

Chin Med J (Taipei) 1997; 59:325-33.

## Temperature Effect on the Sensitivity of ELISA, PA and WB to Detect Anti-HIV-1 Antibody and Infectivity of HIV-1

Gong-Ren Wang<sup>1,2</sup>, Jyh-Yuan Yang<sup>2</sup>, Tsuey-Li Lin<sup>2</sup>, Hour-Young Chen<sup>2</sup>, Chi-Byi Horng<sup>2</sup>

<sup>1</sup>Department of Medicine, Taipei Medical College; <sup>2</sup>National Institute of Preventive Medicine, Department of Health, Executive Yuan, Taipei, Taiwan, R.O.C.

---

### Abstract

**Background.** This study is designed to resolve the problem of whether temperature or freeze/thaw cycle will have any impact on the sensitivity for detection of anti-HIV-1 antibody by particle agglutination (PA), enzyme-linked immunosorbent assay (ELISA) and western blotting (WB). To reduce potential risk for laboratory personnel exposed to HIV-infection, it will be useful to determine the temperature effect on HIV infectivity.

**Methods.** Testing sera were incubated at different temperatures or treated with several cycles of freeze and thaw. PA, ELISA and WB were used to detect anti-HIV-antibodies, whereas syncytia formation assay and polymerase chain reaction (PCR) were applied to detect HIV-infection.

**Results.** The data showed that certain temperature points (no treatment, 25°C for 1hr, 2hrs and 4 hrs, 37°C for 30 minutes and 60 minutes, 56°C for 30 minutes and 60 minutes, 65°C for 15 minutes and 30 minutes) had no impact on the testing results of ELISA, PA and WB in detection of anti-HIV-1 antibody. In addition, testing results of 50 normal human serum samples which had been heated to 56°C for 30 minutes were still negative by ELISA and PA. Only the samples incubated at 65°C for 60 minutes had slight differences in results. Freeze and thaw treatments of the serum did not alter anti-HIV testing results, either. Treatments of supernatant of HTLV-III<sub>B</sub> culture at 56°C for 30 minutes and 60 minutes, 65°C for 15 minutes and 30 minutes could eliminate the syncytia formation caused by HIV-infection. Further analysis of the samples by PCR was able to detect HIV-specific sequences in all the treatments.

**Conclusions.** Anti-HIV antibody is quite stable in serum, even when it is pre-heated to 56°C for 30 minutes. Freeze and thaw treatment of serum samples up to seven cycles did not change the results, either. In addition, to minimize the potential risk of laboratory personnel exposed to HIV infection, pre-treatment of serum samples with heat at 56°C for 30 minutes or 60 minutes can reduce HIV infectivity. However, laboratories still must

emphasize the importance of universal precautions rather than heat-inactivation of serum to prevent occupational transmission of HIV.

*[Chin Med J (Taipei) 1997;59:325-33.]*

**Keywords:** acquired immunodeficiency syndrome, enzyme-linked immunosorbent assay, human immunodeficiency virus, particle agglutination, western blotting

**Received:** August 1, 1996.

**Accepted:** April 21, 1997.

**Address reprint requests to:** Gong-Ren Wang, 161, Kun Yang St., Nan Kang Dist., Taipei, Taiwan, R.O.C.

---

## Introduction

Encouraging progress has been made in the understanding the pathogenesis of human immunodeficiency virus (HIV) infection, started from the initial isolation of causative agents in 1983 [1] to the more recent development of potential therapeutic approaches via antiviral drugs and vaccination trials. Nevertheless, the resolution toward eventual control of HIV still seems far away and acquired immunodeficiency syndrome (AIDS) remains untreatable. To date, no drug or vaccine has become available to cure or stop the disease [2,3]. However, the development of sensitive and specific tests for antibody to HIV, the causative agent of AIDS, has progressed rapidly [1]. These tests have been used for various purposes, including clinical diagnosis of HIV infection, for symptomatic and asymptomatic patients who are in counseling and testing programs, for seroprevalence surveys and for blood-donor screening.

Laboratory testing in clinical practice is performed to provide information and guidance to the physician in treating the individual patient. Erroneous reports, positive or negative, may be distressing to the patient and misguide the physician. Testing for antibodies against HIV is especially complicated by the social and medical implications of false reports. A false positive report to a patient is potentially tragic, but largely preventable. A false negative report has serious potential public health significance because the person may then continue to infect others through high-risk sexual or drug-using practices, or by serving as a blood donor; and further available effective medical treatments would not be sought.

Accurate results from diagnosis of HIV infection will assure the safety of blood banks, provide information for physicians to take appropriate steps to treat patients and monitor the epidemiological trend of HIV infection. For example, owing to a shortage of labor, many foreign workers have been imported into Taiwan, and precautionary steps must be taken toward surveillance of any infectious diseases, including HIV infection, they may carry. To monitor the accuracy of HIV-testing results performed by clinicals and hospitals

in Taiwan, the Department of Health, Executive Yuan asked the National Institute of Preventive Medicine (NIPM) to implement a systematic proficiency testing program to evaluate laboratory test results. The program has indicated that accurate results could best be determined by sensitivity of the detection kits used and the skills of laboratory assistants. However, some of the participating laboratories complained that the ice needed to maintain stability of tested specimens had melted when samples were received. Thus the concern is raised that unfavorable conditions such as high temperature during the sample delivery process or too many cycles of freeze/thaw in the laboratories probably can deviate the test results.

Currently, in Taiwan, the most popular way to screen HIV-1 infections is either by enzyme linked immunosorbent assay (ELISA) or particle agglutination (PA). Once any positive case has been identified, the infections need to be confirmed by western blotting (WB). This study is designed to resolve the problem of whether the temperature or freeze/thaw cycle will have any impact on the sensitivity of ELISA, PA and WB used to detect anti-HIV-1 antibody [4]. Heat-inactivation is an effective means of destroying HIV-1 and is used to prepare therapeutic blood products [5]. To minimize the potential risk of laboratory personnel's exposure to HIV infection, it was also of interest to find out how temperature affected HIV-1 infectivity. By analysis of this information, determination can be made of the best temperature point at which HIV-1 infectivity is inactivated, yet the integrity of anti-HIV-1 antibody remains sufficiently intact to allow detection by ELISA, PA or WB.

## Materials and Methods

A panel of three serum specimens, kindly provided by the Chinese Blood Service Foundation and previously determined as anti-HIV-1 positive by the methods of ELISA, PA and WB was used as testing samples for evaluation throughout this study. The characteristics of the serum samples are listed on [Table 1](#). According to the results of WB, Samples I and II were weakly positive, whereas Sample III was strongly positive ([Figure 1](#)).

Fifty normal human serum samples were obtained for anti-HIV-1 detection by ELISA and PA tests from the Division of Epidemiology of this institute.

### Detection Kits

Three different types of ELISA kits, manufactured by Abbott, Pasteur, and General Biologicals, among the most commonly applied in Taiwan, were used for evaluation of the testing. PA kit for detection of HIV-1 antibodies was purchased from Fujirebio Inc., Japan (Serodia HIV-1). WB kit for detection of antibodies to HIV-1 was manufactured by Cambridge Biotech, Worcester, MA, USA. The experimental procedures of ELISA, PA and WB followed manufacturers' instructions.

### Syncytia Formation Assay

Infectious HIV was measured by determining the ability of heat-treated or freeze/thaw HIV-1 (HXB2 strain) fluids to infect with SupT1 cells. Briefly, cell-free supernatant was serially diluted in individual wells of a 96-well microtiter plate, and immediately  $2 \times 10^5$  exponentially growing SupT1 cells were added to each well. The wells were examined daily for the presence of syncytia. The first sign of syncytia formation could be seen by 24 hours, with complete syncytia developing by 48 hours and final results read at 96 hours.

