Part 6, Inactivation Methods Grouped by Virus

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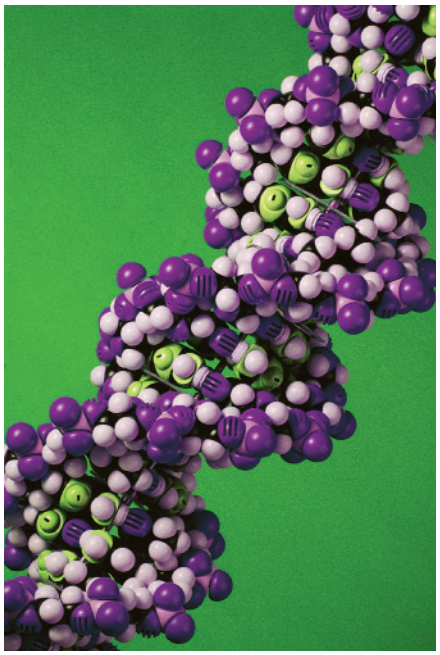

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In previous articles of this viral inactivation series (1–6), the virus inactivation methods described in the recent literature were sorted by test article (bone, red blood cells, and plasma, for example). In this final article, the data from the literature and from the extensive BioReliance databases have been sorted by virus to enable us to draw conclusions on effective inactivation methods for a range of viruses. (Data not referenced to a literature citation came from BioReliance databases).

We selected parvovirus, reovirus (Reo), and hepatitis A (HAV) as models for the nonenveloped viruses, and murine leukemia virus (MuLV) and pseudorabies virus (PRV) for enveloped viruses. These viruses were selected to encompass both single- and double-stranded RNA and DNA viruses and to provide a range of resistance to physicochemical inactivation.

Articles in the literature often use a variety of criteria when performing viral clearance studies and interpreting the data. Log reduction values are often reported as "greater than" (>), and in other cases, they are reported as "greater than or equal to" (≥). The latter implies complete inactivation, yet > is also used (in the literature) to designate complete inactivation. Furthermore, the log reduction values reported are influenced by multiple factors in the viral clearance studies. Reported log reduction values are limited by the starting titer of the virus used in the study, the virus-to-load spiking ratio, and the sensitivity of the assay used to detect the virus.

Although the effect of the sample matrix and exact operating ranges must always be evaluated, these data can provide the reader a starting point for assessing inactivation techniques that might be incorporated in a process or for providing decontamination. (Unless specified, ambient inactivation temperatures were 18°–25°C.)

Nonenveloped Viruses

Single-Stranded DNA viruses

Diameter: 18–24 nm; **Shape**: Icosahedral.
Resistance to physicochemical treatments: Very high.
Parvovirus models: Murine minute virus (MMV), porcine parvovirus (PPV), canine parvovirus (CPV), and bovine parvovirus (BPV).

Although these parvoviruses (MMV, PPV, CPV, and BPV) are classified in the same family, there is some variability even within this classification in susceptibility to inactivation conditions. For example, a recent publication indicates that PPV is less likely than human parvovirus B19 (PV-B19), for which it is often a model, to be inactivated during pasteurization of human serum albumin (HSA) (7). Some of the more commonly used virus inactivation methods for these rather hardy viruses include heat, pH, irradiation, and chemical treatments.

Heat. Heating at 58–60°C for 10 hours generally provides a $\log_{10}$ reduction that ranges from a low of 1.0 to at most 3.9 for MMV and PPV. In one case, PPV was not inactivated by treatment at 60°C for 15 hours. Treatment of PPV with higher temperatures (80°–82°C) for 72 hours appears equally as effective as the 58°–60°C, 10-hour treatment. In one case, treatment at 74°C for 90 minutes provided a $\log_{10}$ reduction of 8.4 — complete inactivation. These data from BioReliance are consistent with those found in the literature. For example, pasteurization at 60°C for 10 hours provided a $\log_{10}$ reduction of 3.4 for PPV (8). Ten hours is considered a requirement for PPV inactivation at 60°C (9). After 10 hours, 3.2 $\log_{10}$ of MMV were inactivated (10).

