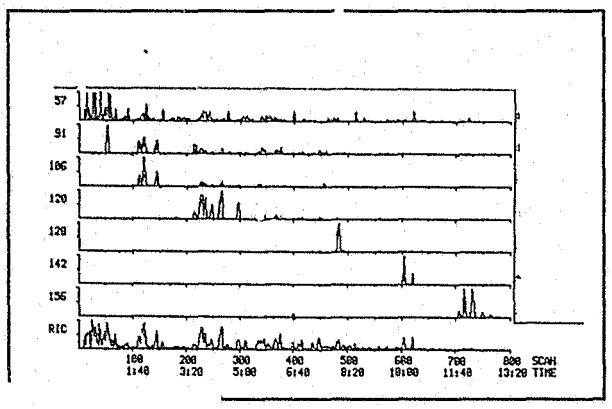
January 1987
Volume 14, No. 1
U.S. Department of Justice
Federal Bureau of Investigation

Crime Laboratory Digest

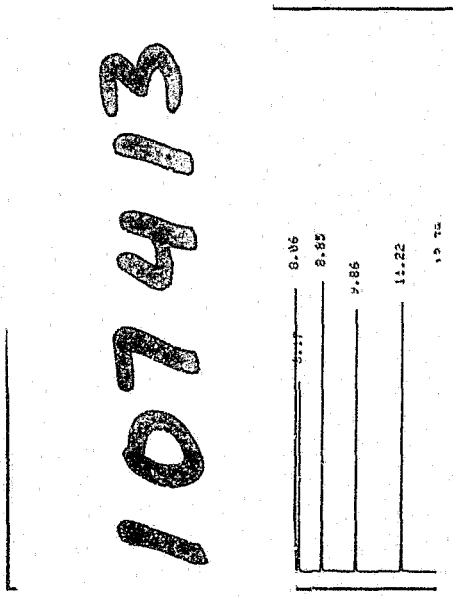
Review Article In This Issue

Fire Investigation
Part 2: Laboratory Investigation



Retention time (RT) in minutes and some compounds from a standard mixture

RT	Compound
3.20	Toluene
3.64	n-Octane
5.04	Ethylbenzene
5.26	m&p-Xylenes
6.00	n-Nonane
7.25	n-Propylbenzene
8.06	Psuedocumene
8.17	n-Decane
8.85	Indane
11.22	n-Dodecane
	n-Tetradecane
	n-Pentadecane
	n-Heptadecane
	n-Octadecane
	n-Heneicosane
	n-Tricosane



CRIME LABORATORY DIGEST

PUBLICATION POLICY

The Crime Laboratory Digest is published quarterly by the FBI Laboratory in cooperation with the American Society of Crime Laboratory Directors (ASCLD). It is intended to serve as a rapid means of communication between crime laboratories, permitting information of interest and value to be disseminated among crime laboratory scientists.

Inclusion of an article in the Crime Laboratory Digest in no way represents an endorsement or recommendation of any part of that article by the Federal Government, the Department of Justice or the FBI. Contributing authors assume total responsibility for the contents and accuracy of their submission. Questions or requests concerning an article should be directed to the contributing agency.

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Crime Laboratory Digest - Editor
FSRTC, FBI Academy
Quantico, Virginia 22135

