Part 3a, Plasma and Plasma Products (Heat and Solvent/Detergent Treatments)

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Viral clearance (inactivation and removal) is an intricate process that requires drug developers to find the right antiviral method for a particular virus — without harming the biological product of interest. Part 1 of this series discussed inactivation of viruses in skin, bone, and cells other than platelets and red blood cells (1). Part 2 reviewed the literature on inactivation of viruses in red blood cells and platelets (2). Part 3 addressed plasma and plasma products. Of the approximately 460 references entered into the literature review database, those dealing with plasma and plasma products are, not surprisingly, the most numerous. We have chosen to divide Part 3 into two sections: Section 3a covers heat and solvent/detergent (S/D) treatments.

Several virus inactivation methods have been used for plasma and plasma products. These methods include, but are not limited to, dry-heat, vapor heating, pasteurization, S/D, methylene blue phototreatment, irradiation, beta-propiolactone/UV treatment, acid pH, and treatment with iodine. Many of these methods can be used during plasma product purification. Heat is commonly used, and analytical methods are used to evaluate modifications made to proteins during exposure to heat. One of these methods is electrospray ionizing mass spectrometry (3). Dry heat and vapor heating are used in several final products, and pasteurization is used in the final albumin container. Some of the specifics of these techniques are proprietary and may require a license fee.

Plasma fractionators have their own unique subtleties for performing inactivation. For example, different fractionators rarely, if ever, apply S/D treatments the same way. In spite of this variability (and the various method subtleties that may not have been published), some commonalties and combinations inactivate both lipid-enveloped viruses and those without lipid envelopes. Multiple factors affect the efficiency of these viral inaction techniques. Temperature and treatment duration are common variables in almost all of the methods. The advantages and important factors to consider for some of these techniques are provided in a review from 2000 (4).

Heat

Dry heat. Table 1 lists references for viruses treated with dry heat; Table 2 presents temperature, time, and $\log_{10}$ reduction for viruses in various coagulation factors. The effectiveness of freeze-drying and dry heat treatments (80°C for 72 hours) in the inactivation of CPV and BPV in two different coagulation factor VIII (FVIII) concentrates was variable and dependent on both the virus and the product. The FVIII products were made at two different facilities. In one FVIII, BPV was most resistant to inactivation by heat treatment. In the second FVIII product, however, there was no significant difference in resistance between the two parvoviruses. The researchers suggested that the variability might have been influenced by differences in ionic composition, the freeze-drying process, or the moisture content (5).

Moisture content influenced the effectiveness of dry heat (80°C for 72 hours) 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. For PPV, with a moisture content ⩾0.8%, the average $\log_{10}$ reduction was 3.72, and it was 2.5 for a moisture content ⩽0.8%. The authors concluded that a minimum moisture content greater than or equal to 0.7% is necessary for virus reduction of four $\log_{10}$ for HAV, PPV, and PRV (6).

Two high-purity coagulation factors, FVIII and plasma factor IX (FIX) were treated with dry heat. It was found that after 24 hours at 80°C, HAV infectivity was reduced by ⩾4.3 $\log_{10}$. That same reduction was achieved after two hours and before six hours at 90°C. HAV was also inactivated during freeze-drying by approximately two $\log_{10}$, whereas CPV was unaffected by lyophilization. Residual CPV was undetected after 48 hours at 80°C or 10 hours at 90°C (7).

FVIII of intermediate purity was spiked with parvovirus B19, freeze-dried, and heated at 80°C for 72 hours. Parvovirus B19 was reduced but not always eliminated, and was detectable by PCR (8).

