Inactivation of Human Immunodeficiency Virus in Serum Specimens as a Safety Measure for Diagnostic Immunoassays

P. Ukkonen¹*, J. Korpela¹, J. Suni², K. Hedman¹

Since the human immunodeficiency virus (HIV) may be transmitted accidentally to laboratory personnel analyzing patient sera, the efficiency of a non-ionic detergent, Triton X-100, in inactivation of HIV in human serum as a safety measure was studied. Semliki Forest virus, an enveloped togavirus, was used as a model virus to create optimal treatment conditions. In the presence of 50% serum, complete inactivation (i.e., no residual virus detected, >7 log reduction of virus titre) was achieved by incubation with 0.2% Triton X-100 for 1 h at 37°C. Under these conditions HIV was also completely inactivated (i.e., no residual infectious virus detected, >5 log reduction of virus titre). Both treated and untreated serum specimens were also tested with several enzyme immunoassays used in virological laboratories to determine whether the inactivation treatment interfered with the assays. The treated specimens, further diluted as recommended for each assay, were subjected to 15 enzyme immunoassays for microbial antibodies and antigens (HIV IgG, hepatitis A IgG and IgM, hepatitis B s, c, and e antigens and antibodies, cytomegalovirus IgG, mumps virus IgG, poliovirus IgG, rubellavirus IgM, toxoplasma IgG, and chlamydia IgG). Clearly decreased sensitivity was found only with two hepatitis B tests (e antigen and antibody to the surface antigen). It is concluded that safe inactivation of HIV in serum is achieved by 0.2% Triton X-100, but the treatment may decrease the sensitivity of some tests in which low specimen dilution is used.

Laboratory personnel performing tests with human serum specimens may be exposed to viruses such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV) present in some patient sera. Transmission of these viruses may occur by accidental needle stick or through mucous membranes or eczematous skin. Recently it was reported that a laboratory worker became infected by HIV through occupational exposure, although the exact mode of transmission remained unclear (1, 2). Therefore, to improve safety in the laboratory, serum specimens should be subjected to an inactivation procedure which effectively kills the potential pathogens but does not interfere with the results of subsequent laboratory tests such as enzyme immunoassays for viral antigens and antibodies. The optimal treatment should be simple to perform using an inactivating chemical non-toxic to humans.

Several treatments and agents have already been tested for the inactivation of HIV, including heat, detergents, beta-propiolactone, ethanol, sodium hypochlorite, phenol, paraformaldehyde, glutaraldehyde and formalin (3–6). However, in most previous studies the inactivation was not done in the presence of serum, which decreases the efficiency of some inactivators. Also, the interference of inactivation with various immunological tests was not usually studied.

The two most important viral pathogens in human serum, HIV and HBV, are viruses with a lipid-containing envelope. Non-ionic detergents such as Triton X-100 and NP-40 have been successfully used to disrupt enveloped viruses (7). It has been shown recently that both Triton X-100 and NP-40 inactivate HIV (5, 6). In the present study we examined the variables of virus inactivation by Triton X-100 (temperature, Triton X-100 concentration, and presence of serum) using Semliki Forest virus as a model virus, confirmed the results with HIV, and determined the effects of the Triton X-100 treatments of sera on several enzyme immunoassays used in microbiological laboratories.

Materials and Methods

Model Virus: Semliki Forest Virus. Semliki Forest virus, an enveloped alpha virus, was grown in baby hamster kidney fibroblasts (BHK-21 cells) as previously described (8). Cell
culture supernatant containing about 10^10 plaque forming units of Semliki Forest virus per ml was used in the inactivation experiments. The virus was incubated for 1 hour with varying concentrations of Triton X-100, in the presence and absence of 50% human serum, and at two temperatures, room temperature (about 29°C) and 37°C. The amount of residual infectious virus in specimens after the inactivation treatment was determined by inoculating 0.2 ml of 10-fold dilutions (dilutions 10^-1 to 10^-9, diluted in Minimum Essential Medium containing 0.2% bovine serum albumin) of specimens in BHK-21 cells on 24-well plates (Linbro, Flow Laboratories, UK). After adsorption for 1 hour at 4°C, 1 ml of culture medium was added and the cells were incubated at 37°C for two days. The cells were examined by microscope, and the reciprocal of the highest specimen dilution giving a cytopathic effect was taken as the virus titre (tissue culture infectious dose, TCID) of the specimen. When necessary, demonstration of Semliki Forest virus glycoproteins in the cultured cells by indirect immunofluorescence was used to confirm the result (differentiation between toxic and virus-induced changes). Triton X-100 contained in the treated specimens was not toxic to the BHK-21 cells as growth of Semliki Forest virus was shown in cells inoculated with 10^-1 dilution of the specimen treated with 0.2% Triton X-100 at 20°C (Figure 1).