Dry heat treatments also produced variable inactivation. Not surprisingly, inactivation increased with increasing temperature, but the effect of moisture can be significant. As noted in one publication, dry heat (80°C for 72 hours) with a moisture content ≥0.8% provided an average $\log_{10}$ reduction of 3.72 for PPV, but it was less (2.5 $\log_{10}$) with a moisture content ≤0.8% (11).

Vapor heating has been used to inactivate MMV. A 4.8 $\log_{10}$ inactivation was obtained by 10 hours of 60°C treatment followed by 1 hour at 80°C (10).

The effectiveness of freeze-drying and dry heat treatment (at 80°C for 72 hours) in the inactivation of CPV and BPV in two different factor VIII (FVIII) concentrates was found to be variable and depended on both the virus and the product. The FVIII products were made by two different facilities. In one FVIII, BPV was more resistant to inactivation by heat treatment than CPV. In the second FVIII product, however, there was no significant difference in the resistance of the two parvoviruses. Some factors that were suggested as influences for the variability were ionic composition, differences in the freeze-drying processes, and moisture content (12).

Freeze-drying and heating at even higher temperatures (for example at 100°C for 30 minutes on the final container of FVIII concentrate) was found to inactivate most viruses to below their detection limits. BPV, however, showed strong resistance to the dry heat treatment (13).

pH. Inactivation of PPV by low pH is variable. For one test article at BioReliance, complete inactivation was found within 6 hours at pH 2.0. However, for that product, it was observed that pH treatments from 2 to 5 were ineffective. Complete inactivation by low pH was found for another product, but this required a contact time of 6 hours, and a pH of less than 2.0. Pepsin and low pH treatment were not very effective for parvovirus inactivation.

Alkaline conditions increased inactivation with increasing concentrations and contact time, ranging from no inactivation up to 5.8 $\log_{10}$. The 5.8 $\log_{10}$ reduction was achieved by also raising the temperature to 37°C. NaOH (0.1 M) at 60°C was an effective sanitization system for the inactivation of viruses that are found in plasma. Two minutes of contact with 0.1 M NaOH at 60°C was sufficient to inactivate CPV below detection limits in these studies (14). Another study, however, found that at 60°C, 0.25 M NaOH inactivated >3.5 $\log_{10}$ of CPV in 30 minutes, but inactivation was not complete (≤6 $\log_{10}$) (15).

Irradiation. UV irradiation is very effective for parvoviruses. In one case, it provided a

5.5 $\log_{10}$ reduction of PPV, which was complete inactivation. BPV in fetal calf serum (FCS) was completely inactivated by UV irradiation (16).

UVC completely inactivated PPV (17,18). UVC also inactivated of both BPV and PPV with $\log_{10}$ reduction factors of 8 for BPV and 5.5 for PPV (19). Lyophilization combined with gamma irradiation has provided a significant $\log_{10}$ reduction of parvoviruses. In one publication, gamma irradiation was reported to inactivate 4 $\log_{10}$. The amount of gamma irradiation ranged from 23 kGy to 30 kGy, depending on the presence of specific plasma proteins (20).

In another article, it was observed that a 6 $\log_{10}$ reduction of PPV could be obtained by gamma radiation. The amount of gamma radiation required can vary from 30 kGy to 100 kGy, depending on the laboratory where the work was performed and the starting titer (21). At lower radiation doses, however, PPV may not be inactivated. One publication reported that in the range of 20–25 kGy, PPV was not inactivated in FCS (16). More recently, a dose of 34 kGy was found to be necessary for reducing infectivity by 4 $\log_{10}$ for BPV in frozen suspensions representative of the process used for preparation of human cortical bone transplants (22).

Electron-beam irradiation has been evaluated for inactivation of PPV and provided $\log_{10}$ reduction of 4.6 in one case. It is reported that extrapolated data indicate a 6 $\log_{10}$ reduction of PPV with a dose of 35 kGy for liquid samples or 55 kGy for frozen samples (23).