For another FVIII product, virus inactivation validation during freeze-drying and treatment with 80°C heat for 72 hours reduced SIN infection by ⩾7.6 $\log_{10}$ and HIV-1 by ⩾6.4 $\log_{10}$ (9). SIN was reported to be inactivated by greater than six $\log_{10}$ by lyophilization followed by heating at 80°C for 72 hours in another high-purity FVIII concentrate (10). A study of 26 hemophilia patients who received FVIII and FIX products that were lyophilized, then heat treated at 100°C for 30 minutes, indicated that the dry heat was sufficient to inactivate HAV, but insufficient for parvovirus B19, which still contaminated the coagulation factor concentrates (11).

Freeze-drying and heating of a final container of FVIII concentrate at 100°C for 30 minutes inactivated HAV to below the detection limit (>5.3 $\log_{10}$) after a few minutes. That heat treatment also inactivated other RNA viruses below detection: HIV >6.6 $\log_{10}$, BVDV >6.6 $\log_{10}$, and VSV >5.8 $\log_{10}$. PRV and reo-3 were inactivated by 5.7 and 6.0 $\log_{10}$ respectively. SV-40 and BPV, however, showed strong resistance to dry heat treatment (12). Heat treatment of an FVIII concentrate at 100°C for 30 minutes was sufficient to inactivate both HAV and polio-1 by approximately five $\log_{10}$ after four minutes (13).

A 1991 review summarized data on dry heat inactivation at lower temperatures (60°C and 68°C for 30 hours or more) and provided for data on combining 60°C heat and heptane and 68°C heat and chloroform (14). Log reduction

factors for HBV, HCV, and HIV by dry heat treatment at 60°C, 68°C, and 80°C in various commercial products were presented by FDA in 1990 (15).

Vapor heating of six different coagulation factors (FVIII and FIX concentrates, fibrin sealant, lys-plasminogen, and fibrinogen) was evaluated for its capacity to inactivate HAV. Vapor heating conditions were variable and depended on the stability of the different products, but all tests included a 10-hour, 60°C treatment with pressures ranging from 190 to 385 mbar. The protocol for fibrinogen used the most intense method, employing 200 mbars pressure, followed by a three-hour heat treatment at 80°C with pressure at 385 mbars. 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 $\log_{10}$ (16).

Vapor heating of a FVII concentrate in another study (which used 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) was evaluated for its ability to inactivate HAV, HIV-1, TBEV, PRV, and MVM. HAV was readily inactivated within one hour at 60°C (5.9 $\log_{10}$). TBEV required six hours of this treatment for inactivation of 6.6 $\log_{10}$, and three hours were required to inactivate 6.2 $\log_{10}$ of HIV-1 and 6.9 $\log_{10}$ of PRV. MVM, however, was very stable and required 10 hours at 60°C followed by one hour at 80°C to inactivate 4.8 $\log_{10}$ (17).

Heating at 60°C for 10 hours in vapor provided a $\log_{10}$ reduction of greater than 6.0 for HIV in a commercial clotting factor concentrate (15). Vapor heating (also called steam treatment) can be performed following two basic procedures. Stream treatment IM-3 (STIM-3) involves heating at 60°C for 10 hours with a vapor pressure of 1,190 mbar. Steam treatment IM-4 (STIM-4) requires an additional hour of treatment at 80°C at a pressure of 1,375 mbar. STIM-3 provided $\log_{10}$ reduction factors of >6.0 for HIV-1, >7.6 for TBEV, >5.6 for PRV, and >6.6 for ERV-1 in two FVIII concentrates. STIM-4 provided greater $\log_{10}$ reduction factors for products that could tolerate the treatment. Its $\log_{10}$ reductions were >10.6 for HIV-1, >12.1 for TBEV, >11.5 for PRV, and >10.8 for ERV-1 (18).

Pasteurization. Immunoglobulin G (IgG) from dissolved fraction II (Cohn fractionation), was pasteurization at 60°C for 10 hours after the ethanol was removed and 33% sorbitol was added as a stabilizer. The clearance rates were >5.3 $\log_{10}$ for HIV-1, >4.7 for BVDV, >7.3 for BHV, 2.9 for EMC, and 3.4 for PPV. During pasteurization, enveloped viruses were inactivated within one hour at a rate that equaled nearly 90% of the spiked viruses, whereas the nonenveloped viruses EMC and PPV were gradually inactivated over a 10-hour period (19).

