FDA regulations require validated viral clearance of biopharmaceutical products. Yet the potential viral contaminants, the media, and the protein make inactivation unique. So it is important for researchers to know what inactivation methods have already been tried. This article series addresses that concern with information compiled from an extensive literature search on viral inactivation. The results of that search are presented in an organized manner — by skin, bone, and cells (1); red blood cells and platelets (2); plasma and plasma products (heat and solvent/detergent treatments) (3); plasma and plasma products (treatments other than heat and solvent/detergent) (4); and culture media, biotechnology products, and vaccines (5).

This article covers disinfection as a stand-alone topic because of the importance of making equipment and components, such as chromatography resins, safe for subsequent use after possible contact with viruses. This point has been repeatedly emphasized by regulatory authorities, and it is also critical to ensuring worker safety when dealing with high-risk materials.

A disinfectant has been defined as “an agent that frees from infection; usually a chemical agent that destroys disease germs or other harmful microorganisms or inactivates virus” (6). In this article, disinfectants targeted to specific viruses are discussed first, followed by specific approaches reported in the literature for disinfection in plasma and culture media. The full name of viruses abbreviated in this article can be found in the “Virus Abbreviations” sidebar, along with whether those viruses are enveloped or not.

**General Methods of Disinfection**

A variety of disinfection methods are targeted to specific viruses, including the use of high hydrostatic pressure, hydrogen peroxide, and ultraviolet (UV) light; bleach and other chlorine compounds; glutaraldehyde, ethanol, and metal ions; quaternary ammonium compounds; and...
sodium hydroxide or sodium carbonate, among a few others.

**High hydrostatic pressure.** At more than 300 MPa for 10 minutes at 25°C, HSV was reduced by more than 7 log10 and CMV was reduced by more than 4 log10. The HSV and CMV virus envelopes were damaged. This technology was investigated for use in the food processing industry at moderate temperatures (7).

**Hydrogen peroxide.** Microaerosolized hydrogen peroxide (5%) mist completely inactivated the viral poultry pathogen infectious laryngotracheitis virus, Newcastle disease virus, infectious bronchitis virus, and avian influenza virus showed reduced infectivity but were not completely inactivated. Avian reovirus susceptibility varied with the exposure method, and infectious bursal disease virus was highly resistant. At a 10% concentration, however, the hydrogen peroxide mist inactivated even the infectious bursal disease virus (8).

**UV.** HIV inactivation by UV light was investigated and found to be dependent on the strength of the UV light and on the composition of the medium in which HIV was contained. Cell-free HIV suspended in medium was inactivated after 10 minutes and cell-associated HIV after 30 minutes. But exposure of up to 60 minutes did not completely inactivate cell-associated HIV in the presence of blood (9).

**Bleach and other chlorine compounds.** HSV was inactivated by 0.01–0.02% bleach (sodium hypochlorite, NaOCl). Bleach was found to be more effective for HSV inactivation than 50% ethanol (10). At exposure greater than 30 seconds, HIV-1 was completely and consistently inactivated by undiluted household bleach. Inactivation was inconsistent at exposure times of 15 and 25 seconds. A 10% dilution of bleach did not inactivate HIV even after five minutes of exposure (11). Total inactivation of VSV on a hydrocolloid dental impression was achieved by three to 20 minutes exposure to 0.5% sodium hypochlorite (12).

Chlorine dioxide inactivated HIV-1 in the presence of blood and medical supplies such as plastics and paper in medical waste processing (13). Chlorohexidine gluconate coated onto latex gloves was found to rapidly inactivate a model retrovirus, HSV, and HBV by 90–100% (14).

Glutaraldehyde at ≥0.1% was effective against purified poliovirus at pH 7.2 when assayed by a suspension procedure. Glutaraldehyde’s inactivating capabilities were increased at alkaline pH, but at acid pH it was ineffective. Glutaraldehyde was not effective at concentrations up to 1% at pH 7.2 when measured by inactivation assays on polio RNA (15).

Glutaraldehyde (2%) totally inactivated VSV on a hydrocolloid dental impression in less than one minute (12). A 2% solution of glutaraldehyde was demonstrated to effectively disinfect solid surgical instruments, as determined by a DHBV assay (16).