CRIME LABORATORY DIGEST Vol 14, No. 1, January 1987
ISSN NO. 0743-1872

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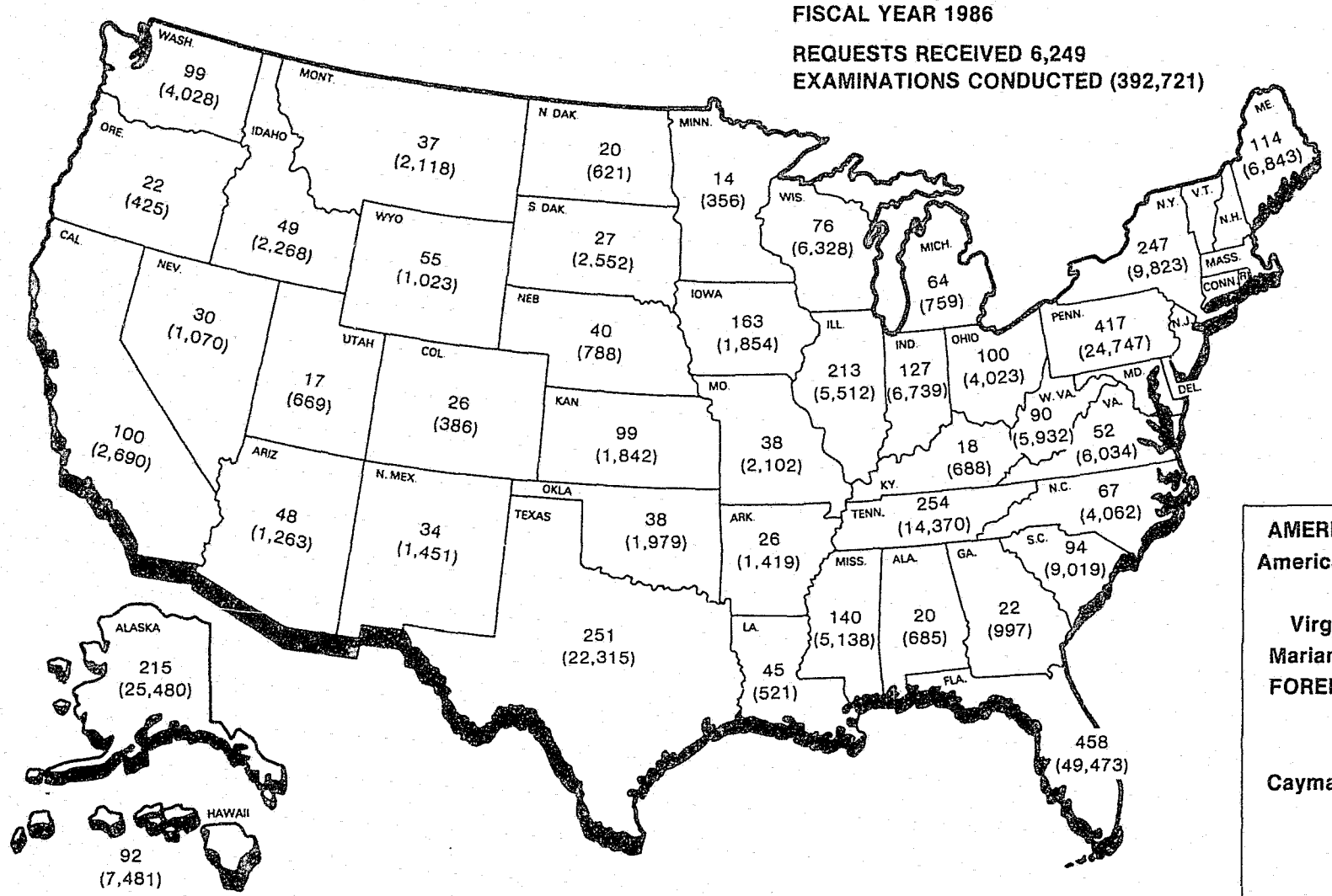
The cover, prepared by the Special Projects Section of the FBI Laboratory, depicts an ion chromatogram, a gas chromatogram and a partial list of the components shown in the gas chromatogram.

FBI LABORATORY

ASSISTANCE TO NON-FEDERAL LAW ENFORCEMENT AGENCIES

FISCAL YEAR 1986

REQUESTS RECEIVED 6,249
EXAMINATIONS CONDUCTED (392,721)



VT	74	(1,020)
NH	90	(2,816)
MA	117	(4.739)
CT	49	(874)
RI	76	(6,257)
NJ	217	(15,997)
DE	167	(14,177)
MD	568	(46,038)
DC	617	(46,883)

AMERICAN POSSESSIONS		
American Samoa	1	(7)
Guam	18	(1,018)
Virgin Islands	75	(4,117)
Mariana Islands	2	(90)
FOREIGN GOVERNMENTS		
Bahamas	3	(10)
Canada	1	(43)
Cayman Islands	12	(604)
Israel	2	(7)
Malta	1	(143)
Sweden	1	(8)

FBI/DOJ

HTLV-III AND THE FORENSIC LABORATORY

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INTRODUCTION

Webster's New World Dictionary partially defines a paradox as "a statement that seems contradictory but may actually be true in fact." This definition may be applied to the statement, "although the possibility of infection of laboratory personnel appears to be remote, evidence bearing the body fluids of a person infected with HTLV-III (AIDS) will not be examined in the forensic laboratory due to the potential hazards associated with the virus." The disjunction of Webster's definition allows us to examine the two aspects of the paradox.