Table 1. Viruses treated by dry heat and the reference reporting on the treatment

Virus	References
BPV	5, 12
BVDV	12
CPV	5, 7
HAV	6, 7, 11–13
HBV	15
HCV	15
HIV	9, 12, 15
Polio	13
PPV	6
PRV	6, 12
Parvovirus B19	8, 11
Reo-3	12
SIN	9, 10
SV40	12
VSV	12

Table 2. Dry heat methods by test article and virus with $\log_{10}$ reduction values

Test Article	Viruses	Temp	Time	$\log_{10}$ Reduction, Variables	References
FVIII concentrates	CPV, BPV	80°C	72 h	Variable, dependent on product	5
FVIII	HAV	80°C	72 h	⩾4.54, moisture content of >0.8% 0.12, moisture content of <0.8%	6
	PPV	80°C	72 h	3.72, moisture content >0.8% 2.5, moisture content <0.8%	
High purity FVIII and FIX	HAV	80°C	24 h	⩾4.3	7
		90°C	2–6 h	⩾4.3	
	CPV	80°C	48 h	Undetectable	
		90°C	10 h	Undetectable	
Intermediate purity FVIII	Parvovirus B19	80°C	72 h	Reduction but not elimination	8
FVIII	SIN	80°C	72 h	⩾7.6	9
	HIV-1			⩾6.4	
High purity FVII	SIN	80°C	72 h	>6	19
FVIII and FIX	HAV	100°C	30 min	No contamination	11
	Parvovirus B19			Contamination	
FVIII	HAV	100°C	30 min	>5.3 after a few minutes	12
	HIV			>6.6	
	BVDV			>6.6	
	VSV			>5.8	
	PRV			5.7	
	Reo-3			6.0	
	SV40			Strong resistance	
	BPV			Strong resistance	
FVIII	HAV	100°C	4 min	~5	13
	Polio			~5	

Inactivation of viruses by pasteurization during the preparation of human intravenous immunoglobulin provided complete inactivation of HIV, CHV, VSV, mumps, vaccinia, ECHO, and HCV when heated for only one hour at 60°C. Viruses were spiked into a 5% IgG solution containing 33% sorbitol (20).

The inactivation of DHBV by pasteurization (60°C for 10 hours) in the chromatographic production process for albumin from plasma was evaluated and found to provide >6.5 $\log_{10}$ reduction (21). Inactivation of HAV by pasteurization in this albumin production process was also evaluated and found to be 4.4 $\log_{10}$ for a 5% albumin solution and >3.9 $\log_{10}$ in a 20% albumin solution (22). Pasteurization (60°C for 10 hours) during the purification process for antithrombin III (ATIII) from plasma provided a $\log_{10}$ reduction factor for HIV-1 of ⩾5.5 (in 15 minutes), ⩾6.6 for BHV (in one to 2.5 hours), ⩾5.0 for BVDV (in 30 minutes), and 6.0 for EMC (in 2.5 hours) (23).

The inactivation kinetics for HAV during pasteurization of a 5% albumin solution were compared with those of two other picornaviruses: polio-1 and ERV-1. The data demonstrated that polio-1 and ERV-1 are much more thermolabile than HAV. Approximately seven $\log_{10}$ of polio-1 were inactivated in 20 minutes and seven $\log_{10}$ ERV-1 were inactivated in 40 minutes. After 60°C treatment for 10 hours, 4.8 $\log_{10}$ of HAV were inactivated (24).