Chemical treatments. Ethyleneimines have been effective for inactivation of PPV. A 4 $\log_{10}$ inactivation of PPV took nine hours in a 5-mM ethylenimine solution and three hours for a 10-mM solution, both at 37°C (23). Binary ethyleneimine (BEI) can also inactivate PPV. A temperature of 37°C is commonly used (24).

Inactine (PEN 110) is an electrophilic compound (positively charged below pH 10), with low molecular weight, from a family of ethyleneimine oligomer substances. It reacts with nucleic acids to permanently modify the virus genome, which prevents virus replication and renders the virus noninfectious. Inactine provided a >5 $\log_{10}$ reduction of PPV in human red blood cell concentrates (25). Iodine treatments have also been effective in inactivating PPV, and Iodine/ Sephadex (not currently available) has delivered iodine to intravenous immunoglobulin in a slow, controlled way, inactivating >4.2 $\log_{10}$ in PPV (20).

Double-Stranded RNA viruses

Diameter: 60–80 nm; **Shape**: Spherical.
Resistance to physicochemical treatments: Medium.
Virus model: Reovirus (Reo).

According to information from the American Type Culture Collection (ATCC), reoviruses are ether-resistant and acid- and heat-stable (26). However, some heating conditions are sufficient for inactivation. Reoviruses are members of the Reoviridae family, which includes blue tongue virus (BTV) that is sometimes used as a model virus. BTV, however, is more sensitive to inactivation than reoviruses. BTV is said to be sensitive to acid pH, detergents, and lipid solvents.

Heat. Heat treatments of 60°C or higher were effective in inactivating Reo. Freeze-drying and heating at 100°C for 30 minutes on the final container of FVIII concentrate was found to inactivate Reo-3 by 6 $\log_{10}$ (13).

pH. Low pH was not very effective for inactivating Reo. At pH 2–4, there were only a few cases in which any inactivation was seen in the BioReliance database. At a pH lower than 2.0 for one hour, some inactivation was seen for two test articles, but in one case a pH of 1.0 was ineffective even after 24 hours. Pepsin treatment at pH 4.4 provided some inactivation, but the $\log_{10}$ reduction values were, at most, 2.3. Adding acetonitrile and lowering the pH enhanced the inactivation. Alkaline conditions were much more effective than low pH treatments and provided reduction values up to 7.0 $\log_{10}$.

Irradiation. UV and gamma irradiation were found to be fairly effective at inactivating Reo. Reo in FCS was reduced by about 4 $\log_{10}$ by UV irradiation (16). UVC (220–280 nm) also provided a 4 $\log_{10}$ reduction (27). Reo in FCS was inactivated, but not very quickly, by gamma radiation in the range of 20–25 kGy (16). Methylene blue photodynamic treatment inactivated 3.80 $\log_{10}$ of Reo-3 (28).

Chemical treatments. Hydrogen peroxide at elevated temperatures (50–55°C) provided a $\log_{10}$ reduction of >4. In the literature, however, avian Reo susceptibility to microaerosolized hydrogen peroxide (5%) mist varied with the method of exposure. A 10% mist was more effective (29).

Formaldehyde provided minimal inactivation of Reo in our tests. In most cases, inactivation was very low or nonexistent. One exception was the treatment with 100 μg of formalin per mL at 37°C for three days.

Urea usually had a significant inactivation effect, with concentration influencing the inactivation. At 4°C and pH 7, 1 M urea was totally ineffective, but urea concentrations of 3–4 M in a pH range of 4.5–8 with exposure times ranging from two to three hours provided reduction values ranging from a low of 2.6 up to 3.6 $\log_{10}$.

Acetonitrile was an effective agent of inactivation for Reo. Other effective inactivating chemicals for this hardy virus include guanidine hydrochloride, ethanol combined with acetic acid, methanol, and bleach. A polyvinylpyrrolidone (PVPP) and iodine complex in a depth filter was used for viral inactivation of Reo-3 in a 2% IgG solution. The filtration speed was 100–500 L 1/h*2, and the temperature was either 40°C or room temperature. Reo was inactivated by 4.9 $\log_{10}$ (the available starting titer) (30).

Single-Stranded RNA viruses

Diameter: 22–30 nm; **Shape**: Icosahedral.
Resistance to physicochemical treatments: High.
Virus model: Hepatitis A (HAV).