### **Polymerase Chain Reaction (PCR)**

In vitro enzymatic amplification was used to identify HIV-1 infection after treatment with different incubation temperatures. Oligonucleotide primers specific for the env and gag were synthesized by Protech Technology enterprise Co., Ltd. Taipei, Taiwan according to the subsequence of HXB2 strain.

The env primers' sequences are 5' AGCAG, CAGGA, AGCAC, TATGG (7347-7366) and 5' CCAGA, CTGTG, AGTTG, CAACAG (7468-7488); gag primers' sequences are 5' ATAAT, CCACC, TATCC, CAGTA, GGAGA, AAT(1097-1124) and 5' TTTGG, TCCTT, GTCTT, ATGTC, CAGAA, TGC (1184-1211). The PCR reaction mixture contained 2 micron g chromosome DNA purified by Genomix Kit (Talent Co, Follateio, Italy), 1.0 micron M each of primers, 200 micron M each of four deoxyribonucleotide triphosphates, 10mM Tris-HCl, pH 7.5, 50mM NaCl, 10 mM MgCl<sub>2</sub>, and 2.5 unit of Taq polymerase (Promega) in a 100 micron l volume. The amplification was carried out with a DNA thermal cycler (Model 480, Perkin Elmer Cetus, USA). The profile for PCR was as follows: denaturing the samples at 94°C for 2 minutes, annealing at 55°C for 2 minutes, then extension at 72°C for 1 minute totally for 30 cycles. The PCR products were 141 bps and 114 bps in length after amplification with env and gag primers, respectively and they were analyzed by electrophoresis on 3% agarose gels.

### **Virus Strain**

HTLV-IIIB/H9 [6] is a HIV-1 (IIIB) permanent productive cell line which was established from the concentrated culture fluids of peripheral blood or bone marrow from several patients with AIDS or related diseases in a cloned permissive neoplastic T cell line H9. This virus appears to be well adapted for in vitro culture in T cells and replicates less in fresh human macrophage.

### **SupT1 Cells**

Non-Hodgkin's T cell lymphoma was isolated from the pleural effusion of an eight-year-old boy, and subcloned on soft agar [7]. High levels of surface CD4 molecules were expressed and can be useful in studies of cell fusion and cytopathic effect (CPE) of HIV infection. They express pan T antigens and lack sheep erythrocytes receptors. SupT1 cells were used for syncytia formation assay as an indicator cell line; they can be maintained in RPMI 1640 plus 10 % fetal bovine serum (FBS).

## Treatment Strategy

To evaluate the temperature effect on the stability of anti-HIV antibody, results obtained by ELISA, PA, and WB from samples with different treatments were compared. These treatments included incubations of samples at room temperature (25°C) for 1 hour, 2 hours, and 4 hours, respectively; 37°C for 30 minutes and 60 minutes; 56°C for 30 minutes and 60 minutes; 65°C for 30 minutes and 60 minutes; 75°C for 15 minutes and 30 minutes; 95°C for 5 minutes and 15 minutes. Also the samples were frozen at -70°C, then thawed at 37°C for one, three, five and seven cycles, respectively. When the serum samples were heated to 75°C or above 75°C in this study, they became gel-like in structure and were no longer in a liquid state. Thus only those samples still in liquid state were investigated.

## Results

### PA Results

The data obtained by PA are presented in [Table 2](#). It was found that temperature had no effect on the detection of anti-HIV-1 antibody by PA. Even when the serum sample was heated to 65°C for 60 minutes, the results remained the same as for the untreated samples. The freeze and thaw treatment of samples, up to seven cycles, did not change results either. In addition, temperature and freeze/thaw treatments did not change the setting pattern of particles for PA which still showed a defined large ring with a rough multiform outer margin and peripheral agglutination. Additional tests of fifty normal serum samples treated with heat showed no sign of agglutination (data not shown).

### ELISA Results

Three different manufacturers' ELISA kits were used to detect the anti-HIV-1 antibody ([Tables 3-5](#)). Samples I and II had lower optical density (OD) than Sample III. This finding is consistent with the WB result which suggested that Samples I and II are weak positive and Sample III is strong positive.

With reference to the detection kit of General Biological, none of the heating treatments had any apparent effect on the results except at 65°C for 60 minutes of Samples I and II, both of which were weakly positive ([Table 3](#)). Treatment at 65°C for 60 minutes did reduce the OD values of Sample I from 0.64 to 0.34 and Sample II from >3 to 1.62, respectively, but the final outcomes were still the same. Over all, increasing temperature did not alter the OD value in any regular patterns. Freeze/ thaw cycles did not change the results either.

For the kit of Abbott ([Table 4](#)), most of the heating treatments did not change the OD values except treatment at 65°C for 60 minutes did turn the result of Sample I negative (from 0.165 to 0.098, cut off value: 0.118). OD value of Sample II was reduced from 0.332 to 0.13 (still positive). This treatment had no effect on Sample III (strongly positive). Freeze and thaw treatments had no effect on the three testing samples.

Different heating treatments did not have any effect on the ELISA results obtained by Pasteur ([Table 5](#)). The OD values did reduce slightly from 1.7 to 1.39 of Sample I and from 1.79 to 1.63 of Sample II by the treatment at 65°C for 60 minutes, however, both results were still much greater than the cut off value 0.107. Once again, freeze and thaw treatments had no effect on the testing results by Pasteur.

Centers for Disease Control (CDC) have issued a warning about the problems of giving false positive results, problems created by heat-inactivation of serum specimens before HIV-1 antibody testing [4]. Therefore, fifty normal human serum samples were randomly selected for treatment with heat at 56°C for 30 minutes before testing by ELISA (Welcome and Abbott); all of them were negative (data not shown).

### **WB Results**

The band pattern of sample I was shown in [Figure 1A](#). The p24, gp41 and gp160 remained unchanged throughout the various temperature treatments except at 65°C for 30 minutes. In that condition, the gp160 became almost invisible, but it was still detectable on the blot under the treatment; p41 band could not be seen very clearly on the picture, but was detectable on the blot. The p41 band of Sample II almost disappeared on the picture after the treatment at 65°C for 60 minutes ([Figure 1B](#)). However, this band could be seen clearly on the blot after treatment. Bands of p24 and gp160 of Sample II were still very clear on the blot after the treatments. For Sample III, intensity of all the bands, including p15, p24, p31, p51, p55, p66, gp120 and gp160, did not alter after the treatments ([Figure 1C](#)). Freeze/thaw treatment of Samples I and III had no effect on the band patterns of WB ([Figure 1D](#)).

### **Syncytia Formation Results**

The CPEs induced by HIV-infection (syncytia formation) of the treated groups and that of the control (untreated) group were shown in [Table 6](#). Syncytia formation could be detected when virus supernatant from HTLV-IIIB/H9 was incubated at room temperature for 1, 2 and 4 hours; and at 37°C for 30 and 60 minutes whereas incubation of the virus at 56°C for 30 and 60 minutes and at 65°C for 15, 30 and 60 minutes can eliminate the syncytia formation induced by HIV infection. Five and seven cycles of freeze and thaw treatment of the virus can reduce the syncytia formation on Day 3 to some extent when compared to the untreated samples, but it is still detectable.

### **PCR Results**

The DNAs were purified from Day 3 cultures without syncytia formation and subjected to PCR analysis. After amplification by env primers, the products were analyzed on a 3% agarose gel. Fragments of predicted size (141 bps) were detected on the samples with incubation at 56°C for 30 and 60 minutes and at 65°C for 30 and 60 minutes, respectively ([Figure 2](#)). When another set of primers specific for gag region of HIV-1, the same results emerged (data not shown).