Inactivation kinetics for HAV, HIV-1, TBEV, PRV, and MVM by pasteurization (60°C) of an antithrombin III product were investigated. HAV was found to be much more thermostable than HIV-1 and TBEV. HAV was inactivated by 5.8 $\log_{10}$ after five hours at 60°C. Within 20 minutes, 6.2 $\log_{10}$ HIV-1 were inactivated. Thirty minutes were required to inactivate 6.6 $\log_{10}$ PRV, and 7.3 $\log_{10}$ TBEV were inactivated in three hours. After 10 hours, only 3.2 $\log_{10}$ MVM were inactivated (25). Inactivation of HAV by pasteurization (at 60°C for 10 hours) was investigated with a FVIII product. HAV was substantially inactivated, but the stabilizers used in the manufacturing process delayed the inactivation, and residual infectious HAV was

Table 3. Log_{10} reduction values and inactivation times reported by Nowak et al. in 1992 for 7s intramuscular and 5s intravenous immunoglobulins (30)

Virus	7s Ig[a] LRV[b]	Inactivation Time (in hours)	5s Ig[a] LRV[b]	Inactivation Time (in hours)
BVDV	>5.0	4	>6.3	4
HIV-1	>6.0	1.5	>6.0	2
HIV-2	>7.8	0.5	>7.8	0.5
HSV-1	>6.3	2	>6.4	8
Polio-3	>4.7	1	>4.6	4
TBEV	>7.3	4	>7.5	4
YFV	>5.9	6	>6.4	6

[a]Ig = immunoglobulin [b]LRV = $\log_{10}$ reduction value

Table 4. Viral inactivation of plasma and plasma products using pasteurization, showing inactivation for HIV by test article and treatment, giving the $\log_{10}$ reduction values for each

Test Article	LRV[a]	Conditions[b]	References
IgG from Cohn fraction II	>4.7	33% sorbitol	19
IVIG process intermediate	Complete[c]	5% IgG, 33% sorbitol	20
IgG	⩾6.0	Stabilizers	31
ATIII process intermediate	⩾5.5	15 minutes	23
ATIII	6.2	20 minutes	25
FVIII process intermediates	⩾9.9	63°C	36
FVIII process intermediates	>4.5	63°C, amino acids and sugars	37
FVIII	Complete[c]	four hours, sucrose and glycine	27
FVIII concentrate	Complete[c]	Stabilizers	31
Alpha$_1$-proteinase inhibitor	Complete[c]	one hour, stabilizers	32

[a]LRV = log10 reduction value
[b]Unless otherwise noted, treatment was at 60°C for 10 hours
[c]Complete is ⩾8.5

Table 5. Viral inactivation of plasma and plasma products using pasteurization, showing inactivation for HAV by test article and treatment, giving the $\log_{10}$ reduction values for each method; data from different testing laboratories also introduces variables related to virus titer and assay method as illustrated.

Test Article	LRV[a]	Conditions[b]	References
Albumin process intermediates			22
5% albumin	4.4		
20% albumin	>3.9		
Albumin, 5%	4.8		24
AT III	5.8	five hours	25
FVIII process intermediate	>5.6	63°C	36
FVIII process intermediate	>4.5	63°C, amino acids and sugars	37
FVIII	residual HAV	stabilizers	26
FVIII	inactivation	stabilizer	27
Isotonic buffer solution	residual HAV		27
Undefined	6	two hours, test lab 1	38
50 mg/mL albumin in serum	~3[c]	20 hours, test lab 2	38
Saline	~3[d]	20 hours, test lab 3	38
Fraction V products	2.8	pH 6.5	39
Fraction V products	3.4	pH 7.0	39
Fraction V products	4.6	pH 7.5	39
5% albumin	3.8	pH 6.4	39
5% albumin	2.6	pH 6.9	39
5% albumin	2.5	pH 7.4	39

[a]LRV = log10 reduction value
[b]Unless otherwise noted, treatment was at 60°C for 10 hours.
[c]$TCID_{50}$ assay
[d]Plaque assay

found even after a 10-hour heat treatment in the stabilized preparation (26).