Many published studies as well as leading medical and epidemiology experts advise that the probability of the transmission of HTLV-III in clinical laboratory specimens is remote, as is the possibility of acquiring the disease through casual contact with AIDS patients. Experts from the Centers for Disease Control (CDC) in Atlanta, Georgia, have advised the FBI Laboratory that our employees are at "low risk" of acquiring a laboratory borne HTLV-III infection. Thus, it appears concern regarding the acquisition of HTLV-III by forensic laboratory workers from evidence is contradictory to that advice. However, there is still the possibility of laboratory workers acquiring an HTLV-III infection.

What are the risks of handling evidence bearing body fluids and stains derived from persons with AIDS or those testing positive for the HTLV-III antibody? Regardless of the fact that forensic laboratory workers are in the "low risk" category, is any risk acceptable?

Little research has been conducted concerning the transmission of the virus in forensic specimens and the long-term virulence of the virus in body fluid stains. Unlike other pathogenic microorganisms normally encountered in forensic specimens, such as hepatitis B, HTLV-III has two rare attributes. The first is that there is no vaccine against the virus, and if the infection results in the acquired immune deficiency syndrome, the disease is fatal. The second unusual trait is the extraordinary and unknown length of the incubation period before symptoms of the disease appear following exposure to the virus. This phenomenon could result in a laboratory worker becoming infected and unknowingly transmitting the virus to his or her spouse. It is feasible that an infected male laboratory worker could transmit the virus to his pregnant wife, who would then infect her unborn child by transplacental infection during delivery, or even through later breast feeding (Lapointe *et al.* 1985; Ragni *et al.* 1985; Thiry *et al.* 1985; Ziegler *et al.* 1985). Because many techniques employed in the forensic laboratory are unique and therefore not performed in clinical laboratories, this paper will address some aspects of HTLV-III and its transmission. Also discussed are means to deal with potential cases of HTLV-III contamination and other pathogenic microorganisms in forensic laboratories, as well as the policy of the FBI Laboratory for accepting evidence containing known HTLV-III viruses.

THE VIRUS AND ITS MECHANISM

Human T-lymphotropic virus type III (also designated lymphadenopathy-associated virus and abbreviated HTLV-III/LAV) is the etiologic agent of the acquired immune deficiency syndrome (AIDS) and AIDS-related complex (Barre-Sinoussi *et al.* 1983; Gallo *et al.* 1984; Levy *et al.* 1984; Centers for Disease Control 1986a; Coffin *et al.* 1986). The virus infects helper/inducer T-lymphocytes and possibly other cell types, with a direct cytopathic effect and/or indirect effects on cells involved in cellular and humoral immunity (Dalgleish *et al.* 1984; Fauci *et al.* 1984; Klatzmann *et al.* 1984; Montagnier *et al.* 1984; Popovic *et al.* 1984). The virus, described as a "retrovirus", utilizes the enzyme reverse transcriptase to incorporate the genetic information of the viral RNA into the DNA of the host cell.

The origin of the virus is uncertain.

According to Dr. Robert Gallo, Chief of the Laboratory of Tumor Cell Biology at the National Cancer Institute, the virus probably entered man 20 years ago. It is hypothesized that the virus was transmitted by humans in Africa who were bitten by African green monkeys. During the mid-1970's there was a cultural exchange of some 10,000 people between Haiti and Zaire, and the virus may have crossed the Atlantic in that exchange. It then may have moved from Haiti to New York when the island became a popular vacation spot for male homosexuals, according to Dr. Peter Fischinger of the National Cancer Institute (Thompson 1985).

Once the virus invades the human body, it utilizes the antigenic sites of its protein coat to adhere to a T-helper lymphocyte, the control cell in the immune system. It then injects its RNA into the T-lymphocyte, and by using reverse transcriptase, incorporates its genetic code into the DNA of the host cell. At this point, the infected T-lymphocyte may continue to function normally, with no symptoms of AIDS being seen. At some point, for reasons unknown, the viral genome activates itself and causes the lymphocyte to divide and produce new viral RNA. The new viral particles then invade other T-lymphocytes until few remain uninfected, and the host's immune system fails. When this occurs, the victim has AIDS and eventually dies. Death in AIDS patients, however, is not caused by the HTLV-III directly; it is caused by secondary weak pathogens that can no longer be immunologically defeated (Davis *et al.* 1980; Thompson 1985).