### **Discussion**

Given the medical and social significance of a positive test for HIV antibody, test results must be accurate and interpretations of the results must be correct. For these reasons, the Department of Health has requested that an individual be considered to have serological evidence of HIV infection only after an ELISA test is repeatedly reactive or a PA test is positive and another confirmatory test such as WB has been performed to validate the results.

Appropriate storing and shipping conditions of specimen are the key points of accurate testing results for detection of infections. For viral infections, the detection of corresponding antibody responses is easy and reliable [8]. However, because the major composition of an antibody is amino acid which is heat liable, high temperatures may cause denaturation of proteins and results in false negative outcome. Therefore, it is important to ensure that the samples to be tested are at good quality and under good conditions.

According to the results here, high temperature up to 65°C had no obvious effect on the sensitivity of PA and WB in detection of anti-HIV-1 antibody. In ELISA, only the treatment of 65°C for 60 minutes did turn the result to negative by the Abbott ELISA kit but not by the other two ELISA kits. Shirazian et al. [9] found that the washed samples of residual blood from needles and broken pieces of glass that had been exposed to HIV-1-seropositive sample and left at room temperature for one hour, one day and one week still resulted in positive tests for HIV-1 antibody by ELISA, immunofluorescence (IF) and WB. Their results are consistent with the findings here; both indicated that HIV-1 antibody is quite stable in serum.

On the other hand, results here differed from the results of experiment performed by Evans et al. [10] and McBride et al. [11], those reports indicated that heat-inactivation of serum specimens before screening by ELISA for HIV antibody can give false positive results and can also interfere with WB analysis. The major reason for the difference may be the detection kits used in this study are much more advanced than the old generation kits.

An extra fifty normal serum samples were tested by ELISA (Wellcome and Abbott) and PA. All those testing results were still negative when the serum was pre-heated to 56°C for 30 minutes. New generation kits have improved significantly in quality, specificity and sensitivity over the old kits used several years ago. Therefore, the false positive problem caused by the heating process may have been resolved or improved already.

Currently, only WB is used as confirmatory test in Taiwan. Although all WB interpretations are based on detecting antibodies against specific viral proteins, different official agencies or institutions have promoted the use of different sets of criteria for interpreting HIV-1 band patterns in the WB test. So far, CDC and World Health Organization (WHO) interpretive criteria [12,13] are those most accepted by the testing laboratory. Both WB interpretive criteria consider a WB test that has no bands as nonreactive for HIV antibody, and WB band patterns that do not meet the specific criteria for reactive are termed "indeterminate". For CDC criteria, any two bands of p24, gp41 or gp120/160 are considered as reactive; for WHO criteria, any two of the env proteins (gp41, gp120 and gp160) are regarded as positive. Therefore, p24, gp41, gp120 and gp160 should deserve more concern during the processing of samples, because if one of these bands has

disappeared, the result may become "indeterminate". Data here suggested that the experimental treatments did not lessen the intensity of the bands. The serum samples used for the HIV-1 antibody proficiency test were delivered by this staff and kept carefully in container packed with ice. Even the worst case, they would not have been left at room temperature for more than two to three hours. Therefore, participating laboratories doing proficiency tests of HIV-infection should not complain again that poor storage conditions have influenced their testing results.

For purposes of public health, people usually pay much more attention to false negative results for HIV infection. Factors that were or might have been responsible for the appearance of false negatives are (1) assay insensitivity; (2) window period [14] and a late stage of AIDS with no antibody response yet, and antibodies saturated by an overburden of HIV antigens, respectively; (3) incorrect original designation of the specimen as positive or continuing uncertain serostatus; (4) deterioration of the specimen in storage, and (5) technical error. To avoid false negative results, the Department of Health has issued different policies or strategies such as strict regulations on detection kits before their marketing, regular check of the detection kits already on the market, proficiency test for detection of HIV-infection, training programs for laboratory technicians and others. Even through all the efforts have been made, the problem of a window period of HIV infection can not be overcome. When whole-virus-lysate ELISA (old generation kit) was used to screen blood donations from 1985 to 1990 in the United States, the average length of the window period was 45 days [14]. The average window period of the new generation kit, which can detect both IgG and IgM HIV-1 and HIV-2 antibodies, is 25 days [15,16]. During the acute period of infection, tests for p24 antigen can detect HIV infection earlier than antibody tests [17]. p24 antigen, the core structural protein of HIV, is detectable two to three weeks after HIV infection. On an average, p24 antigen is detected an estimated six days before antibody tests become positive [18,19]. However, the detection kits can not be depended upon totally to resolve problems caused by a window period. Even through the sensitivity of detection kits has been improved significantly, the answer to window period still remains unsolved. An improvement in donor interviewing about the behaviors associated with risk for HIV infection is one of the best ways to further secure safety of blood bank and public healths.

Since temperature has no effect on PA, ELISA and WB sensitivity in detecting HIV-1 antibody, an attempt was made to heat the serum samples before the process of detection to inactivate the virus and reduce potential risk of exposure to HIV-infection. Both syncytia formation assay and PCR were applied to detect HIV infection. The HIV-infected cells can attract uninfected T cells bearing CD4 nearby to form a giant cell, a so-called "syncytia", which is very easy to observe under the microscope. PCR is an extremely sensitive method to pick up any signal (DNA or RNA) existing in samples. In this study, no sign of HIV-infection was observed by syncytia formation assay after a three-day incubation. Usually HIV-infected culture in vitro can induce CPE in two to four days when co-cultured with uninfected CD4+ T cells. Further analysis of the samples by PCR indicated that the HIV-1 signal can be detected. However, if the virus loads are extremely low, it may take longer for syncytia to form. Thus, the testing samples may not have been incubated long enough to see the CPE induced by HIV, but its genomic signals could still be picked up by PCR. High temperature can cause dissociation of the non-covalent bonds between gp41 and gp120,

which in turn will reduce the syncytia formation ability of HIV. In addition, it is possible that syncytium-inducing (SI) property of HIV changes to non-SI by treatment with heat. All of these factors might contribute to the opposite results seen in CPE and PCR.

The report of Stromberg et al. [20] indicated that mild heat treatment (45°C) is not an adequate process for viral inactivation of red cell products. Putting the data together, results here suggested that HIV infectivity can be reduced to some extent by heating to 56°C for 30 minutes and 60 minutes or 65°C for 30 and 60 minutes, but it is very difficult to totally destroy HIV-infectivity by such treatments.

The virus titer was not determined in this study; however, it should be higher in the supernatant collected from HTLV-III<sub>B</sub>/H9 cell culture than in the serum of HIV-positive individuals. Thus, it is reasonable to predict that after heat treatments of samples at 56°C or 65°C, the virus titer in any suspected serum should drop to even lower levels.

Only fifty samples were tested by two different ELISA kits with no apparent dissimilitude, therefore, the possibility of false positive results caused by heating of serum samples could not be excluded. Based on these findings, it is suggested that serum samples could be heated first under some circumstances such as to achieve an efficient proficient testing program to inactivate the virus or to reduce its infectivity before the process of detection of the HIV antibody. But laboratory workers are still urged to follow the universal precautions, with the basic assumption that all blood should to be considered to be potentially infective [21,22].

#### Acknowledgements

We thank Dr. Chin Li-Te at the Chinese Blood Services Foundation and Mr. Yeh Jane Shiann at Provincial Tao Yung Hospital for performance of the ELISA analyses.