Kinetics of inactivation by pasteurization (at 60°C) in plasma products were compared with those for isotonic solutions. In the presence of a FVIII preparation, the inactivation was slower, but both HSV-1 and polio were completely inactivated within four hours. Data for inactivation of HIV-1 and HIV-2 in FVIII solution were extrapolated, and it was calculated that the log reduction value (LRV) at 10 hours would be >30 $\log_{10}$. TBEV in intravenous immunoglobulin was completely inactivated in four hours; YFV in FVIII in one hour and YFV in intravenous immunoglobulin six hours; BVDV in FVIII in six hours and four hours in intravenous immunoglobulin. HAV was efficiently inactivated in FVIII; however, in a buffer solution, it was more stable than polio (27).

Inactivation of parvovirus B19 in plasma at 60°C was found to be time-dependent. Viral B19 protein was not detected after 12 minutes. The authors conceded, however, that this did not mean that the heat treatment completely inactivated the virus (28).

Inactivation by heating at 60°C of YFV, TBEV, and BVDV in the presence of various stabilized plasma proteins (FVIII, ATIII, intramuscular immunoglobulin, and intravenous immunoglobulin) showed that all three viruses were completely inactivated within six hours. Slight variations, however, were observed depending on the protein sample in which the viruses were inactivated. BVDV was found to be more stable than TBEV or YFV (29).

Inactivation of viruses by pasteurization was reported for a 7s intramuscular immunoglobulin and a 5s intravenous immunoglobulin. Table 3 shows LRVs and inactivation times (30).

Inactivation of HIV-1 and HIV-2 in a stabilized FVIII concentrate was slower than inactivation in cell culture medium, but complete inactivation was achieved after only one hour. In an IgG preparation with stabilizers, 60°C temperature for 10 hours was found to inactivate ⩾6.0 $\log_{10}$ of HIV-1 and ⩾7.8 $\log_{10}$ HIV-2 (31).

Pasteurization of stabilized liquid alpha$_1$-proteinase inhibitor from plasma with 60°C heat was effective in inactivating viruses to below the detection limit. For HIV, HSV, VSV, and CMV, one hour of treatment was required. Polio required a five-hour treatment for inactivation, and vaccinia required five to 10 hours. Ten hours were required for PPV. Visna virus was inactivated in a half-hour (32). Data for inactivation of HIV-1 by pasteurization at 60°C for stabilized aqueous preparations of albumin, antihemophilic concentrate, ATIII, cholinesterase, FVIII, FIX, FXIII, fibrinogen, and prothrombin complex were also reported (33).

Pasteurization (at 60°C for 10 hours) in the presence of guanidine hydrochloride was shown to be an effective method for inactivation of parvovirus and picornavirus. Both model viruses were inactivated below the limit of detection when spiked into apolipoprotein AI (apoA-I) (34).

A modified pasteurization process (at 63°C for 10 hours) was employed for virus inactivation of process intermediates of a FVIII plasma product. Enveloped (HIV, SIN, HSV, PRV) and nonenveloped (polio, coxsackievirus, HAV) were inactivated. Virus reduction factors ($\log_{10}$) were ⩾9.9 for HIV, ⩾8.4 for SIN, ⩾7.7 for HIV-1, ⩾5.3 for PRV, ⩾5.6 for HAV, ⩾9.8 for polio-1, ⩾4.7 for coxsackievirus-B6, and ⩾1.1 for SV40. The temperature selected was 63°C, rather than 60°C, because earlier reports in the literature suggested that temperatures greater than 60°C were required to inactivate picornaviruses (35,36).