OCCURRENCE AND FREQUENCY

The HTLV-III virus has been isolated from blood, bone marrow, lymph nodes, brain tissue, semen, cell-free plasma, vaginal secretions, cervical secretions, tears and human milk (Groopman *et al.* 1984; Zagury *et al.* 1984; Salahuddin *et al.* 1985; Thiry *et al.* 1985; Vogt *et al.* 1986; Wofsy *et al.* 1986). According to CDC, the highest incidence of both infection and transmission of the virus appears in active homosexual males and intravenous drug users. Although the disease is primarily seen in homosexual males and transmitted through sexual intercourse by homosexual males, hemophiliacs that receive infected blood, nonhomosexual intravenous drug users injecting themselves with contaminated needles, and Haitians display increased rates of AIDS infections. Several cases have been reported where the virus has been transmitted by unusual means. In one case, a male hemophiliac who had been infected with the virus from blood products transmitted the virus to his wife, who then infected her child during the birth process (Ragni *et al.* 1985). In another instance, a woman delivered her child by Caesarean section and received a contaminated blood transfusion after the birth. Through consumption of human milk or some other means of transmission, the child was infected and exhibited symptoms of AIDS several months later (Ziegler *et al.* 1985). Four of eight women in Australia who were artificially inseminated with semen from AIDS carriers became positive for the AIDS antibody (Stewart *et al.* 1985). Other fetuses/infants have been positive for the HTLV-III antibody received from their mothers (Thiry *et al.* 1985). Prompt *et al.* (1985) reported the transmission of the virus by renal transplantation. To date, all published reports and data from CDC show that casual contact with any of the previously mentioned body fluids is not the cause of transmission of the AIDS virus.

Data from CDC shows that 1,758 health care workers in the United States have been studied for the presence of the HTLV-III antibody. Twenty-six (1.5%) were positive for the HTLV-III antibody, and all but three belonged to groups recognized to be at increased risks (Abbott Laboratories 1985). These cases probably represent occupational transmission of HTLV-III due to parenteral (injection by sharp instrument) exposure. A health care worker in Great Britain is believed to have developed HTLV-III antibody following parenteral exposure to the blood of an AIDS patient (Abbott Laboratories 1985). According to CDC, there have been and likely will be more instances of transmission of the virus by heterosexual intercourse.

The incidence of AIDS cases is increasing exponentially in the United States.

Dr. James W. Curran of CDC estimates that from 500,000 to 1 million people have been infected with the virus, but only approximately 20% of those infected have developed actual symptoms (Abbott Laboratories 1985). It is estimated that this number will increase, perhaps to 30%, in the future. From June 1, 1981 through September 30, 1985, CDC received reports of 13,611 cases of AIDS. Of those patients, 6,944 have died. AIDS transmitted by transfusions accounted for 236 cases; hemophiliacs with AIDS numbered 105 cases. Three states, North Dakota, Montana and Idaho have reported no cases, and 31 states have each reported more than 25 cases. The cumulative number of reported AIDS cases from Jan 1, 1986 through July 13, 1986 for selected geographical areas, many of which contribute a significant number of cases to the FBI Laboratory is as follows: Massachusetts (196), New York (1,765), New Jersey (401), Pennsylvania (172), Michigan (186), Maryland (101), the District of Columbia (114), Florida (334), Texas (330), California (1521) and Alaska (9). The total number of AIDS cases reported to CDC for this period in the U.S. was 6,146 (Centers for Disease Control 1986a).

From October 1, 1985 through March 31, 1986, the U.S. Department of Defense tested 308,076 recruit applicants for serological evidence of AIDS infection. Antibodies against the virus were found in 1.5 per 1,000 recruits. The rates varied among men, women and racial groups. It was higher among the 265,361 men of all ages (1.6/1,000) than among the 42,715 women (0.6/1,000). Prevalence varied with race. For the 237,568 whites tested the rate was 0.9/1,000, and for the 55,185 blacks it was 3.9/1,000 (Centers for Disease Control 1986b).

MANIFESTATIONS OF AIDS

Persons infected with the AIDS retrovirus may present a variety of manifestations ranging from asymptomatic infection to severe immunodeficiency and life threatening secondary infectious diseases or cancer. The CDC classifies AIDS patients in one of four categories ranging from acute infections to those classified as "other conditions". Manifestations of the disease can include a mononucleosis-like syndrome with or without meningitis, lymph node enlargement that persists for months (not caused by other disease states), cancers and myelopathy (diseases of the spinal cord). Secondary infections and death can occur from such microorganisms as *Pneumocystis carinii*, which produces a severe form of pneumonia, to unusual forms of tuberculosis (Centers for Disease Control 1986a).

