Inactivation of Human Immunodeficiency Virus Type 1 and Herpes Simplex Virus Type 2 by Commercial Hospital Disinfectants

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Human Immunodeficiency Virus type 1 (HIV-1) survives drying on environmental nonporous surfaces at 35°C for at least 45 minutes at 40–60% relative humidity. This viral infectivity is readily destroyed by a wide range of commercial hospital disinfectants unless the amount of organic material present, e.g., blood, approaches 20%.

HIV-1 is the etiologic agent of acquired immunodeficiency disease syndrome (AIDS). The U.S. Public Health Service currently estimates that the number of HIV infections in the United States is between 945,000 and 1.4 million. The Public Health Service projects that, by the end of 1992, the cumulative number of diagnosed AIDS cases will total 365,000, with 263,000 deaths. In 1992 alone, there are expected to be 80,000 newly diagnosed cases of AIDS, and 65,000 deaths, primarily of persons diagnosed in previous years.1 Viral pathogenesis is dependent upon direct entry of the virus into the blood stream where T-cell CD-4 receptor sites act as points of viral attachment. Infection is possible through transfusion of contaminated blood; however, it now appears that the primary route of transmission is through sexual activities with an infected partner or by the sharing of contaminated needles by intravenous-drug users. Health care professionals, not in any of the above primary risk groups, have become infected with AIDS because of occupational exposure to HIV-1-infected blood.2 These case reports suggest that direct exposure of HIV-1-contaminated blood to mucous membranes or to skin that is chapped, abraded, or otherwise compromised, as with eczema, may result in HIV-1 infection. Thus, the possibility of environmental transmission of HIV-1 exists in areas where spills of contaminated fluids may occur.3 Therefore, it is necessary to have disinfectants available that are known to inactivate HIV-1. The United States Environmental Protection Agency (EPA) is empowered to regulate microbial disinfectants in terms of microbial efficacy and safety.4 We report evidence of complete inactivation of HIV-1 by various hospital disinfectants using an EPA-approved protocol.5 In addition, we report the observation that virucidal activity can be diminished in the presence of a relatively high organic load, e.g., blood serum. Finally, the results of a comparative evaluation of several commercial disinfectants for inactivation of HIV-1 on an environmental surface and HSV-2 on medical instruments are presented.

Materials and Methods

HIV-1: EPA-Type Virucide Assay

Host Cells and Virus Propagation. T lymphoma (H-9) cells were propagated in RPMI 1640 medium supplemented with up to 20% fetal calf serum. H-9 cell suspensions (approximately \(10^6\) cells/ml) were cultured in 25 cm² screw-capped tissue culture flasks and incubated at 37°C in 5% CO₂ in air with daily observations and refedings at least twice per week.

HIV-1-negative H-9 cells were inoculated with an HIV-1 culture supernatant and incubated at 37°C in 5% CO₂ in air and refed as necessary. Cultures were monitored for viral infectivity by detection of p24 core protein by the enzyme-linked immunosorbent (ELISA) antigen capture assay as well as by microscopic observations for cytopathic effect (CPE) with a phase contrast microscope. A call-free supernatant was obtained by pelleting the cells in a Sorvall RC-2B centrifuge fitted with an HB-4 rotor at 1500 rpm for 10 minutes. The supernatant was assayed for viral titer and stored at -70°C in a mechanical freezer.

0.5 ml of a previously prepared viral pool containing 10% fetal calf serum was spread onto the entire surface of individual glass surfaces (60-mm Petri dishes) and allowed to dry to a film at 35–37°C for 45 minutes. Each disinfectant was diluted to its recommended use concentration with either deionized or 500 ppm (as CaCO₃) synthetic hard water.6 5.0 ml of the use-dilution of the disinfectant was spread over the dry viral film. After 10 minutes contact at 20–25°C, the virus-disinfectant mixture was removed by pipet and decimally diluted in RPMI 1640 medium (Hazleton Laboratories) or trypsin case soy broth (BBL) containing 20% fetal calf serum as a neutralizer. The virus dilutions were then inoculated into H-9 cells in suspension and incubated for up to 14 days at 37°C in 5% CO₂ in air with frequent observations of suspension morphology, viability, cytotoxicity, cell numbers, and metabolism. The virus-disinfectant mixture represented 10⁻³ dilution of virus in the presence of neutralizer.
virucide. The p24 antigen capture method used for detection of infectivity has been approved by the EPA.  