#### References

1. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S. Isolation of a T-lymphotropic retrovirus from a patient at risk to acquired immune deficiency syndrome (AIDS). *Science* 1983;220:867-71.
2. Richman DD. HIV therapeutics. *Science* 1996;272:1886-8.
3. Bloom BB. A perspective on AIDS vaccine. *Science* 1996;272:1888-90.
4. Centers for Disease Control. Problems created by heat-inactivation of serum specimens before HIV-1 antibody testing. *MMWR* 1989;38:407-13.
5. Martin LS, McDougal JS, Loskoski SL. Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy-associated virus. *J Infect Dis* 1985;152:400-3.
6. Popovic M, Sarngadharan MG, Read E, Gallo RC. Isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 1989;224:497-500.

7. Smith SD, Shatsky M, Cohen PS, Warnke R, Link MP, Glader BE. Monoclonal antibody and enzymatic profiles of human malignant T lymphoid cells and derived cell lines. *Cancer Res* 1984;44:5657-60.
8. Sarngadharan MG, Popovic M, Bruch L, Schupbach J, Gallo R. Antibodies reactive with human T lymphotropic retrovirus (HTLV-III) in the serum of patients with AIDS. *Science* 1984;224:506-8.
9. Shirazian D, Herzlich BC, Mokhtarian. F, Grob D. Detection of HIV antibody and antigen (p24) in residual blood on needle and glass. *Infect Cont Hosp Ep* 1990;11:180-4.
10. Evans RP, Shahson DC, Mortimer PP. Clinical evaluation of Abbott and Wellcome enzyme-linked immunosorbent assays for detection of serum antibodies to human immunodeficiency virus (HIV). *J Clin Pathol* 1987;40:552-5.
11. McBride JH, Howantiz PJ, Rodgerson DO, Miles J, Peter JB. Influence of specimen treatment on nonreactive HTLV-III sera. *AIDS Res Hum Retrov* 1987;3:333-40.
12. Centers for Disease Control. Interpretation and use of the western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. *MMWR* 1989;262:3395-7.
13. World Health Organization. Acquired immunodeficiency syndrome (AIDS) proposed WHO criteria for interpreting results from western blot assays for HIV-1, HIV-2, HTLV-1/HTLV-II. *Wkly Epidem Rec* 1990;65:281-8.
14. Petersen LR, Satten GA, Dodd R, Busch M, Kleinman S, Grindon A, Lenes B. Duration of time from onset of human immunodeficiency virus type one infectiousness to development of detectable antibody. *Transfusion* 1994;34:283-9.
15. Busch MP, Lee LLL, Satten GA, Henrard DR, Farzadegan H, Nelson KE, Dodd RY, Petersen LR. Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. *Transfusion* 1995;35:91-7.
16. Lackritz EM, Satten GA, Aberle-Grasse J, Dodd RY, Raimardi VP, Janssen RS, Lewis WF, Notari EP, Petersen LR. Estimated risk of transmission of the human immunodeficiency virus by screened blood in the United States. *N Engl J Med* 1995;333:1721-5.
17. Alter HJ, Epstein JS, Swenson SG, VanRaden MJ, Ward JW, Kaslow RA, Menitove JE, Klein HG, Sandler SG, Sayers MH, Chernoff AI, Hewlett IK. Prevalence of human immunodeficiency virus type 1 p24 antigens in US blood donors-an assessment of the efficacy of testing in donor screening. *N Engl J Med* 1990;323:1312-7.
18. Munde Y, Kamtorn N, Chaiyaphruk, Nantachit N, Ness PM, Nelson KE. Infectious disease markers in blood donors in northern Thailand. *Transfusion* 1995;35:264-7.
19. Centers for Disease Control. U.S. public health service guidelines for testing and counseling blood and plasma donors for human immunodeficiency virus type 1 antigen. *MMWR* 1996;45(RR-2):1-9.
20. Stromberg RR, Kuypers FA, Sawyer L, Friedman L, Cole M, Tran K, Hanson CV. Loss of red blood cell viability associated with limited thermal inactivation of extracellular HIV-1. *Vox Sang* 1994;67: 260-6.
21. Centers for Disease Control. Recommendations for preventive of HIV transmission in health-care settings. *MMWR* 1987;36(suppl 2):3-18.

22. Centers for Disease Control. Update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR* 1988;37:377-88.

[Vox Sang.](#) 1992;62(1):12-20.

[Related Articles.](#)

[Links](#)

**High-temperature short-time heat inactivation of HIV and other viruses in human blood plasma.**

[Charm SE](#), [Landau S](#), [Williams B](#), [Horowitz B](#), [Prince AM](#), [Pascual D](#).

Charm Bioengineering, Inc., Malden, MA 02148.

An ultra-short-time heating system was used to process blood plasma spiked with various viruses (HIV, vesicular stomatitis virus, encephalomyocarditis virus). Virus reduction and recovery of plasma proteins were measured at various temperatures from 65 to 85 degrees C. Processing at 77 degrees C and 0.006 s resulted in a high level of virus kill, including greater than or equal to 4.4 log<sub>10</sub> HIV, while maintaining protein structure and activity essentially intact.

PMID: 1374578 [PubMed - indexed for MEDLINE]

[Unfallchirurg.](#) 1997 May;100(5):375-81.

[Related Articles.](#)

[Links](#)



**[Inactivation of HIV-1 in human femur heads using a heat disinfection system (Lobator SD-1)]**

[Article in German]

[von Garrel T](#), [Knaepler H](#), [Gurtler L](#).

Klinik für Unfallchirurgie, Phillips-Universität Marburg.

The use of allogenic bone transplants in surgery has been greatly diminished owing to the risk of transmitting infectious diseases. This risk can be reduced by the use of a thermal disinfection system (Lobator SD-1). This is achieved by increasing the temperature to 80 degrees C, inactivating a number of bacterial and viral agents. In this study the decay of HIV at high temperature in the Lobator SD-1 was researched. In the center of human femoral heads 100 microliters of a highly concentrated suspension of free and cell-bound HIV (10<sup>10</sup>) was exposed to the thermal process at intervals of 5, 10, 20, 30, 40, 50 and 62 min. For the recultivation HUT-78 cells were used through titration of the virus suspension in ten-fold dilutions over ten dilution steps and incubation up to a maximum of 21 days. Evidence of the virus was checked through observing giant cell formations and

quantitative determination of p24 antigen using an Elisa test. Linear virus inactivation was found based upon the time the virus was exposed to heat. After a treatment of 40 min in the disinfection system, total virus inactivation was achieved. The normal disinfection process time using Lobator SD-1 is 92 min. A temperature of 80 degrees C is reached after approximately 45 min. The results prove that this system totally inactivates HIV in human femoral heads.

PMID: 9297246 [PubMed - indexed for MEDLINE]

## Novel method of Inactivation of HIV-1 by the freeze pressure generation method (FPGM).

Int Conf AIDS 2004 Jul 11-16; 15:(abstract no. C10237)  
 Otake T, Kawahata T, Mori H, Kojima Y, Hayakawa K  
*Osaka Prefectural Institute of Public Health, Osaka, Japan*

**BACKGROUND:** HIV infection through blood transfusion is still possible if donated blood collected during the so-called window period is used for the transfusion. We already reported that high-pressure (over 600 MPa) treatment at room temperature inactivates HIV-1, and have recently shown that high pressure generated by the expansion of water due to freezing (freeze pressure generation method, FPGM) has an inactivating effect on bacteria.

**METHODS:** Seven HIV-1 strains were used: 4 T cell-tropic strains and 3 macrophage-tropic strains including clinical isolates. A plastic tube containing a virus suspension was placed in a pure-water-filled stainless pressure-tight container, which was left in a freezer for 24 hours. The infective titer of the virus was determined using MT-4 and MAGIC5A cells. Reverse transcriptase (RT) activity was measured using a nonradioisotope RT activity assay kit. In addition, the viral envelope function was tested. A concentrated virus sample was reacted with MT-4 cells, which were reacted with anti-gp120 antibody to measure the amount of virus bound to the cells.

