven different polymorphic enzymes and serum proteins by continuous zone electrophoresis and isoelectric focusing (phosphoglucomutase [PGM] [*17*], esterase D [EsD] [*18*], glyoxalase I [GLO I] [*18*], erythrocyte acid phosphatase [EAP] [*19*], adenosine deaminase [ADA] [*20*], adenylate kinase [AK] [*20*], haptoglobin [Hp] [*21*], group-specific component [Gc] [*22*], transferrin [Tf] [*23*], hemoglobin [Hb] [*24*], and peptidase A [PEP A] [*25*]).

Semen samples were analyzed for acid phosphatase [*26*], examined microscopically for choline (Florence reagent) [*27*] and spermatozoa, analyzed for P-30 by enzyme-linked immunosorbent assay technique (ELISA) [*28*], grouped by microtiter adsorption inhibition for ABH blood group substances (BGS) [*24*], and examined for PGM [*30*] and PEP A [*31*] activity by electrophoresis.

Saliva stains were examined for amylase activity and ABH blood group substances by adsorption inhibition [*32*].

Blood alcohol determination was made with a Perkin-Elmer Sigma 2 Gas Chromatograph.

The bacterial cultures were examined for viability by standard bacteriological methods [*33*].

Irradiation Methods

All gamma irradiation was accomplished using a cobalt-60 source in water at a temperature of 24°C. For the 300 000-rad experiment, the dose rate-to-water was 6.57 krad/min. For the megarad experiment the dose rate was 6.55 krad/min.

All irradiation using X-rays was conducted with a Phillips, Model MCN 321 X-ray generator with a nominal voltage of 320 kV with a continuously rated current of 10 mA at 4.0-mm focal spot size, oil cooled with a beryllium window. Calibration of the X-ray output of the machine was accomplished by the use of transfer dosimetry packets supplied by NIST. Each packet contained five calibrated FTW-60-00 radiochromic dosimeters (NIST Batch 169), held between 0.864-mm-thick blocks of polystyrene and sealed inside two thin polyethylene pouches. After irradiation, the dosimeters were analyzed using a Cary Model 219 spectrophometer. Dosimeter film thicknesses were read using a Mitutoyo Model DGS-E digital gauge. Values of increase in optical absorbance per unit thickness ($\Delta A/t$, where $\Delta A = A_2 - A_1$) were determined. Absorbed dose interpretations were made from a calibration (that is, $\Delta A/t$ as a function of dose) of this batch of radiochromic dosimeters performed in June 1987. Results are shown in Table 1. Irradiation with X-rays of HIV and other samples was accomplished at a distance of 25.72 cm from the source at 300 kV and 10 mA (see Table 2).

HIV (HTLV-IIIB) Methods

The AIDS virus HTLV-IIIB was obtained from the culture supernatant of virus producing H9 cells. Cell clone H9, an $OKT4^+$ T-cell line that is permissive to HTLV-III replication but partially resistant to its cytopathic effect, was maintained in RPMI-1640 medium containing 20% fetal calf serum. The cells were seeded at a concentration of 4×10^5 cells/mL of culture media.

The percentage of cells expressing p24 and p17 group-specific antigen (gag) proteins of HTLV-III was used as a measure of infectivity and determined by indirect immuno-

TABLE 1—*Calibration data for X-ray machine.*

Dosimeter Numbers	NIST Absorbed Dose in H_2O, krad	Amount of Time Irradiated, min	Distance, cm
536–540	32	90	50
541–545	47	180	50
546–550	73	360	50
551–555	0	(control)	. . .

All Dosimeters (except control) were irradiated at 300 kV and 10 mA. Analysis of the dosimeter response consists of determining the mean ΔA/t *for each group of five dosimeters irradiated together and the sample standard deviation of each group as shown below.*

Dosimeter Numbers	Mean $\Delta A/t$, 605 nm	% Std. Dev., s_{n-1}, 605 nm
536–540	0.2779	5.30
541–545	0.5773	2.91
546–550	1.0890	1.93

TABLE 2—*Parameters used for calibrated X-ray machine to irradiate HIV samples.*

Rads	Voltage, kV	Current, mA	Distance, cm	Time, min
10 000	300	10	25.72	12.970
25 000	300	10	25.72	32.444
50 000	300	10	25.72	64.889
75 000	300	10	25.72	97.332
100 000	300	10	25.72	129.777

fluorescence microscopy with the use of mouse monoclonal antibodies to HTLV-III p17 and p24 [*34–36*]. The positive cells were visualized by treatment with fluorescein-labeled goat antimouse immunoglobulin G (IgG) (Cappell Laboratories, Cochranville, Pennsylvania).