Heat treatment at 63°C for 10 hours was also effective for virus inactivation of a high-purity FVIII concentrate stabilized by von Willebrand factor. In-process production samples were stabilized using amino acids and sugars before spiking. HIV, HSV, PRV, and the nonenveloped viruses polio, coxsackievirus, and HAV were

Table 6. Inactivation of HAV by pasteurization by six different manufacturing processes (A–F) presented by H. Wilkommen of the Paul-Erlich Institut (40)

	Reduction Factor (Log_{10})					
Protein Concentration	**A**	**B**	**C**	**D**	**E**	**F**
Four or five percent	⩾6.6	2.9	3.0	5.7	3.8	4.5–5.8
20% to 25%		3.8	3.8	2.5	4.1	4.3
		⩾6.6		⩾5.8		

inactivated by greater than 4.5 $\log_{10}$ (37). Tables 4 and 5 list the pasteurization methods for HIV and HAV by test article and treatment, giving the LRV for each method.

Data from different testing laboratories also introduces variables related to virus titer and assay method (see Table 5). In an industry–FDA forum, a company presented data that it had gathered from the industry: Clearance of HAV varied from a $\log_{10}$ reduction of six within two hours to a $\log_{10}$ reduction of only three after 20 hours of treatment. The presence of albumin or saline alone or the use of the $TCID_{50}$ or plaque assays left the outcome unaltered (38).

The effect of pH on pasteurization for inactivating HAV and BVDV was found to be variable in products similar to fraction V (5% human plasma protein solution and 5% plasma protein fraction). An increase in pH from 6.4 or 6.5 to 7.5 increased HAV inactivation. But in 5% albumin, inactivation was more effective at pH 6.4 compared with other tests at pH 6.9 or 7.4. For BVDV, however, pH 6.4 to 7.5 had little effect on inactivation in a solution of 5% plasma protein fractions. The authors concluded that predicting how variations in process parameters will affect viral clearance is difficult, stating that results will vary depending upon the virus and the product being tested (39).

Further information illustrating the variability found during inactivation of HAV by pasteurization is illustrated in Table 6. H. Wilkommen of the Paul-Erlich Institut (www.pei.de) virus safety section presented the data for albumin manufactured according to the *European Pharmacopoeia* (www.pheur.org) monograph (40). Table 7 lists the viruses discussed in the pasteurization section and includes the references that pertained to each.

Other heat treatments studied and published include heat with n-heptane; heat treatment at 56°C; treatment at high temperature, short time (HTST); and 60°C treatment with chloroform.

Heat treatment with n-heptane. Incubation with n-heptane at 60°C for 20 hours was shown to inactivate at least 8.6 $\log_{10}$ of HIV-1 in a FIX preparation. Virus was spiked into the FIX before freeze-drying or the heptane heat treatment (41,15,42).

Heat treatment at 56°C. HIV-infected plasma was heated for one hour at 56°C, and ⩾5.9 $\log_{10}$ of HIV were inactivated (43).

HTST. Plasma spiked with HIV, VSV, or EMC was treated at various temperatures from 65°C to 85°C. Heating at 77°C for 0.006 seconds resulted in HIV inactivation by ⩾4.4 $\log_{10}$. The duration of the total process was 0.250 seconds with a hold-temperature time of 0.006 seconds. EMC was completely inactivated (⩾5.8 $\log_{10}$) at 72°C and above, and VSV was completely inactivated (⩾4.4 $\log_{10}$) at 75°C and above (44).

Heat treatment with 60°C and chloroform. HIV inactivation at ⩾6.0 $\log_{10}$ was achieved with a commercial clotting factor concentrate (15).

Solvent/Detergent (S/D)

LRVs achieved with an S/D treatment of 1% w/v Tween and 0.3% tri(*n*-butyl)phosphate (TNBP) during the manufacture of a high-purity FIX were 4.5 for HIV-1, 5.1 for SIN, 6.1 for VSV, 5.1 for BVDV, and 5.3 for PRV (45). A 2000 S/D inactivation review article stated that the most frequently used combination of S/D is 1% TNBP and 1% Triton X-100 (46). In that reference, Pamphilon provides a list of viruses and the publications that provide LRV data. Table 8 is adapted from the Papillon article (46) and from the material in a Horowitz article (47). In all of the enveloped viruses tested and described by Horowitz (47), viruses were completely killed. HBV, HCV, and HDV were inactivated regardless of the TNBP–detergent pair or protein solution used. Combinations included: 0.3% TNBP, 0.2% sodium cholate at 24°C for six hours; 0.3% TNBP and 0.2% sodium cholate at 30°C for six hours; 0.3% TNBP and 1% Tween 80 at 24°C for six hours; 2% TNBP at 37°C for four hours; and 1% TNBP and 1% Triton X-100 at 30°C for four hours (47).