**RESULTS:** The virus infective titer of all HIV-1 strains decreased to approximately 1/100 after treatment at -10degreesC (100MPa), and below the detection limit after treatment at -20degreesC (200 MPa) or -30degreesC (250 MPa). The RT activity of HIV-1 remained unchanged at -10degreesC, but decreased to approximately 1/10 at -20degreesC or -30degreesC. In addition, we confirmed that the ability of the virus to bind to the surface of cells was lost by treatment at -20degreesC.

**CONCLUSIONS:** Several HIV-1 strains including clinical isolates became completely inactivated by FPGM at -20degreesC (200 MPa) to -30degreesC (250 MPa). The mechanism of the virus inactivation may involve virus enzyme damage and changes in virus envelope proteins. FPGM seemed to be simple and convenient method for the detoxication of blood preparation.

**Keywords:** AEGIS, HIV-1, HIV Envelope Protein gp120, RNA-Directed DNA Polymerase, HIV Infections, Pressure, Virion, Macrophages, T-Lymphocytes, immunology, methods

Keywords: Keywords: W. Lyman, et l, AIDS, Electric Current, lymphoblastoid cell lines (H9 and CEM-SS), HIV-1, AIDS, tritiated thymidine ( $^3\text{H-TdR}$ ), HBWSS, Syncytium-formation assay, varicella, The RF Strain, (SDH), H+(ATP).

## BIOCOMPATIBLE ELECTRIC CURRENT ATTENUATES HIV-I INFECTIVITY

---

William D. Lyman, Irwin R. Merkatz  
 William C. Hatch and Steven C. Kaali  
 Departments of Pathology,  
 and Obstetrics & Gynecology  
 Albert Einstein College of Medicine,  
 1300 Morris Park Ave., Bronx, N.Y.10461

Running title: Electricity reduces HIV-1 infectivity

Correspondence: Dr, Wm.. D. Lyman  
 Department of Pathology  
 Albert Einstein College of Medicine  
 1300 Morris Park Avenue  
 The Bronx, NY 10461  
 (212) 430-2171

### SUMMARY

In this report, we present the results of double-blinded studies on the use of direct electric current to alter the infectivity of HIV-1 for susceptible cells *in vitro*. Two lymphoblastoid cell lines (H9 and CEM-SS) were exposed to aliquots of the RT strain of HIV-1 treated with direct current. Results of these studies show that virus treated with currents from 50 to 100 microamperes ( $\mu\text{A}$ ) has a significantly reduced infectivity for susceptible cells.

These experimental currents were equal to 3.85 and  $7.7 \mu\text{A}/\text{mm}^2$  current densities respectively. The reduction of infectivity was dependent upon, the total electric charge ( $\mu\text{A} \times \text{min}$ ) passing through the chamber to which the virus was exposed. Viral infectivity was determined by two independent measures: a syncytium-formation assay which can be used to quantify the production of infectious particles; and, a reverse transcriptase assay which is an index of viral protein production. Additional experiments demonstrated that the currents employed were biocompatible. Uninfected H9 cells were exposed to the same conditions used for the viral aliquots.

There was no significant change in the percentage of viable uninfected cells exposed to any of the currents tested. Therefore, because biocompatible direct electric current attenuates the infectivity of cell-free virus, this treatment may allow development of new strategies to prevent transmission of HIV-1 through either treating the general blood supply or developing alternative barrier contraceptive devices. Additionally, biocompatible electric current may be applicable for the direct treatment of AIDS patients by utilizing either extracorporeal systems or self contained indwelling electrodes. Lastly, because the virus is being attenuated, electric current may also render treated HIV-1 suitable for vaccine development.

Key words: HIV-1, AIDS, treatment, suppression of infectivity, electricity

## INTRODUCTION

The number of individuals infected by the human immunodeficiency virus type-1 (HIV-1) continues to increase on a world-wide basis (1). A significant percentage, if not all, of these individuals will eventually develop the acquired immunodeficiency syndrome (AIDS) (2)- While horizontal transmission in the homosexual population may be contained or decreasing (3), heterosexual transmission and infection through contaminated blood supplies continues to increase (4). Additionally vertical transmission from infected females to their fetuses is also on the rise with a resultant increase in the number of children with AIDS (5). New strategies, therefore, must be devised in order to limit more effectively the spread of this virus.

In this regard, three principal approaches are currently being investigated. In order to decrease susceptibility to the consequences of infection, vaccines are being sought which will induce the production of protective antibodies (6). As treatment modalities, the use of soluble antagonists to block the receptor for HIV-1 is being studied (7) as are pharmacologic agents such as nucleic acid analogs which can interfere with the transcription of viral genomic sequences (8). Each of these systems has----- and limitations and to date none has proven completely effective.

Because heat or light in combination with drugs and dyes can inactivate viruses including HIV-2 in vitro (9), others have suggested the use of these forms of energy to treat .. AIDS patients. The results of studies using heat have not been peer- reviewed and are therefore impossible to evaluate. The use of light with drugs ["photopheresis"] (10) appears to be efficacious although this treatment may be limited by drug toxicity and the potential long-term effects of ultraviolet radiation on blood cell nucleic acids. Also, by its nature, this last system may not be suitable for the treatment of tissue-associated virus.

As a result of our interest in the use of electric current to alter biological systems, we focused our investigations on the ability of direct electrical current at biocompatible levels to alter the infectivity of HIV-1 for susceptible CD4 positive cells in vitro.

## MATERIALS AND METHODS

### Electrical treatment of HIV 1:

The RF strain of HIV-1 (AIDS Reagent Program) was cryopreserved prior to treatment at  $-70^{\circ}\text{C}$ . For treatment, a sample of virus was thawed and maintained on ice at  $4^{\circ}\text{C}$ . Ten microliters ( $\mu\text{l}$ ) of HIV-1 at a concentration of  $10^5$  infectious particles per ml were placed into a chamber which included a pair of platinum electrodes 1mm apart permanently mounted into a well 1.56mm in length and 8.32mm in depth equal to  $12.9 \mu\text{l}$  volume capacity. The chamber was connected to a power supply capable of creating constant direct current. The viral aliquots were exposed to direct currents ranging from 0 microamperes ( $\mu\text{A}$ ) for up to 12 minutes to  $100 \mu\text{A}$  for up to 6 minutes. Intermediate currents of 25, 50 and  $75 \mu\text{A}$  were used to expose similar viral aliquots. Under these conditions, for example, 0, 50 and  $100 \mu\text{A}$  represent 0, 3.85 and  $7.7 \mu\text{A}/\text{mm}^2$  current densities respectively. The current was monitored throughout the experiment. A matrix of current and time employed is shown in Table 1.

After the exposure of virus to electric current, the contents of the chamber were removed and placed into sterile microtubes. Five  $\mu\text{l}$  of each sample were removed and diluted with  $95 \mu\text{l}$  tissue culture medium supplemented with 10% fetal calf serum (FCS) for subsequent assays.

### Syncytium-formation assays:

This assay was performed as previously described by Nara *et al* (11). Briefly,  $10^5$  CEM-SS cells were dispensed into poly-L-lysine coated microliter wells. Thereafter, tenfold dilutions of H9 cells incubated with the treated HIV-1 samples were co-cultured in triplicate for up to 4 days with the CEM-SS cells. Identical wells were prepared with control uninfected and infected cells. The wells were examined for syncytium formation at 2 and 3 days and quantified using an inverted microscope.