PREVENTION AND CONTROL

There currently exists no vaccine or cure for the AIDS virus. One promising drug, azidothymidine or "AZT" is currently being used experimentally to treat AIDS patients (Yarchoan *et al.* 1986). The only other preventive measure for the transmission of AIDS is for those individuals who are infected with the virus to voluntarily cease such activities that further spread the disease.

The control and disinfection of HTLV-III on environmental surfaces such as laboratory bench tops and instruments is relatively simple. Ordinary bleach solutions (0.5% sodium hypochlorite) and 70% alcohol will destroy the virus within 1 minute, and the virus is susceptible to high heat (Spire *et al.* 1984; Spire *et al.* 1985; Resnick *et al.* 1986). Drying does not necessarily inactivate the virus. A study done by Resnick and coworkers showed that HTLV-III can survive in liquid and dried bloodstains for a minimum of 15 days at room temperature, and it concluded that "....infectious virus can persist in a liquid or dried state for prolonged periods of time, possibly even at elevated temperatures" (Resnick *et al.* 1986). In another study, the virus was inactivated by heating at 56° C for 30 minutes but was not inactivated by 2×10^5 rad of gamma irradiation or 5×10^3 J/m^2 ultraviolet irradiation (Spire *et al.* 1985). The use of heat for disinfection of HTLV-III is certainly appropriate with respect to inanimate objects that are not for serological examination. However, heat will denature the proteins of interest and render the specimens unsuitable for most serological and possibly other

forensic examinations. Dr. Phillip D. Markham participated in Resnick's study and has indicated the virus could be deep frozen with little effect when thawed (Markham, personal communication). Freezing prolongs the longevity of most viruses, including HTLV-III. It is standard practice in the FBI Laboratory to freeze all items of evidence prior to examination, with the exception of liquid blood samples and items to be examined for latent fingerprints. Therefore, this practice as well as refrigeration will prolong the pathogenicity of HTLV-III.

SAFETY AND HANDLING

CDC recommends that biosafety level 2 practices, containment equipment and facilities be utilized when working with any known or potentially infectious body fluid and tissues (Richardson and Barkley 1984). Additional containment equipment and special research activities involving HTLV-III-related viruses and virus-producing cell lines require biosafety level 3 practices. These recommended biosafety level practices, containment equipment and facility safeguards, which are too lengthy to be discussed in this paper, are described in detail in "Biosafety in Microbiological and Biomedical Laboratories" published by CDC (Richardson and Barkley 1984). The Advisory Committee on Dangerous Pathogens (ACDP) in Great Britain, in its AIDS Interim Guidelines published in December, 1984, recommends that laboratory workers should not be expected to receive specimens from AIDS patients without prior consultation. It recommends that laboratory staff should follow a written protocol for work with HTLV-III, and procedures with viable viruses must be done in a containment level 3 laboratory. The ACDP (1985) has advocated that a full-scale postmortem examination should not be done to confirm the cause of death when AIDS has already been established.

The World Health Organization (WHO) has placed emphasis on education of the public to the risk of AIDS infection and ensuring that health care workers are informed about management of the disease. The WHO recommendations focus on proper precautions when caring for AIDS patients and handling specimens from these patients. The agency suggests that laboratory personnel be screened periodically for the antibody to HTLV-III (Abbott Laboratories 1985).

AIDS AND THE FORENSIC COMMUNITY

The forensic crime laboratory performs examinations on items of evidence that are foreign to the clinical laboratory setting. The forensic serologist not only performs analyses of liquid blood samples in cases of violent crimes but also examines numerous items of evidence bearing dried body fluid stains. Often, large items such as blankets, sheets and carpets are suspended and scraped vigorously. This action could create potentially infectious airborne particles. Other items, such as those examined at crime scenes, are processed outside the laboratory and involve potential injury to the skin by sharp and possibly contaminated objects. In one case submitted to the Serology Unit of the FBI Laboratory, an individual received a deep puncture wound to the knee from a wrapped package concealing numerous sharp carpet nails. The nails were attached to a blood soaked carpet which was saturated during the violent stabbing death of a known homosexual. The Serology Unit and the Chemistry-Toxicology Unit also often examine hypodermic syringes and needles for the presence of blood and illicit drugs. This evidence presents potential hazards since users of drugs are in the high risk category of persons infected with both hepatitis B and AIDS. Other Units in the FBI Laboratory as well as the Latent Fingerprint Section frequently examine body fluid stained evidence and occasionally body parts potentially contaminated by HTLV-III or other human pathogens. The nature of the violent criminal and his or her personal habits increases the possibility of laboratory workers coming in contact with materials contaminated with HTLV-III.