**Human Immunodeficiency Virus.** The HIV-1 strain (HTLV-III) grown in 9H cell line was obtained from Dr. R. C. Gallo CDC, USA. For inactivation experiments, the virus was concentrated by pelleting in a ultracentrifuge. Concentrated HIV was then incubated for 1 hour with and without 0.2% Triton X-100 in the presence of 50% human serum. The amount of residual infectious virus in serum specimens after the inactivation treatment with Triton X-100 was determined as follows. Ten-fold dilutions (10^-1 to 10^-6, diluted in RPMI 1640 supplemented with 10% foetal bovine serum) of specimens were added to polybrene pre-treated 9H cells (8 x 10^5 cells/ml). After incubation for 24 hours at 37°C, the supernatant was discarded, the cells were washed once with RPMI 1640 medium, and fresh culture medium was added. Every five days up to 35 days post-infection, a 2 ml culture medium sample was harvested for reverse transcriptase assay (9), and a cell specimen was taken for immunofluorescence staining in which a monoclonal antibody against the p24 protein of HIV (Labsystems, Finland) was used as the first antibody and a fluorescein-conjugated anti-mouse IgG/Fab2 (Cappel, USA) as the second antibody. The reciprocal of the highest specimen dilution giving a positive result by immunofluorescence and/or reverse transcriptase after 35 days in culture was taken as the virus titre (TCID). To determine the toxicity of Triton X-100 to H9 cells, the cells were first incubated for 2 hours at 37°C with mixtures of Triton X-100, human serum and culture medium, corresponding to the respective concentrations when different dilutions of Triton X-100 treated HIV specimens were tested for infectivity. The cells were then washed and HIV was added to the cultures. Growth of HIV was demonstrated as described above. The results showed that the amount of Triton X-100 contained in the 10^-1 specimen dilution was toxic to the cells (most cells died, no virus antigen detectable). Therefore, dilutions starting from 10^-2 could be used in the infectivity titration of Triton X-100 treated specimens.

**Tests for Microbial Antigens and Antibodies.** To study the interference of the inactivation treatment of serum specimens with various immunoassays, one part serum was mixed with one part PBS or with 0.4% Triton X-100 in PBS, and the mixture incubated for 1 hour at 37°C. This specimen (1:2 diluted serum) was then diluted to the final serum dilution recommended for each assay with the diluents provided with the kits. Therefore, the final concentrations of Triton X-100 varied considerably (from 0.2% to 0.0001%) in the different assays. The possible interference of the inactivation procedure was tested on the following enzyme immunoassays (EIA): EIA for antibodies to HIV (Vironostika anti-HTLV-III, Organon Teknika, Holland, and Wellcozyme anti-HTLV-III, Wellcome Diagnostics, UK); antibodies to the envelope and core proteins of HIV (HTLV III confirmatory EIA, Abbott Laboratories, USA); hepatitis B surface antigen, HBsAg (Auszyme monoclonal, Abbott); hepatitis B e antigen, HBeAg (Abbott-HBe, Abbott); antibodies to hepatitis B core antigen, anti-HBc (Corzyme, Abbott); antibodies to hepatitis A virus, Anti-HAV (Havab, Abbott); IgM class antibodies to hepatitis A virus (Havab-M, Abbott); IgG antibodies to poliovirus type 1 (method modified from references 10 and 11); IgG antibodies to mumps virus (12); IgM antibodies to rabies virus (Rubazyme-M, Abbott); IgG antibodies to cytomegalovirus (Cytomegalovirus IgG EIA, Labsystems, Finland); IgG antibodies to Chlamydia trachomatis (Chlamyset antibody EIA, Orion Diagnostica, Finland); and IgG antibodies to Toxoplasma gondii (Toxoplasma gondii IgG EIA, LabSystems). Western blotting (DuPont HTLV-III western blot, Biotech Research Laboratories, USA), immunofluorescence microscopy with slides containing HIV infected H9 cells (AIDS-IFA slides, I.A.F. Production, Canada), and fluorescent-conjugated anti-human IgG conjugate (Dakopatts, Denmark) were used as confirmatory tests for anti-HIV antibodies.

**Results.**

**Optimization of Inactivation by Triton X-100 with Semliki Forest Virus as Model Virus**

The inactivation of Semliki Forest virus by Triton X-100 was remarkably less efficient in the presence of serum or at 20°C than in the absence of serum or at 37°C.