Enteroviruses used to test the efficacy of disinfectants for medical devices included two wild type strains. The wild type strains showed less sensitivity to glutaraldehyde than the reference strain of echovirus type 25 JV-4 and polio (17).

**Ethanol.** Disinfection using 70% ethanol was evaluated for HIV in suspension and HIV dried onto surfaces in the presence of both high and low concentrations of protein. In suspension, high titers of HIV were inactivated rapidly regardless of protein load. When the virus was dried onto a glass surface, however, the rate of inactivation decreased when high levels of protein were present (18).

**Metal ions.** Copper (II) and iron (III) ions were shown to inactivate five enveloped or nonenveloped, single- or double-stranded DNA or RNA viruses. Adding peroxide enhanced the effectiveness of the ions in a procedure intended for liquid disinfection of medical devices. Mixtures of copper (II) ions and peroxide were more effective than glutaraldehyde. Viruses used were Fru174, T7, HSV, HBV, and Junin (19).

Quaternary ammonium compounds and sodium hydroxide or sodium carbonate.

Didecyldimethylammonium chloride (DDAC, 0.1%) with 0.1 N sodium hydroxide (NaOH) was somewhat effective in inactivating SVDDV. At 4°C, greater than 3.1 log10 were inactivated after two hours (20). Data for HAV inactivation with 0.1% DDAC and 0.1 N NaOH indicate log10 reduction factors of greater than 3.8 and greater than 5.3 (21).

In a study on decontamination of stainless steel surfaces, FCV was not effectively inactivated at concentrations recommended by the manufacturer of a combination disinfectant consisting of a quaternary ammonium compound and sodium carbonate. At twice the recommended concentration, however, it completely inactivated FCV (22).

**Other disinfectants and reviews.** A thorough review of disinfectant inactivation of HIV was published in 1991 (23). Cytopathic effect (CPE) and p24 antigen were measured to assess the effectiveness of glutaraldehyde, phenolics, iodine, chlorine, quaternary ammonium compounds, and ethanol in inactivating cell-free and cell-associated HIV. The data are presented in Table 1. Cell-associated HIV was more resilient. The presence of blood also decreases the efficacy

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### Table 1. Data from review of HIV inactivation by various disinfectants, including information on plasma and various plasma products (23)

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Concentration (percentage)</th>
<th>Contact Time (in minutes)</th>
<th>Test Article</th>
<th>Inactivation (log10 reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hypochlorite</td>
<td>0.5</td>
<td>1, 5</td>
<td>50% plasma</td>
<td>&gt;7.0</td>
</tr>
<tr>
<td>β-Propionylactone</td>
<td>0.14</td>
<td>240</td>
<td>4% gamma globulin</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>70</td>
<td>1, 5</td>
<td>50% plasma</td>
<td>&gt;7.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>water-saturated</td>
<td>15–240</td>
<td>lypophilized FVIII</td>
<td>0</td>
</tr>
<tr>
<td>Quaternary ammonium compound-1</td>
<td>0.08</td>
<td>1</td>
<td>50% plasma</td>
<td>&lt;6.0</td>
</tr>
<tr>
<td>Nonidet p-40</td>
<td>0.5</td>
<td>1,5,10,15</td>
<td>50% plasma</td>
<td>&gt;8.0</td>
</tr>
<tr>
<td>TNBP plus sodium cholate in varying concentrations</td>
<td>0.3</td>
<td>150</td>
<td>FVIII concentrate</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>150</td>
<td>FVIII concentrate</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>20–300</td>
<td>FVIII concentrate</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>20–300</td>
<td>FVIII concentrate</td>
<td>&lt;2.5</td>
</tr>
</tbody>
</table>

*1.536% octyldecyl(dimethyl ammonium chloride, 0.768% dixoyldimethyl ammonium chloride, 0.768% N-dioxyl(dimethyl ammonium chloride, and 12.288% alkyl(dimethyl benzyl ammonium chloride*
of disinfectants. Cell-associated HIV in whole human blood was completely inactivated by glutaraldehyde, iodine, and 75% ethanol after one minute and by hypochlorite after a 10-minute exposure (9).

Lysing reagents and fixatives were evaluated for their ability to inactivate HIV-infected H9 cells in whole blood preparations. All of the commercial lysing and fixing reagents inactivated cell-associated HIV by 3–5 log10. Ammonium chloride had little effect (24).