**Cytotoxicity Assay.** Dilutions of the disinfectants were inoculated directly into flasks of RPMI 1640 medium with 20% serum and decimally diluted in RPMI 1640. The dilutions were inoculated into H-9 cell suspensions, and the cells were observed for cytotoxic effects, as judged by microscopic observations, pH changes, and trypan blue (0.04%) exclusion.

**Virus Control.** Virus films (0.3 ml) were prepared as in the virucide assay. Each film was rehydrated with 3.0 ml of sterile RPMI 1640 with 10% fetal calf serum and titrated for the 50% tissue culture infective dose (TCID₅₀). Viral replication was detected by ELISA antigen capture, and the TCID₅₀ was calculated by the method of Reed and Muench. The ELISA system was employed rather than reverse transcriptase because of its greater sensitivity.

**Herpes Simplex Virus, Type 2 (HSV-2): ASTM-Type Suspension Test**

The viral assay employed was a suspension test in contrast to the "dried film" EPA-type assay used for HIV-1. This test is recommended for special applications of virucides, such as disinfection of uncleaned reusable medical materials.

The disinfectant active ingredients were prepared in water. Equal volumes of the disinfectant active ingredient and serum were mixed together and allowed to remain in contact for 10 minutes.

Monolayers of human epithelial (H.Ep.2) cells were propagated in 3 ml of RPMI 1640 supplemented with 5% calf serum. The 80–100% confluent monolayer was infected with 0.1 ml of the virus-disinfectant reaction mixture and incubated at 37 ± 1°C in 5% CO₂ in air. Cultures were monitored for up to 14 days for evidence of infection by cytopathic effect (CPE) employing phase contrast microscopy (magnification ×100). Cytotoxicity assays with H.Ep.2 cells were performed as above.

**Disinfectants**

Four quaternary ammonium disinfectants were tested.

Product A contained 3.8% n-alkyl (60% C₁₄, 30% C₁₆, 5% C₁₈, 5% C₂₀) dimethylbenzylammonium chloride, and 5.8% n-alkyl (68% C₁₃, 32% C₁₄) dimethyltetrahydrobenzylammonium chloride yielding 469 ppm active quaternary ammonium chloride at the use dilution. Product B contained 3.0% n-alkyl (60% C₁₄, 30% C₁₆, 5% C₁₈, 5% C₂₀) dimethylbenzylammonium chloride and 7.28% n-alkyl (68% C₁₃, 32% C₁₄) dimethyltetrahydrobenzylammonium chloride yielding 469 ppm active quaternary ammonium chloride at the use dilution. Product C contained 1.5% octydicyclohexidinium chloride, 0.76% diocyldimethylammonium chloride, 0.763% didecylidimethylammonium chloride, and 12.28% alkyl (67% C₁₄, 25% C₁₆, 7% C₁₈, 1% C₁₉, C₂₀) dimethylbenzylammonium chloride yielding 600 ppm active quaternary ammonium chloride at use dilution. Product D contained 6.00% n-alkyl (60% C₁₄, 30% C₁₆, 5% C₁₈, 5% C₂₀) dimethylbenzylammonium chloride, 6.00% n-alkyl (68% C₁₃, 32% C₁₄) dimethyltetrahydrobenzylammonium chloride, yielding 469 ppm active quaternary at the use dilution. Also tested were Product E, a phenolic disinfectant, containing 4.9% potassium orthocresol, 5.3% potassium ortho-benzyl chlorophenate, and 0.9% potassium para-toluidinephenate with a use dilution of 1:28; product F, an acid-based cleaner-disinfectant containing 16.4% phosphoric acid and 4.6% citric acid with a use dilution of 1:2; and product G containing 70% ethyl alcohol and other alcohols in proprietary form.