HAV is a picornavirus; rhinoviruses, which are more sensitive to acid, are also included in this family. Most of the picornaviruses inhabit intestinal tracts, and not all picornaviruses will grow in tissue cultures. In addition to HAV, other members of the family include encephalomyocarditis (EMC), bovine enterovirus (BEV), coxsackieviruses, echoviruses, and polioviruses.

Heat treatment in solution at temperatures from 58°C to 100°C effectively inactivated HAV, from a low of >2.7 up to 6.5 $\log_{10}$. Inactivation times ranged from zero to 16 hours. HAV appears to be more heat-stable than polioviruses and rhinoviruses. The inactivation kinetics for HAV during pasteurization of a 5% albumin solution were compared to those of two other picornaviruses. Approximately 7 $\log_{10}$ of polio type-1 were inactivated in 20 minutes, but after 60°C for 10 hours, only 4.8 $\log_{10}$ of HAV were inactivated (10). HAV was efficiently inactivated in FVIII; however, in a buffer solution, it was more stable than polio (31).

Protein concentration and stabilizers can also affect heat treatment in solution. Inactivation of HAV by pasteurization in one albumin production process was evaluated and found to be 4.4 $\log_{10}$ for a 5% albumin solution and >3.9 $\log_{10}$ in a 20% albumin solution (32). Inactivation of HAV by pasteurization (60°C for 10 hours) was investigated in a FVIII product. HAV was substantially inactivated, but the stabilizers used in the manufacturing process delayed the inactivation, and residual infectious HAV was found even after a 10-hour heat treatment in the stabilized preparation (33).

A modified pasteurization process (63°C for 10 hours) was employed for virus inactivation of process intermediates of a FVIII plasma product. Polio, coxsackievirus, and HAV were

inactivated. Virus reduction factors (log_{10}) for HAV were ≥5.6, for polio-1 ≥9.8, and for coxsackievirus-B6 ≥4.7. The temperature of 63°C was selected rather than 60°C because earlier reports in the literature stated that temperatures greater than 60°C were required to inactivate picornaviruses (34). Heat treatment at 63°C for 10 hours was also effective for virus inactivation in a high-purity FVIII concentrate stabilized by the von Willebrand factor. In-process, production samples were stabilized using amino acids and sugars before spiking. In this case, polio, coxsackievirus, and HAV were inactivated by >4.5 log_{10} (35).

Dry heat appears to be an effective inactivation method; however, in one case, no inactivation was found at 100°C for one hour. Two high-purity coagulation factors (FVIII and FIX), subjected to dry heat treatment for 24 hours at 80°C, reduced HAV infectivity was reduced by ≥4.3 log_{10}. That reduction was also achieved after two hours and before six hours at 90°C. HAV was also inactivated during the freeze-drying process by approximately 2 log_{10} (36).

A study of 26 hemophilia patients receiving FVIII and FIX products that had been heat treated at 100°C for 30 minutes following lyophilization indicated that the dry-heat treatment is sufficient to inactivate HAV (37). Freeze-drying and heating at 100°C for 30 minutes on the final container FVIII concentrate was found to inactivate HAV to below the detection limit (>5.3 log_{10}) after a few minutes (13).

Heat treatment at 100°C for 30 minutes was sufficient to inactivate HAV and polio-1 by approximately 5 log_{10} after four minutes in a FVIII concentrate (38). Moisture content influences the effectiveness of dry heat (80°C, 72 hours) for viral inactivation in lyophilized FVIII. When the moisture content was ≥0.8%, the average log_{10} reduction for HAV was ≥4.54; when moisture content was ≤0.8%, however, the average log_{10} reduction was 0.12 (11).