Phenolic compounds at two to four times the manufacturer’s recommended concentrations were shown to be effective in inactivating FCV on stainless steel surfaces (22).

Iodophor (ProMedyne-D) diluted to provide 75 ppm titratable iodine required three to 10 minutes of exposure on a hydrocolloid dental impression for inactivation of VSV (12).

As an alternative to glutaraldehyde, electrolyzed acid water (EAW) was evaluated as a disinfectant. DHBV infectivity was completely lost after incubation of the inoculum with 100 volumes of EAW for seven minutes or with 500 volumes for one minute (25).

Formaldehyde, β-propiolactone, and Virkon S (a peroxymonosulfate-based compound) were effective in inactivation of an astrovirus. The astrovirus, however, was resistant to heat inactivation, low pH, detergent, and phenolic, quaternary ammonium, or benzalkonium chloride–based products (26).

**Disinfection in Plasma Products**

Methods found in the literature for disinfection in the presence of plasma or plasma products include sodium hydroxide and heat, sodium hypochlorite and sodium dichloroisocyanurate, and others.

**Sodium hydroxide and heat.** Although not performed in the presence of plasma or plasma products, 0.1 M NaOH at 60°C was found to be an effective clean-in-place system for the inactivation of viruses present in plasma. Treatment of HAV with up to 1.0 M NaOH at 15°C did not lead to rapid inactivation. However, a two-minute contact time with 0.1 M NaOH at 60°C was sufficient to inactivate HAV, CPV, PRV, and BVDV (27). In another study, it was found that at 60°C, 0.25 M NaOH inactivated greater than 3.5 log10 of CPV and HAV in 30 minutes, but inactivation was not complete (<6 log10) for either virus. For HAV, only 2.7 log10 were inactivated after exposure to 0.1 M NaOH at 25°C for two hours. CPV was inactivated by more than 3.5 log10, but inactivation was also incomplete. A two-hour exposure to 0.5 M NaOH at 4°C provided a reduction of more than 5 log10 of CPV, but for HAV it was only a reduction of 2.4 log10. Even for the CPV, inactivation was incomplete (21).

The data for HAV inactivation from these two studies are summarized in Table 2.

| Sodium hypochlorite (NaOCl) and sodium dichloroisocyanurate (NaDCC). A two-minute exposure of DHBV- and HBV-rich plasma to NaOCl (domestic bleach 3,600 ppm and industrial bleach 3,180 ppm) and NaDCC (3,000 ppm available chlorine) was sufficient for inactivation. Inactivation was found to be concentration dependent but independent of contact time. (28).

**Other disinfectant studies.** HBV-infected plasma was exposed to various disinfectants. Sodium hypochlorite at 1,000 ppm free chlorine provided only minimal inactivation, and povidone–iodine at 9.5, and 3.6% provided no measurable inactivation. Sodium hypochlorite at 4,700 ppm free chlorine, 1% glutaraldehyde, and an iodophor–detergent disinfectant containing 3.6% povidone–iodine reduced viral titers by more than 3–4 log10 (29).

A review of HIV inactivation by various disinfectants included information on plasma and various plasma products. The data are summarized in Table 1. The differences in inactivation for the FVIII concentrate seem to be related to the inoculum size. For the two test articles with 150-minute contact times, the inoculum was 4.5 log10 TCID50. For the two with 20–300 minutes of contact time, the inoculum was 6 log10 TCID50 per mL (23).

**Culture Media**

Cell-free HIV in RF-10 culture medium (10% fetal calf serum [FCS] in culture medium RPMI 1640) was inactivated within one minute by various disinfectants, as determined by CPE. The supernatants, however, contained p24 antigen levels in the samples treated with glutaraldehyde, potassium monopersulphate-based products, and ethanol. The final concentrations of the active ingredients in those disinfectants were 0.5% glutaraldehyde, 0.5% potassium monopersulphate, 2,500 ppm hypochlorite, 1:40 quaternary ammonium compounds, 0.8% iodine, 1:100 phenolics, and 35% ethanol. Cell-associated HIV was completely inactivated by glutaraldehyde, hypochlorite, iodine, phenolics, and 75% ethanol after a one-minute exposure. Cell-associated HIV was not completely inactivated by 35% ethanol, a potassium monopersulphate-based product, or quaternary ammonium product even after five minutes. Elevated p24 levels were found, without CPE, in glutaraldehyde, iodine, 75% ethanol, and phenolic compounds (9).