All disinfectants were tested in the presence of 10% organic soil (Table I). Additional experiments were performed with greater amounts of organic soil to assess the possible quenching of virucidal activity that might occur in the hospital environment where blood spills and other high organic loads are encountered. For these data, an EPA-type carrier HIV-1 test (Table II) and a serum-suspension test with HSV-2 (Table III) were used.

**Results and Discussion**

The commercial products were used according to label directions. The six products tested fully inactivated high concentrations of dried HIV-1 within a 10-minute exposure period (Table I). Inactivation of HIV-1 was achieved even in the presence of 10% serum. The toxicity to H-9 cells as a result of exposure to dilutions of the disinfectants was expressed as a TCD₅₀ of 10⁻²⁵. Because of this degree of cytoxicity, neither survival nor inactivation of the HIV-1 could be determined at dilutions less than 10⁻¹⁰, except for ethyl alcohol. This is a common problem of virucidal disinfectant tests that utilize cell culture systems. The activity of various disinfectants against HIV-1 in the presence of 10% serum is shown in Table I and the effect of increasing to 20% serum is shown in Table II.

As can be noted in Table I, the disinfectant formulations studied were effective against HIV-1 in a carrier test in the presence of 10% serum. In all cases, at least a 3-log reduction (99.9%) was detected and at least a 4-log reduction (99.99%) was detected when 70% ethyl alcohol was used. Thus, representative commercial disinfectants (mixed aryl-alkyl quaternary ammonium salts, phenolics, and acids) cannot inactivate HIV-1 in the presence of serum as stipulated by the EPA for one-step surface disinfection. This activity can be partially diminished when the serum concentration is raised to 20%, as shown in Table II.

Spire et al. reported that the AIDS virus survives
TABLE I
Inactivation* of Dried HIV-1 by Six Hospital Disinfectants

<table>
<thead>
<tr>
<th>Disinfectant Product</th>
<th>ppm Active</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt; Virus Control</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt; Cytotoxicity Control</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt; Virucidal Test&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Log&lt;sub&gt;10&lt;/sub&gt; Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>469</td>
<td>10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>≤10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>≥3.0</td>
</tr>
<tr>
<td>Product B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>469</td>
<td>10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>≤10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>≥3.0</td>
</tr>
<tr>
<td>Product C&lt;sup&gt;a&lt;/sup&gt;</td>
<td>600</td>
<td>10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>≤10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>≥3.0</td>
</tr>
<tr>
<td>Product D&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
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<tr>
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<td>10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>≤10&lt;sup&gt;-5&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Product G&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>≤10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>≥4.0</td>
</tr>
</tbody>
</table>

*EPA-type experiment showing complete inactivation using 10% serum as an organic load and allowing for a 10-minute exposure to disinfectant at 20–25°C. Ratio of disinfectant to virus was 10:1.

<sup>a</sup>Products are defined in Materials and Methods.

<sup>b</sup>Tissue culture infectious dose as measured by antigen capture method of supernatant from H-9 cell cultures.

<sup>c</sup>Tissue culture infectious dose of disinfectant-treated virus as measured by antigen capture method.

<sup>d</sup>A-33, dry, one premixed packet per gallon of deionized water (1:256).

<sup>e</sup>A-33, liquid, diluted 1:84 in deionized water.

<sup>f</sup>A-500, one premixed packet per gallon; 500 ppm water (1:256).

<sup>g</sup>Omgo, diluted 1:256 in deionized water.

<sup>h</sup>STAT III diluted 1:128 in deionized water.

<sup>i</sup>Springthorpe diluted 1:2 in deionized water.

<sup>j</sup>Triple A, complete inactivation in 80 seconds.