Vapor heating (lyophilization, followed by adding water to achieve homogenous moistening, then heating at 60°C for 10 hours, followed by heating at 80°C for one hour in an airtight container under nitrogen gas atmosphere) of a FVII concentrate provided HAV inactivation within one hour at 60°C (5.9 log_{10}) (10). Vapor heating was evaluated for its capacity to inactivate HAV in six different coagulation factor concentrates, fibrin sealant, lys-plasminogen, and fibrinogen. Vapor heating conditions were variable and depended on the stability of the different products, but all included a 10-hour, 60°C treatment and pressures ranging from 190 mbar to 385 mbar. The most intense protocol was for fibrinogen, and it employed a pressure of 200 mbar and a three-hour treatment at 80°C with a pressure of 385 mbar. One-step vapor heating procedures had the capacity to inactivate between 5.9 and >6.3 log_{10}. The two-step procedure inactivated between >8.7 and 10.4 log_{10} (39).

pH. The effect of pH on the effectiveness of pasteurization for inactivating HAV was found to be variable in fraction V–like products (5% human plasma protein solution). Increasing the pH from 6.4 or 6.5 to 7.5 increased the HAV inactivation. But in a 5% albumin solution, inactivation was more effective at pH 6.4 than 6.9 or 7.4. We concluded that predicting how variations in process parameters will affect viral clearance is difficult and that results will vary depending on the virus and the product (40).

Sodium hydroxide at concentrations of 0.01 N to 1.0 N has provided a maximum inactivation of >2.7 log_{10} at low temperatures. It was reported that 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 (14). At 60°C, 0.25 M NaOH inactivated >3.5 log_{10} of HAV in 30 minutes, but inactivation was incomplete (≤6 log_{10}). Only 2.7 log_{10} were inactivated after exposure to 0.1 M NaOH at 25°C for two hours. A 2-hour exposure to 0.5 M NaOH at 4°C reduced HAV only 2.4 log_{10} (15).

Demineralization (decalcification) of bone in HCl has been shown to inactivate HAV (41).

Irradiation. Electron beam, UV, and UVC have been shown to be effective techniques for inactivating HAV. A fibrinogen solution containing rutin to protect the fibrinogen was irradiated with UVC (254 nm at 0.1 J per cm^2). HAV was inactivated by ≥6.5 log_{10} (17). UVC irradiation was also used for virus inactivation in plasma and in a FVIII concentrate. HAV in the FVIII concentrate and in the plasma was inactivated to undetectable levels (≥6.3) (18).

The antigenicity of HAV was shown to be almost unaltered after UV treatment in a study to determine the suitability of UV inactivation for vaccine preparation. UV doses up to 920 J/m^2 were applied. UV treatment preserved antigenicity at levels comparable to treatment with formalin at 250 μg/mL, but formalin requires up to 15 days to achieve inactivation (42).

Chemical treatments. Iodine at 37°C reduced HAV by 3.8 log_{10} after six hours. An HAV vaccine treated with 0.2 mg/mL formaldehyde for five days at 32°C was found to be safe and immunogenic in experimental models (43). HAV inactivation with 0.1% didecyldimethylammonium chloride (DDAC), a quaternary ammonium compound, and 0.1 N NaOH was evaluated for sanitization studies (15).

HAV and polio-2 were inactivated by 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 (44).

Enveloped Viruses

Single-stranded RNA viruses

Diameter: 80–110 nm; **Shape**: Spherical.
Resistance to physicochemical treatments: Low.
Retrovirus models: Murine leukemia virus (MuLV) and xenotropic murine leukemia virus (XmuLV).

Within the retrovirus family are the avian type C retroviruses, foamy viruses, the human T-lymphocyte and bovine leukemia viruses (HTLV–BLV), lentiviruses, mammalian type-B oncoviruses, and the viruses related to murine leukemia viruses (MLV). The type-C MuLV — the most commonly used model virus — is particularly susceptible to inactivation.

Heat. At 56–100°C in solution, inactivation was generally very effective. In one case, however, stabilizers were required. Without the stabilizers, there was no inactivation at 60°C. Except for this unusual case, log_{10} reduction values ranged from 3.6–9.2.

pH. At pH values below 3.0, inactivation was usually instantaneous; however, the log_{10} was as low as 4.1 and as great as 7.1. Acetic acid (1 M) inactivated >4 log_{10} at either 2°C to 10°C or 18°C. At a pH of 3.5, the lowest log_{10} reduction observed was 3.5. At pH 3.7, instantaneous inactivation was sometimes obtained, but for one test article the reduction was only 1 log_{10}. In another study on pH 3.7 inactivation, however, the log_{10} reduction was 5.6. At pH 3.8, log_{10} reduction values were as high as 7, but in two cases only a 1.7 log_{10} reduction was found.