### Reverse transcriptase assay:

Uninfected H9 cells, were pelleted at 1,000 rpm for minutes at room temperature, the supernatant was decanted and the cells were resuspended in  $100 \mu\text{l}$  treated viral sample. The cells were incubated for up to 6 hours with the viral samples. At the end of the incubation

time, the viral/cell suspensions were centrifuged at 1,000 RPM for 5 minutes and the supernatant decanted. The cell pellet was then resuspended in 5ml of RPMI, 10% FCS and placed into a T25 tissue culture flask and maintained at 37°C, 5% CO<sub>2</sub> in a humidified chamber. At 2 day intervals (beginning at day 2), 1ml of the cell suspensions was removed from each sample and centrifuged at 1,000 rpm for 5 minutes in order to pellet the cells. The supernatant was subsequently centrifuged at 14,000 RPM for 15 minutes. The pellet was resuspended in suspension buffer and assayed using standard methodology employing Mg<sup>++</sup> as the divalent cation poly (rA) oligo d(T) 12-18 as template primer, and tritiated thymidine (<sup>3</sup>H-TdR) which comprise the reaction mixture. Known HIV positive and negative control samples were included in each assay for reference. Thirty  $\mu$ l of the reaction mixture were added to each 10  $\mu$ l viral sample and incubated at 37 °C for 60 min. Samples were then incubated with 1ml of cold quench solution on ice for 15 minutes and filtered through a Millipore manifold. Chimneys were rinsed first with wash solution and followed by cold 95% ethanol. The filters were dried by vacuum and counted in scintillation fluid. Reverse transcriptase activity is expressed as counts per minute (cpm) and is considered positive only if cpm are at least five times greater than the cpm obtained with HIV negative control samples.

### Biocompatibility of electric currents/time:

To determine if the electric currents used were in a biocompatibility range of energy, uninfected H9 cells were exposed to distinct currents for different amounts of time. The H9 cells were washed two times in Hanks Balance Salt Solution (HBSS). Thereafter, the cells were resuspended in RPMI, 10% FCS at a concentration of 10<sup>6</sup> cells per ml, Ten  $\mu$ l of the cell samples were placed into the reaction chamber. The cell samples were then exposed to 0, 50 or 100  $\mu$ A for 0, 3 or 6 minutes. At the end of each test, the cell sample was removed from the chamber and approximately 10  $\mu$ l of the sample was mixed with 90  $\mu$ l of trypan blue. The number of viable cells was determined by trypan blue exclusion using a hemocytometer and light microscope. Results are expressed as percentage of viable cells from the total of all cells. At least 200 cells per field were counted.

### Statistical analysis:

Results of the syncytium-formation and reverse transcriptase assays were tested for statistical significance by the Student's t test and analyses of variance.

## RESULTS

### Syncytium-formation assay:

Using this index of HIV-1 infectivity, it was determined that exposing virus to direct electric current suppressed its capacity to induce the formation of syncytia. Figure 1 shows a representative experiment and Table 2 shows the Group data for 3 separate experiments. As can be noted in Figure 1, a statistically significant ( $p < 0.001$ ) reduction in syncytium number was observed and this reduction was dependent upon the current applied to the viral isolate. At three different viral dilutions, there were analogous results in that a total charge of 200  $\mu\text{A} \times \text{min}$  (25  $\mu\text{A}$  for 8 minutes) reduced the number of syncytia from 50 to 65% while a charge of 300  $\mu\text{A} \times \text{min}$  (50  $\mu\text{A}$  for 6 minutes, 75  $\mu\text{A}$  for 4 minutes or 100  $\mu\text{A}$  for 3 minutes) resulted in 90% reduction.

### Reverse transcriptase assays:

The direct electric currents to which HIV-1 was exposed also reduced reverse transcriptase activity. Five separate experiments were conducted and a representative experiment is shown in Figure 2 and the group data are included in Table 3. As can be seen in Figure 2, there was a significant decrease in the amount of reverse transcriptase activity after exposure of the virus to either 50  $\mu\text{A}$  for 3 or 6 minutes. An equivalent reduction in reverse transcriptase activity was also noted with exposure to, 100  $\mu\text{A}$  for 3 minutes and almost ablation of reverse transcriptase activity was seen with exposure of the viral isolate to 100  $\mu\text{A}$  for 6 minutes. The group data (Table 3) show that after exposure to 50  $\mu\text{A}$  for 6 minutes, there was a 44% reduction in activity and treatment of virus with 100  $\mu\text{A}$  for 6 minutes resulted in a 94% reduction. An analysis of variance indicates that the decrease in reverse transcriptase activity was statistically significant ( $p < 0.0001$ ).

### Biocompatibility of the electric currents/time:

The results of a viability analysis using trypan blue exclusion criteria applied to uninfected cells exposed to the different currents and times used for these studies are shown in Table 4. The viability of H9 cells, after exposure to 100  $\mu\text{A}$  for either 3 or 6 minutes, did not show a significant decrease when compared to the 0 Current control. After maximum treatment at 100  $\mu\text{A}$  for 6 minutes, cell viability was 93%. Interestingly, in other preliminary experiments in which HIV-infected H9 cells were used, the results show that at 100  $\mu\text{A}$  there may have been a significant decrease in the number of viable cells. That is, while an instantaneous pulse of 100  $\mu\text{A}$  did not affect the viability of infected cells, at 3 and 6 minutes of exposure to 100  $\mu\text{A}$ , a decrease in viability was noted. This decrease was time dependent in that exposure to 100  $\mu\text{A}$  for 3 minutes resulted in a viability of 83% while 100  $\mu\text{A}$  for 6 minutes resulted in a viability of 80%. Although these data are provocative, they only represent a preliminary experiment and require further investigation.

With respect to the possibility that the electric current was transduced into heat, the calculated rise in temperature within the chamber was determined to be less than 1°C. In order to verify this, a temperature microprobe was introduced into the chamber containing tissue culture medium alone. Results of these studies are shown in Table S. Similar results were obtained when H9 cell-containing medium was placed in the reaction chamber. The data indicate that for the currents and times used for these experiments, there was no alteration in the temperature of the chamber.

## DISCUSSION

The results reported here demonstrate that HIV-1 treated with direct electric currents from 50 to 100iA has a significantly reduced infectivity for susceptible cells in vitro. This reduction of infectivity correlates with the total electric charge passing through the chamber. Although extrapolation of these data predicts that ablation of HIV infectivity may be possible, and additional preliminary data support this prediction, the expectation that some virions may still escape the electrical effect cannot be discounted. Nevertheless, the therapeutic potential of electric current may reside in its ability to lower the viral titer to subclinical significance or in its incorporation into a strategy analogous to that of other therapies in which repeated cycles of treatment eventually achieve remission or cure.

The data presented in this report are based on both quantitative and qualant determinations of viral infectivity. Although the syncytium-formation assay can be used to quantify the number of infectious viral particles, this use with respect to HIV-1 may be abridged because of the ability of free fusogenic peptide (gp41) to induce syncytia by itself. Therefore, while syncytia were observed at some dilutions of electrically-treated virus, this may simply represent the presence of soluble gp41 in the tissue culture medium. We believe that the correlation between total charge and reduction in syncytium number more adequately reflects the ability of direct electric current to reduce HIV-1 infectivity.

This belief is also supported by the results of the reverse transcriptase assays.

Although a decrease in HIV-1 reverse transcriptase does not assure reduced infectiousness of this virus for Susceptible cells; we feel that, taken together with the syncytium-formation data, the results indicate that significant attenuation of HIV-I infectivity is achieved by treatment with direct electric currents.

With respect to the biocompatibility of the electric currents and total charges reported here, two separate sets of evidence are applicable. The first has to do with the results showing that, by trypan blue exclusion, no significant cytotoxicity was induced in by any total charge tested. The other evidence is obtained from reports which clearly indicates that

the amount of electricity used for these experiments is significantly below presently used therapeutic electric currents which are in the milliamperage range (12-16).