Results

Using highly concentrated levels of HIV (10^{11} viral particles per millilitre), it was determined that between 50 000 and 75 000 rads of X-rays were required to inactivate the viruses completely based on analyses of p17 and p24 viral core proteins. However, using less concentrated serial dilutions of the virus to the lowest detectable level (5×10^7 viral particles per millilitre), it was determined that 25 000 rads of X-ray irradiation completely inactivated the virus (see Table 3). The amount of HIV in the test samples far exceeded the amount normally found in a person with frank AIDS (approximately 200 viral particles per millilitre).

With the exception of a liquid blood sample irradiated with one megarad of gamma radiation, no damaging effects were observed in any substance of serological interest or blood alcohol determination following irradiation with any dose of gamma or X-ray radiation. The liquid sample appeared to be hemolyzed following irradiation at 1 megarad as a result of the reaction with the biomaterial and the resulting extreme damage. However, liquid blood samples irradiated at 300 000 and 25 000 rads showed no hemolysis.

TABLE 3—*Evaluation of HIV irradiated at different doses.*

Dose, rads	Virus Particles per mL	HIV Expression % Inhibition	
		p17	p24
10 000	5×10^8	67	67
	1×10^8	75	75
	5×10^7	70	70
25 000	5×10^8	94	88
	1×10^8	94	97
	5×10^7	100	100
50 000	5×10^8	100	100
	1×10^8	100	100
	5×10^7	100	100
100 000	5×10^8	100	100
	1×10^8	100	100
	5×10^7	100	100

When the liquid blood sample irradiated at 1 megarad was dried and analyzed the same as any laboratory-received dried bloodstain, no detrimental effects were observed. All electrophoretic banding patterns after irradiation, regardless of doses, appeared to be more distinct than before irradiation, presumably because of a lack of bacterial degradation. The bacterial cultures were sterilized at all doses of X-ray and gamma radiation exceeding 25 000 rads which could explain the banding pattern phenomenon.

Discussion

The radiation dose (25 000 rads) in this study required to inactivate the AIDS virus completely is a ten-fold decrease in the amount previously reported by Spire et al. [*9*], who failed to determine the threshold dose of radiation required to inactivate HIV in serial dilutions.

This procedure cannot be used on blood, semen, and other specimens that will be subjected to DNA typing since high levels of X-rays or gamma rays produce free radical reactions that can significantly alter DNA. In cases where liquid body fluids are encountered from persons infected with HIV or from persons at risk for HIV infection, an alternate procedure has been established in the FBI Laboratory. The samples for DNA typing are removed and dried for 72 h at room temperature before typing, and the remainder, if in sufficient quantity, will then be irradiated and tested by conventional serological means.

Improperly managed ionizing radiation can be dangerous or lethal. A dose of approximately 1000 rads of ionizing radiation is lethal in humans [*6*]. Individuals who use radiation sources must learn the fundamentals about radiation, dosimeters, shielding, federal exposure limits, and operation of radiation sources. Only those certified/trained or licensed to operate these sources should be allowed to carry out the required radiation treatment.

Conclusion

Our studies indicate that an X-ray dose of 25 000 rads is sufficient to inactivate HIV in blood specimens and body fluids that may be submitted to a forensic science laboratory for examination and analyses. The development of this technique allows a forensic science laboratory to inactivate HIV in liquid blood samples and other liquid body fluids thereby reducing the risk of possible transmission of a lethal virus in blood derived from persons with AIDS, persons infected with HIV, and those in the traditional "high risk"categories of being potentially infected. It could be argued that this technique is not necessary if normal precautions are taken when handling these types of fluids, that is, by using gloves, masks, coats, and eye protection. However, any reasonable and available means to minimize risk is acceptable when dealing with lethal viruses, especially if no harmful effects to evidence are produced by such treatment.

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Address requests for reprints or additional information to
Paul D. Bigbee, M.S.
Forensic Science Research and Training Center
FBI Academy
Quantico, VA 22135