One study presented at a conference described pH 3.5–4.0 to be effective at 18°C to 26°C, and very little difference was seen in inactivation kinetics between pH 3.7 up to 4.1. At 2°C to 8°C, however, pH 4.1 inactivation was slower and required up to an hour; only about 30 minutes were needed for pH 3.7 (45).

At pH 4, log_{10} reduction values ranged from 3.2 to 4.9. At pH 4.5 for 45 minutes, log_{10} reduction values ranged from a low of 1.1 up to 4.7. In one case, however, two hours at pH 4.5 produced no measurable inactivation. A 16-hour cold-room treatment at pH 4.5 provided a 4.8 log_{10} reduction, and 48 hours at 2–20°C gave a log_{10} reduction greater than 5.9 for one test article. At pH values greater than 5,

inactivation is typically low. In one case with low conductivity, a $\log_{10}$ reduction of approximately 3 was obtained at a pH of 6.9.

In addition to the influence of time and temperature on pH inactivation, it has been observed that different products and different protein concentrations affect the ability of low pH treatments to inactivate MuLV. Abujoub described an inactivation time for one product of 60 minutes and 120 minutes for another (45). Furthermore, in one product, X-MuLV was not completely inactivated even after 120 minutes. Protein concentration also affected the inactivation kinetics. In buffer only, X-MuLV was inactivated in 120 minutes. The addition of protein prevented complete inactivation with the same pH, temperature, and exposure time.

Under alkaline conditions, $\log_{10}$ reduction values ranged from 4.1 to >6.2 at NaOH concentrations ranging from 0.01–1 N. Temperature influenced the effectiveness of the inactivation. Not surprisingly, at lower temperatures, inactivation by alkaline conditions was reduced.

Irradiation. Electron beam treatment gave a $\log_{10}$ reduction of 1.3–1.9, and gamma irradiation produced a $\log_{10}$ reduction of >5.5. Methods such as electron beam and gamma irradiation are being applied to new technologies such as tissue therapies, and the database of inactivation information is expected to expand in this area.

Chemical treatments. Ethanol at 20% provided no inactivation, but concentrations of ethanol ranging from 30–70% always gave a $\log_{10}$ reduction >4. Inactivation by methanol and hexane was not as effective. Log_{10} reduction values ranged from <1–2.5. Trifluoroacetic acid (TFA) at a low pH gave good inactivation, but at a neutral pH or at lower temperatures (2–8°C), there was no inactivation. Acetonitrile was effective in all cases, providing a 3.8 to >5.1 $\log_{10}$ reduction at times ranging from zero to four hours. Bleach was also found to be effective. A 4.5 $\log_{10}$ reduction was observed at time zero with 600 ppm hypochlorite.

Urea is an effective inactivating agent for MuLV, either with or without acetonitrile. Concentrations of 2–6 M urea were used. Guanidine hydrochloride (4 M at pH 7) provided a 3.8 $\log_{10}$ reduction at time zero.

Detergents alone may not be sufficient to inactivate lipid-enveloped viruses such as MuLV. In some cases, we have observed that MuLV was not sufficiently inactivated by 0.1% to 1% Tween: Sometimes, there was no inactivation at all. From a review of the data, Tween 20 appears to be more effective than other Tweens, even at low temperatures, and Triton X-100 was also highly effective in most cases.

In one study, it was shown that Triton X-100 (0.5% at 4°C) completely inactivated Friend murine leukemia virus (Fr-MuLV) within four hours without influencing the binding capacity of a number of monoclonal antibodies. Log_{10} reduction values were ≥3.8 for Fr-MuLV (46).

A review of viral safety discusses the successful use of S/D. S/D has been shown to completely inactivate MuLV (≥6.0) (47). Complete inactivation was also reported for a recombinant blood factor (48). In S/D inactivation of MuLV, Tween from vegetables has been found to be as effective as Tween derived from animals.