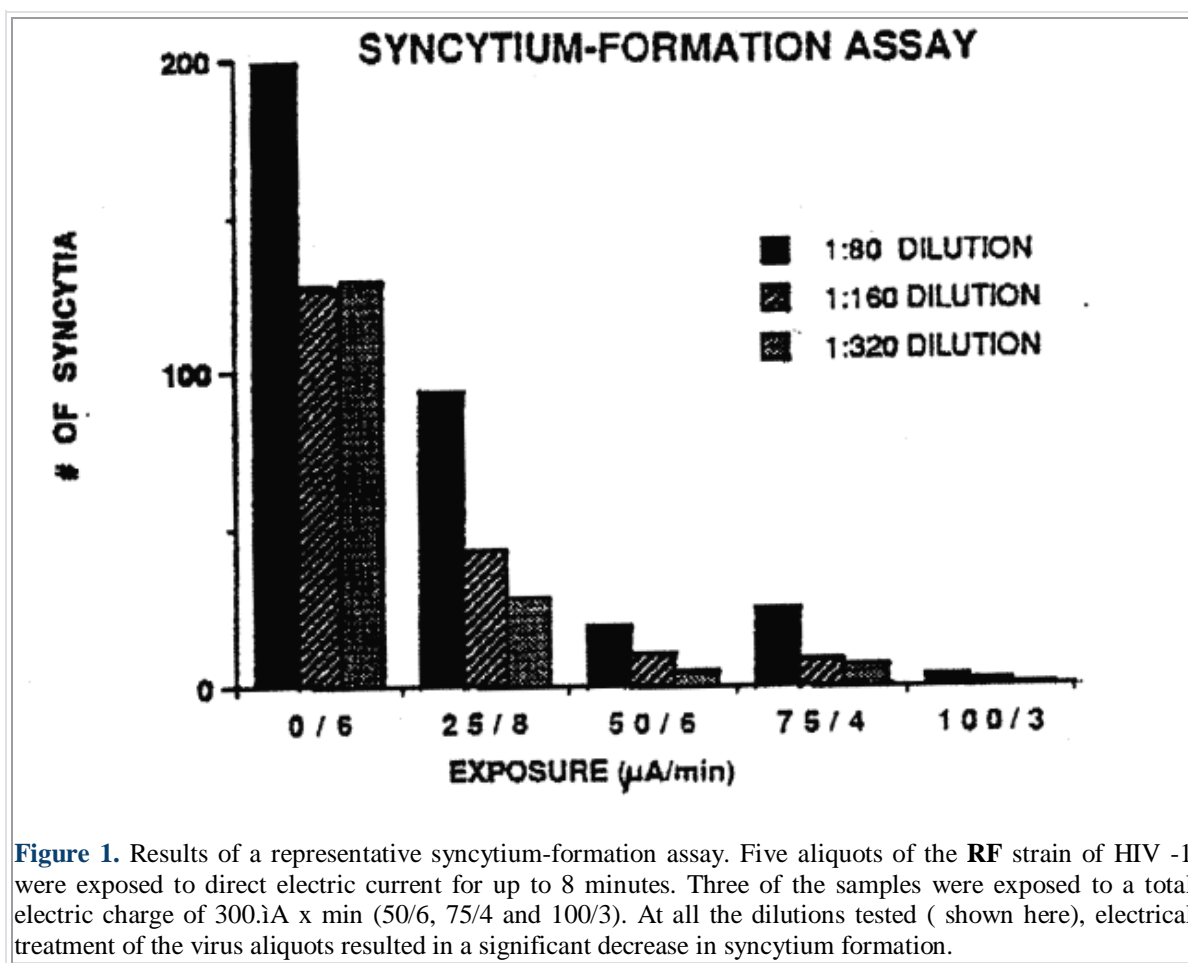
Rather than negative effects, exposure of cells to electric current may actually have positive consequences for resistance to infection in that important cellular electrochemical changes correlate with enhancement of specific enzymatic activities. In particular, a facilitation of succinate dehydrogenase (SDH) and ATPase activity has been observed (12,15). Both of these enzymes are associated with the oxidative capacity of the cell. Specifically, it has been suggested that an electrochemical reaction occurs between mitochondrial membrane-bound H<sup>+</sup> ATPase and ADP leading to the formation of ATP. Therefore, exposure of cells to direct electric current may directly or indirectly increase energy resources within a cell and facilitate cell metabolism. This, in turn, may actually render a cell less susceptible to the effects of viral infection.

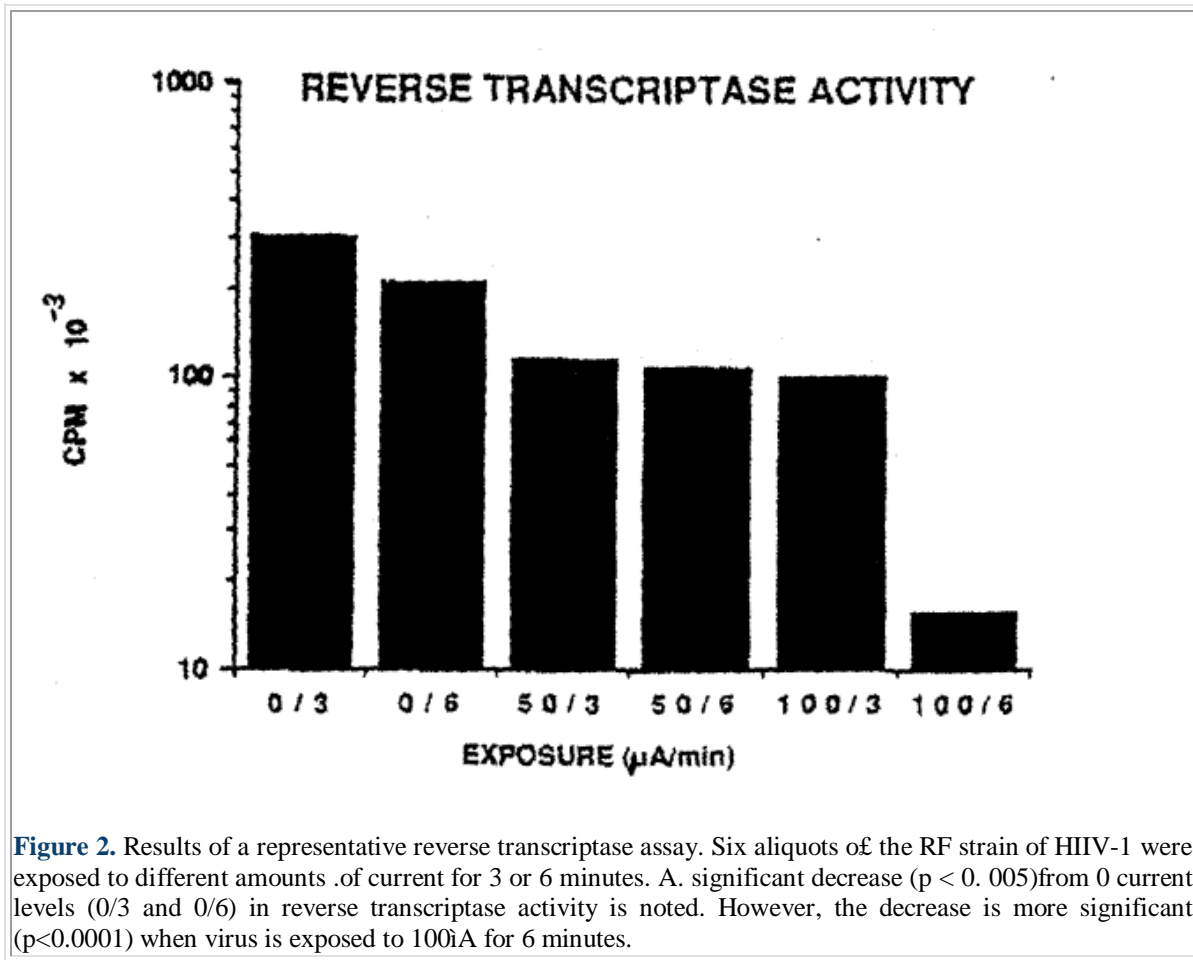
In summary, the data presented here indicate that biocompatible direct electric current significantly reduces the infectivity of HIV-1. Continuing investigations are exploring the mechanisms through which this effect is mediated. The initial focus of these experiments is centered on the potential role which ionic and molecular species generated by electrolysis may have on the virus. However, the complete mechanism by which direct electric current attenuates HIV-1 infectivity is undoubtedly far more complex than simple electrolysis. Nonetheless, and independent of a complete understanding of all of the mechanisms involved in the attenuation of HIV-1 infectivity, the present observations may serve as an initial step for the development of new strategies to treat infection or prevent transmission of HIV-1 through either treating the general blood supply or developing alternative barrier contraceptive devices. It may also be feasible to treat AIDS patients with direct electric current using either extracorporeal systems or self contained indwelling electrodes. Lastly, because viral infectivity is being attenuated, electric current may render treated HIV-1 suitable for vaccine development.