TABLE II
Partial Inactivation* of Dried HIV-1 by Three Classes of Disinfectant Active Ingredients in the Presence of 20% Serum

<table>
<thead>
<tr>
<th>Disinfectant Class</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt; Control</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt; Cytotoxicity Control</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt; Virucidal Test</th>
<th>Log&lt;sub&gt;10&lt;/sub&gt; Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-2.5&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-1.5&lt;/sup&gt;</td>
<td>1.00</td>
</tr>
<tr>
<td>Glutaraldehyde&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-2.5&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-1.5&lt;/sup&gt;</td>
<td>1.70</td>
</tr>
<tr>
<td>Chlorine dioxide&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-2.5&lt;/sup&gt;</td>
<td>&lt;10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>10&lt;sup&gt;-1.5&lt;/sup&gt;</td>
<td>1.70</td>
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</table>

*EPA-type experiment showing incomplete inactivation using 20% serum as an organic load and allowing for a 10-minute period of exposure to disinfectant at 20–25°C; ratio of disinfectant to virus was 1:1.

<sup>a</sup>Ortho-phenylphenol, 1.0% ortho-para-chlorophenol diluted 1:32 in 500 ppm hard water.

<sup>b</sup>Glutaraldehyde previously stressed in an EPA "re-use" test.

for 25 days in the laboratory, thereby emphasizing the need for effective disinfectant procedures to minimize risk to health care workers. These investigators further showed the effectiveness of 125 ppm glutaraldehyde against HIV-1 in a suspension test. In our hands, however, employing the EPA dried-carrier method, we have been unable to reproduce this claim. Furthermore, our data show only a partial effect of 11,000 ppm when tested in the presence of an organic load that exceeds the EPA requirements of 5% (Table II). These data confirm the neutralizing effect of blood serum against glutaraldehyde as reported by Hanson et al. However, we clearly demonstrate the effectiveness of alcohol on a dried carrier against HIV-1 in agreement with the data of Martin et al.

Furthermore, we confirm that chlorine dioxide is effective in destroying HIV-1<sup>a</sup> on inanimate surfaces but that this effect is also diminished by blood serum. We extend the findings of Rasmin et al. in that as little as 469 ppm active quaternary ammonium compounds completely inactivated dried HIV-1 in 10 minutes.

Results on the effect of increasing concentrations of serum on virucidal activity are shown in Tables III and IV. These data show that ethanol, isopropl alcohol, phenol, and 1:10 bleach (5.25% sodium hypochlorite) were refractive to the neutralizing activity of serum. With respect to the glutaraldehyde data, it appears that a dried viral film (Table II) is more difficult to inactivate than when virions are in a suspension (Table III), in agreement with the work of Springthorpe et al. with Rota virus.

These results also confirm the findings of Wallbank<sup>a</sup> that, in practice, disinfectants can be rendered less effective by organic matter such as serum. It is for this reason that the EPA's performance requirement for a "one-step" hospital disinfectant stipulates that the disinfectant must completely destroy a dried film of the virus in the presence of at least 5% organic soil. Accordingly, our results on seven products, representing four classes of hospital disinfectants (quaternary ammonium salt, phenolic, mixed organic-inorganic acid, and alcohol) should provide some assurance<sup>a</sup> of efficacy under typical hospital usage conditions.

Care must be taken to not rely on disinfectants to do more than the labels claim. The results reported here suggest that the AIDS virus is readily inactivated by a variety of commercial disinfectants, although the virus can remain viable under conditions that are likely to be found in hospital settings. As expected, there is an inverse relationship between disinfectant efficacy and concentration of organic matter. Increasing amounts of organic material will diminish virucidal activity of certain classes or concentrations of disinfectants. The inclusion of so-called "inerts" into disinfectant formulations can often mitigate the adverse effects of such environmental conditions.

In the disinfection of laboratory benches, culture and blood spills, pipets, syringes, catheters, etc., use...
TABLE III
Viralicidal Activity as a Function of Serum Concentration on Various Classes of Disinfectants* in a Suspension Test Log₅
Reduction of HSV-2