Double-Stranded DNA viruses

Diameter: 120–2-0 nm; **Shape**: Spherical.
Resistance to physicochemical treatments: Medium.
Virus model: Herpes group, pseudorabies virus.

The herpes group of viruses includes herpes simplex (HSV), bovine rhinotracheitis virus (BRV), cytomegaloviruses (CMVs), and varicella-zoster virus (VZV). PRV is commonly used as the model for herpes virus.

Heat. Rabbit serum heated 30 minutes at 56°C provided fairly effective inactivation for PRV, but the researchers found that PRV is usually resistant to heat in the presence of protein stabilizers (16). Heating in solution at temperatures from 60°C to 80°C can, however, often effectively inactivate PRV by 4.1 to 8.1 $\log_{10}$. Inactivation kinetics of pasteurization (60°C) were studied for an antithrombin III product, and it was found that 30 minutes were required to inactivate 6.6 $\log_{10}$ of PRV (10). A modified pasteurization process (63°C for 10 hours) of process intermediates for a FVIII plasma product provided viral inactivation of PRV at ≥5.3 $\log_{10}$ (34).

Dry heat at temperatures of 59°C to 100°C does not appear to be as effective as heating in solution. Inactivation times for heat treatments ranged from zero to 72 hours. Lyophilization without heat treatment provided no inactivation in some cases and >4.4 $\log_{10}$ reduction in one case.

A minimum moisture content ≥0.7% is necessary for virus reduction in the magnitude of 4 $\log_{10}$ for PRV in lyophilized factor VIII (11). Freeze-drying and heating at 100°C for 30 minutes on a final container FVIII concentrate inactivated PRV by 5.7 $\log_{10}$ (13). Vapor heating of a FVII concentrate inactivated 6.9 $\log_{10}$ PRV in three hours (10). Another article describes a >11.5 $\log_{10}$ for PRV by vapor heating of a coagulation factor concentrate (49).

pH. Low pH (1.7–4.0) inactivation was effective. Increasing the time of inactivation can compensate for low temperatures. For example, at pH 4.0, 2°C to 10°C, a $\log_{10}$ reduction of >5.7 was achieved for a two-day inactivation treatment. At room temperature, the $\log_{10}$ reduction was >5.3 after a 20-minute treatment. In another case, it did not appear to be necessary to extend the time when the temperature was lowered. At pH 3.5–3.7, a 2–8°C temperature produced a >5.7 $\log_{10}$ reduction within one hour. At temperatures of 15°C and higher, $\log_{10}$ reductions of >6.4 were achieved in one hour.

When pH was raised to 5, log reduction values were severely reduced: In most cases, no reduction was found. Even with the addition of phenol and a one-day treatment, there was no inactivation of PRV at pH 5.5.

Low pH was investigated for inactivation of PRV in several monoclonal antibodies produced in either Sp2/0 or NS0 cell lines. Log_{10} reduction ranged from a low of 3.78 to complete inactivation at pH ranging from 3.32 to 3.51 (50).

Virus inactivation with pH 4/pepsin has been used as part of the manufacturing process for intravenous IgG. Factors found to influence the inactivation kinetics include temperature and content of the IgG solution. At 37°C, complete inactivation of PRV required 30 minutes. Below 37°C, there was a marked decrease in virus inactivation with the lowest rates at 4°C. At neutral pH, where no pepsin activity was detectable, virus inactivation was negligible at both 37°C and 4°C. Increasing sucrose concentrations in the IgG solutions inhibited the rate of PRV inactivation of PRV. Increases in IgG concentrations had no clear effect on PRV inactivation (51).

Alkaline conditions were effective at inactivating PRV. At a NaOH concentration as low as 0.1 N, inactivation was effected within a few hours. Inactivation by 0.5 N NaOH for two-hours was effective at temperatures as low as 4°C. One study achieved PRV inactivation by a two-minute contact with 0.1 M NaOH at 60°C (14).

Irradiation. Gamma irradiation was highly effective, providing >5.6 $\log_{10}$ reduction. PRV inactivation by gamma irradiation has also been described by Pruss et al. (22). Light and merocyanine 540 inactivated PRV by 6 $\log_{10}$ in the absence of added protein. But when 16% plasma was added, reduction was only 1.3 $\log_{10}$ (52).