## ACKNOWLEDGMENTS

Thanks go to Mrs. Agnes Geoghan for her excellent secretarial assistance and to Dr. Gabor, Kemeny for important technical help. Additional thanks go to Drs. Frank Lilly and Philip Aisen for their constructive criticism of this manuscript.

## LEGENDS





Table

Experimental

Current (µA). Time (Minutes)

1

Paradigm

0	1 4 8 12
25	2 4 8 12
50	3 4 6 12
75	2 4 8 12
100	1 3 4 12

**Table 2**  
 Effect of ELECTRIC Current on Syncytium Formation<sup>a</sup>  
 % of O Current Control (Ä%)<sup>b</sup>  
 Current (iA) Six Minute Exposure

<b>0</b>	<b>100 (0)</b>
<b>50</b>	<b>50 (-50)</b>
<b>100</b>	<b>35 (-65)</b>

a = Value at 1:160 dilution of virus.  
 b = Value equals the mean of 3 experiments.

**Table 3**  
 Effect of Electric Current on Reverse Transcriptase Activity<sup>a</sup>  
 % of O Current Control (Ä%)  
 Current (ia) Six Minute Exposure

<b>0</b>	<b>100 (0)</b>
<b>50</b>	<b>56 (-44)</b>
<b>100</b>	<b>6 (-94)</b>

a = Value equals the mean of 5 experiments.  
 The standard error of the mean in each case was less than 10% of the mean value.

**Table 4**  
 Effect of Eclectic Current on Viability of Uninfected H9 Cells  
 (% Viable Cells<sup>a</sup>)  
 Length of exposure (Minutes), Current (iA) 0 3 6

<b>0</b>	<b>96 94 6</b>
<b>50</b>	<b>98 95 98</b>
<b>100</b>	<b>96 97 93</b>

a = At least 200 cells counted in hemocytometer field

**Table 5**  
 Effect of Electric Current on Temperature of  
 Tissue Culture Medium<sup>a</sup> (°C) Length of Exposure (Minutes)

Current (iA)	0 3 6
0	19 19 19
50	19 19 19
100	19 19 19

a = The temperature was monitored before, during and after exposure. Results shown are end-point determinations.

## REFERENCES

1. Sato PA, Chin J, Mann JM. Review of AIDS and HIV infection Global epidemiology and statistics. *AIDS* 1989; 3 Suppl.1:S301-7.
2. Centers for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *MMWR* 1987; 1 Suppl. 36:S1-15.
3. Thacker SB, Berkelman RL. Public health surveillance in the United States. *Epidemiol. Rev* 1988; 10:164-90.
4. Klein RS, Friedland GH. Transmission of human immunodeficiency virus type (HIV-1) by exposure to blood: Defining the risk. *Ann Int Med* 1990; 113:729-30.
5. Oxtoby MJ. Epidemiology of pediatric AIDS in the United States. In: *Brain in Pediatric AIDS* (Kozlowski PB, Snider DA, Vietze PM, Wisniewski HM, eds) 1990:1-8
6. Broder S, Mitsuya H, Yarchoan R, Pavlakis GN. Antiretroviral therapy in AIDS. *Ann Int Med* 1990; 113:604-18.
7. Perno CF, Baseler MW, Broder S, Yarchoan R. Infection of monocytes by human immunodeficiency virus I blocked by inhibitors of CD4-gp120 binding, even in the presence of enhancing antibodies. *J Exp Med* 1990; 171:1043-56.
8. Mitsuya H, Weinhold KJ, Furman FA et al. 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/ lymphadenopathy-associated virus in vitro *Proc Natl Acad Sci USA* 1985; 82:7096-100.
9. Quinnan GV, Wells MA, Wittek AE, et al. Inactivation of human T-cell virus, type III by heat, chemicals and irradiation. *Transfusion* 1986; 26:481-3.
10. Bisaccia E, Berger C, Klainer AS. Extracorporeal photopheresis in the treatment of AIDS-related complex: A pilot study. *Ann Int Med* 1990; 113:270-75.
11. Nara PL, Hatch WC, Dunlop NM, et al.: Simple, rapid quantitative, syncytium-forming microassay for the detection of human immunodeficiency virus neutralizing antibody. *Aids Res Hum Retrovirus* 1987; 3:283-302
12. Cheng N, Van Hoof H, Bockx E, et al. The effects of electric currents on ATP generation, protein synthesis, and membrane transport in rat skin. *Clin Ortho Rel Res* 1982; 171:264-72.
13. Frank G, Schachar N, Dittrich D, et al. Electromagnetic stimulation of ligament healing in rabbits. *Clin Ortho Rel Res* 1983; 175:263-72.

14. Eriksson E, Haggmark T. Comparison of isometric muscle training and electrical stimulation supplementing isometric muscle training in the recovery after major knee ligament surgery. *Amer J Sports Med* 1979; 7:159-71.
15. Stanish WD, Valiant GA, Bonen A, et al. The effects of immobilization and of electrical stimulation on muscle glycogen and myofibrillar ATPase. *Can J Appl Sport Sci* 1982; 7:267-71.
16. Pills AA. Electrochemical information transfer at living cell membranes. *Ann NY Acad Sci* 1974; 205:148-70.

Acta Orthopaedica Scandinavica

**Publisher:** Taylor & Francis

**Issue:** Volume 71, Number 5 / October 01, 2000

**Pages:** 508 - 512

**URL:** [Linking Options](#)

**DOI:** 10.1080/000164700317381225

**Inactivation of HIV by application of heat and radiation: Implication in bone banking with irradiated allograft bone**

Philippe Hernigou , Gabriel Gras , Georgette Marinello , Dominique Dormont

**Abstract:**

We developed methods for inactivating the human immunodeficiency virus by heat and ionizing radiation and tested the effects of these treatments on the mechanical strength of bone. Simultaneous use of heat and radiation caused a considerably greater inactivation of HIV than the additive effects of the two separate treatments, but also caused a significant reduction in the maximum load sustained by the bone specimens tested with an Instron machine. Application of the same doses but given in the sequential fashion of radiation followed by heat also caused marked inactivation of HIV and had less effect on the mechanical strength of the bone.