<table>
<thead>
<tr>
<th>Final Concentration of Active Ingredients in Reaction Mixture</th>
<th>% Serum in Reaction Mixture</th>
<th>35% ETOH</th>
<th>35% IPA</th>
<th>Alkaline Glutaraldehyde, ppm</th>
<th>1,250 ppm</th>
<th>NaClO₆</th>
<th>2625 ppm</th>
<th>NaClO₆</th>
<th>1312 ppm</th>
<th>NaClO₆</th>
<th>263 ppm</th>
<th>2.5% Phenol</th>
<th>500 ppm</th>
<th>Log₅ Virus in Serum</th>
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<tr>
<td>2.5</td>
<td>≧4.5</td>
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<td>≧5.5</td>
<td>4.5</td>
<td>4.5</td>
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<td>≧4.5</td>
<td>≧4.5</td>
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<tr>
<td>5.0</td>
<td>≧4.5</td>
<td>≧4.5</td>
<td>≧5.5</td>
<td>4.5</td>
<td>4.5</td>
<td>≧5.5</td>
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<td>≧4.5</td>
<td>6.5</td>
<td>2.5</td>
<td>0.9</td>
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</tr>
<tr>
<td>10.0</td>
<td>≧4.5</td>
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<td>4.5</td>
<td>4.5</td>
<td>≧5.5</td>
<td>0.9</td>
<td>≧4.5</td>
<td>≧4.5</td>
<td>6.5</td>
<td>2.5</td>
<td>0.9</td>
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<td>25.0</td>
<td>≧3.5</td>
<td>≧3.5</td>
<td>≧3.5</td>
<td>4.5</td>
<td>4.5</td>
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<td>3.0</td>
<td>5.5</td>
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<td>37.5</td>
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<td>5.5</td>
<td>2.5</td>
<td>0.9</td>
<td></td>
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</table>

Liquid suspension test. One part of HSV-2 virus pool of 10⁻³ TCID₅₀/0.1 ml mixed with an equal part of disinfectant active ingredient in the presence of serum. The disinfectant-virus serum reaction mixture was incubated for 10 minutes at 20-25°C. The efficacy of the disinfectant is expressed as log₅ reduction relative to the virus in serum control. ETOH = ethyl alcohol, IPA = isopropyl alcohol, NaClO₆ = sodium hypochlorite, BAK = benzalkonium chloride. (All showed 2 logs of cytotoxicity.)

* Percent disinfectants, as active ingredients in 1:2 reaction mixture, not as formulated commercial products.
  1:10 dilution of household bleach added to serum-virus mixture; 2 logs of cytotoxicity were observed.
  1:20 dilution of household bleach added to serum-virus mixture; 1 log of cytotoxicity was observed.
  1:100 dilution of household bleach added to serum-virus mixture; 1 log of cytotoxicity was observed.
  Values in italics are taken to indicate complete inactivation in the system employed considering cytotoxicity and virus control.
  Denotes at least 90% reduction in viral titer but complete inactivation not accomplished.
  _O_ denotes no decrease in viral titer.

TABLE IV
HSV-2 Susceptibility of Disinfectants* to Inactivation by Organic Soil

<table>
<thead>
<tr>
<th>Disinfectant Concentration in Reaction Mixture</th>
<th>% Serum in Reaction Mixture</th>
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<tbody>
<tr>
<td>NaClO₆, 263 ppm</td>
<td>2.5</td>
</tr>
<tr>
<td>NaClO₆, 1312 ppm</td>
<td>25</td>
</tr>
<tr>
<td>Benzalkonium chloride, 500 ppm</td>
<td>25</td>
</tr>
<tr>
<td>Alkaline glutaraldehyde, 1250 ppm</td>
<td>25</td>
</tr>
<tr>
<td>2.5% Phenol</td>
<td>&gt;50</td>
</tr>
<tr>
<td>35% ETOH</td>
<td>&gt;50</td>
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<td>35% IPA</td>
<td>&gt;50</td>
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<tr>
<td>NaClO₆, 2625 ppm</td>
<td>&gt;50</td>
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</table>

* Disinfectants as active ingredients, not as formulated commercial products.
  1:10 dilution of household bleach.
  1:10 dilution of household bleach.

Acknowledgments
The test disinfectants in (Table 1) were supplied by the Airkem™ Professional Products Division of Ecolab, Inc., except for product G, which was supplied by Microgen, Inc. We wish to thank Dale L. Fredell and Dr. Burt Baum of Ecolab, Inc. for their review of this manuscript.

References

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Disinfection of HIV-1 and HSV-2

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