Methylene blue (MB) and dimethylmethylene blue (DMMB) activated by light at a wavelength of 630 nm inactivated PRV by >5.0 $\log_{10}$ (53).

Chemical treatments. Ethanol alone or combined with acetic acid was effective for inactivation of PRV, as were methanol and

guanidine hydrochloride. Urea was effective with one exception: At a temperature of 2–8°C, urea provided only a 1.4 $\log_{10}$ reduction after one hour of exposure. Ether at low temperatures had only a small effect (a 1.4 $\log_{10}$ reduction). Acetonitrile at a 10% concentration and a pH of 7.8 had no effect, but 50% acetonitrile inactivated >4.7 $\log_{10}$ in four hours. Iodine, hydrogen peroxide, detergents, and solvent/detergent treatments were all effective in our studies.

A β-propiolactone concentration of 0.1% (w/w) was used for virus inactivation in a 4% immunoglobulin solution. At 22°C, PRV was inactivated below detection after 80 minutes (54). Inactivation of viruses spiked into an antithrombin III complex containing up to 0.1% human albumin by liquid iodine provided a >4 $\log_{10}$ reduction of PRV (55).

Supercritical fluid extraction has also been evaluated as a viral inactivation method for bone tissue. Hydrogen peroxide (35% w/w for two hours at 40°C), sodium hydroxide (1M for one hour at 20°C), and ethanol (95% for three hours at 20°C) treatments were also evaluated. When all four of these steps were incorporated into the viral inactivation process, a $\log_{10}$ reduction >17.6 for PRV was achieved. The supercritical fluid extraction alone provided a $\log_{10}$ reduction of >4.0 for PRV (56).

Inactine (PEN 110) provided a >5 $\log_{10}$ reduction of PRV (25). Inactivation of PRV by aminomethyltrimethylpsoralen (AMT) was found to be dependent on plasma levels. A plasma level of 14.5% and a concentration of 40 µg/mL of AMT were found to be optimal. A $\log_{10}$ reduction of about 5 was achieved for PRV (57). Binary ethylenimine (BEI) inactivates viruses in a first-order reaction and is reported to be superior in both safety and antigenicity to formalin. Important variables include pH, osmolarity of the virus suspension, and agitation rate. At 37°C, BEI has been found to inactivate PRV (24).

S/D treatment is quite effective for inactivating PRV (58–60). In some cases, inactivation to below the detection limit of PRV has been observed within two minutes (61). Caprylate has been used as an alternative to S/D for the inactivation of PRV in plasma-derived products (62). A recent publication describes the use of octanoic acid to inactivate PRV in immunoglobulin solutions (63). Vegetable Tween has been found to be as effective in S/D treatments as Tween derived from animals.

Inactivation Is Still Idiosyncratic

In searching the literature from 1990 through most of 2001 and from BioReliance databases, variables were sought that affect viral inactivation. These findings are not unique nor are they all-encompassing. Reviews published while these data were being prepared include one that addresses blood components (64), one that addresses plasma derivatives (65), and one prepared by the Council of Europe Expert Committee in Blood Transfusion that addresses labile blood products (66). Examining the data from publications and from our databases reinforces the opinion that the nature of both the inactivation process and the product can strongly influence viral inactivation.

Many variables affecting viral inactivation are well understood, but some results are not readily explained without the details of the study. Even then, results are sometimes difficult to explain. Unknown or unspecified details may have an impact on log reduction variables. Variability in virus titer and virus strain may also influence the log reduction values: There is clearly a need for standardized virus preparations and assays. Variability in measuring devices, calibration, and protocols for preparing inactivation solutions can influence reported log reduction values.

In spite of the variability found, there are some consistencies. For example: a given inactivation treatment will inactivate specific viruses within a specified range of conditions. This article series should provide a starting point when selecting a viral inactivation method. The series should also create greater awareness of the need to fully understand the properties of test articles, viruses being inactivated, and critical control parameters for a selected inactivation method.

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