Data compiled in a review on the inactivation of HIV by disinfectants showed that one to two percent alkaline glutaraldehyde with a contact time of one to 10 minutes was sufficient to completely inactivate a spike of 3 log10 of HIV into tissue culture fluid. Formaldehyde at a concentration up to 2% reduced a log10 spike of 5.27 by more than 4.27 log10 after five minutes. A 50% ethanol solution reduced HIV in tissue culture fluid by more than 3.52 log10 and 35% isopropanol provided greater than 3.78 log10 reduction. Povidone–iodine at a 1:40 solution and Betadine surgical scrub at a 1:60 dilution, both in the presence of 20% FCS, did not inactivate HIV. Quaternary ammonium compounds, however, inactivated more than 3 log10 of HIV in the presence of 10% FCS after 10 minutes. Psoralen plus UV light (365 nm) did not inactivate HIV in tissue culture fluid after a four-minute exposure, but more than 6 log10 were inactivated after two hours (23). UV light inactivation of HIV in culture medium is also discussed by Druce (9).

Benzalkonium chloride inactivated HIV-1 in 0.05% tissue culture fluid (30). The ability of sanitizing agents to inactivate human viruses in a 10% bovine serum albumin and yeast extract mixture was assessed. The sanitizing agents included a

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**Table 2. Data from two studies on HAV inactivation by NaOH (21, 27)**

<table>
<thead>
<tr>
<th>NaOH (M)</th>
<th>Temperature (°C)</th>
<th>Time (minutes)</th>
<th>Log10 Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>60</td>
<td>2</td>
<td>complete</td>
</tr>
<tr>
<td>0.1</td>
<td>25</td>
<td>120</td>
<td>incomplete</td>
</tr>
<tr>
<td>0.25</td>
<td>60</td>
<td>30</td>
<td>incomplete</td>
</tr>
<tr>
<td>0.5</td>
<td>4</td>
<td>120</td>
<td>incomplete</td>
</tr>
<tr>
<td>1.0</td>
<td>15</td>
<td>—</td>
<td>incomplete</td>
</tr>
</tbody>
</table>
Validation

benealkonium chloride-based product, a
chloroxylenol, cetrimide/chlorhexidine, and
povidone–iodine. All of the products were
effective in inactivating HSV and HIV-1,
but a human coronavirus and human
nonenveloped viruses (polio, ADV, and
coxsackie virus) were not inactivated, with
one exception. A benznalum chloride
product (Dettol hospital concentrate) was
able to inactivate the nonenveloped human
coxsackie virus (31).

Bleach (5.25% sodium hypochlorite) at a
1:10 or 1:100 dilution was found to
effectively inactivate RSV in up to 50%
FCS (32).

HIV-1, HAV, RSV, vaccinia, HSV-1, and
polio-2 were used to evaluate the capability
of a hydrogen peroxide gas plasma
sterilization process. The test viruses were
suspended in cell culture medium and dried
on the bottom of sterile glass petri dishes.
This technique was effective against both
lipid and nonlipid viruses. Viral titers were
reduced from 2.5 log10 to 5.5 log10, a
99.68% to 99.999% decrease (33).

Making This Series Dynamic
This article series has presented published
viral inactivation methods from the past
decade and into 2001. Reviews that were
published while preparing this series include
one that addresses blood components (34),
one that addresses plasma derivatives (35),
and one prepared by the Council of Europe’s
Expert Committee in Blood Transfusion
that addresses labile blood products (36). I am
sure that many other valuable publications
were missed because the literature search
was limited to make it manageable. The
reference list will be posted on the
BioPharm International website
(www.biopharm-mag.com), and we
encourage readers to send us publication
information that is not provided in the series
(published articles dating from 1990 and
beyond). You can email additional article
references to associate editor Penny Cass at
pcass@advanstar.com to help make the
database a useful resource for all.

This review on virus inactivation was
suggested by Dr. Jeri Ann Boose and
Dr. Michael Wiebe, and I would like to
thank them for their encouragement. The
final article in this series will discuss
variability for specific viruses culled from a
large database. BPI

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