The FBI Laboratory strives to achieve the safest work environment possible for its employees. Certain practices currently being utilized in the FBI Laboratory have been and will continue to be the focus of criticism by

some individuals as being "knee jerk" and exaggerated responses to potentially pathogenic microorganisms, in particular HTLV-III. For example, the Serology Unit requires that employees handling liquid blood samples wear white laboratory coats, latex gloves, protective masks and protective eyewear. In addition, when handling any evidence in a manner which creates an aerosol or airborne particles, masks are required. While it is recognized that the mask provides limited protection, exposure of the oral and nasal mucosal surfaces to droplet and particulate contamination will be avoided. Latex gloves and laboratory coats are required at all times when handling any liquid or dried body fluid. Further, the FBI Laboratory requires that any case involving items from an individual with an infectious disease be worked in the laminar flow cabinet unless specimen size is prohibitive. These precautions may be considered excessive. However, if the prevention of serious disease results, it is difficult to imagine how any safety measure can be considered excessive.

FBI POLICY ON AIDS

Cases involving evidence derived from persons infected with tuberculosis or AIDS will not normally be accepted for examination by the FBI Laboratory without the contributor first obtaining authorization from the Chief of the Scientific Analysis Section. This policy is in effect because of the potential persistence of the causative agents (*Mycobacterium tuberculosis* and HTLV-III) in liquid or dried body fluid stains examined in the laboratory. Unlike such diseases as hepatitis B, which is curable and can be prevented, HTLV-III is lethal. There is no vaccine against it, and the possibility exists that an asymptomatic infection could be transmitted to others.

The FBI Laboratory will accept AIDS cases only when the following criteria have been met:

1. Prior authorization from the Scientific Analysis Section of the Laboratory has been received by the contributor.

2. The contributor must understand that currently, the submitted evidence will be treated such that the evidence will be rendered unsuitable for serological analyses (autoclaving). Other Units of the FBI Laboratory will then conduct their examinations.

3. Acknowledgement letters from both the prosecuting and defense attorneys must accompany all evidence, advising that they are aware that the serological evidence will be destroyed and that this procedure will not be subject to legal or judicial action in the future.

Contributors will also be advised that the mailing of liquid blood, other body fluids or stains bearing human pathogens is subject to the regulations of Title 42 of the Code of Federal Regulations, Part 72, which governs the interstate shipment of diagnostic specimens. The specimens and evidence are subject to applicable packaging, labeling and shipping requirements for the interstate shipment of etiologic agents. Additional information on the interstate shipment of evidence containing any microorganism that is pathogenic to humans may be obtained by writing or calling the Centers for Disease Control, Office of Biosafety, 1600 Clifton Road, N.E., Atlanta, Georgia 30333, telephone (404) 329-3883 (FTS 236-3883) (Richardson and Barkley 1984; Taylor, personal communication).

It is the goal of the FBI Laboratory to continue to perform examinations as a full service laboratory for its contributors. However, the safety and welfare of its employees is the Laboratory's highest priority and must be considered when accepting evidence. The most logical means of protecting employees against HTLV-III is to destroy or inactivate the virus without destroying or denaturing the proteins and other substances contained in or on evidence. The FBI Laboratory is currently conducting research in conjunction with the National Bureau of Standards and the National Institutes of Health on the sterilization of evidence by more elevated amounts of gamma radiation than has previously been reported. If the sterilization

of evidence can be accomplished without destroying or altering proteins and other substances, the employee will be protected, and the contributor, prosecutors, evidence technicians and any other person subsequently handling the evidence will be protected as well (McKinney, personal communication; Simic, personal communication).

CONCLUSION

The possibility of infection of forensic laboratory personnel by HTLV-III appears remote. However, this possibility still exists, as well as the possible subsequent transmission of the virus to a spouse or other contacted person. Furthermore, there is only experimental treatment for the disease, and if contracted by any means, it is ultimately fatal. In terms of known pathogens, the virus and its infectivity to man has only been recently discovered. A great deal of research must be conducted, especially in the area of forensic science, to solve the paradox of HTLV-III in the forensic laboratory.

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