In contrast to the viruses listed in Table 8, vaccinia virus spiked into intermediates in the production of FVIII and FIX was found to be relatively resistant to inactivation by

Table 7. References for viruses that were tested for inactivation using pasteurization

Viruses	References
BVDV	19, 23, 27, 29, 39
BHV	19, 23
CHV	20
CMV	32
Coxsackievirus	36
DHBV	22
ECHO	20
EMC	19, 23
ERV	24
HAV	22–24, 26, 27, 36–40
HCV	20
HIV	19, 20, 23, 25, 27, 31–33, 36, 37
HSV	27, 32, 36, 37
Mumps	20
MVM	23
Polio	24, 27, 32, 36
PPV	19, 32
PRV	23, 36, 37
Parvovirus B19	28
SIN	36
SV-40	36
TBEV	23, 27, 29
Vaccinia	20, 32
Visna	32
VSV	20, 32
YFV	27, 29

Table 8. The most frequently used S/D combination treatment used is 1% TNBP and 1% Triton X-10. Viruses from two review articles are listed with their LRVs.

Virus	LRV[a]	LRV[b]
CMV	>6.0	⩾6.0
DHBV	⩾7.3	⩾7.3
HBV (in vitro chimpanzee)	⩾6.0	⩾6.0
HCV (in vitro chimpanzee)	⩾5.0	⩾5.0
HIV-1	⩾6.0	⩾11.0
HIV-2	>6.0	⩾6.0
HSV-1	⩾5.8	⩾5.8
MuLV		⩾6.0
PI-1		⩾4.0
PRV	⩾6.5	
Rauscher MuLV, ecotropic		⩾2.0
SIN	⩾3.6	⩾8.8
Sendai		⩾6.0
VEE		⩾6.0
VSV	⩾6.2	⩾9.2
XMuLV		⩾4.0

[a]These values are from the D. Pamphilon 2000 review article (46).
[b]These values are from the B. Horowitz et al. 1993 article (47).

Table 9a. Variables in detergent type and concentration, time, and temperature affecting inactivation of SIN and VSV in coagulation factors FVIII and FIX

LRV	Detergent, Concentration	Time	Temperature (°C)	Reference
Sindbis Virus[a]				
4.9	Triton X-100, 1%	15 seconds	22 or 25	5
>5.6	Triton X-100, 1%	10 minutes	22 or 25	5
4.6	Triton X-100, 1%	5 seconds	Room temperature	48
4.0	Tween 80, 1%	30 minutes	22 or 25	5
7.1	Tween 80, 1%	2 hours	22 or 25	5
Vesicular Stomatitis Virus[a]				
5.8	Triton X-100, 1%	15 seconds	22 or 25	5
>6.3	Triton X-100, 1%	30 minutes	22 or 25	5
CR[b]	Triton X-100, 1%	3 minutes	20	49
3.2	Tween 80, 1%	10 minutes	22 or 25	5
>5.5	Tween 80, 1%	2 hours	22 or 25	5

[a]TNBP 0.3% concentration in all cases [b]CR = complete removal

Table 9b. Variables in TNBP concentration, detergent type and concentration, temperature, and time affecting inactivation of VSV, SIN, hepatitis viruses, and HIV in plasma

LRV	TNBP%	Detergent, Concentration	Temperature (°C)	Time	Reference
Vesicular Stomatitis Virus					
⩾7.5	1	Triton X-100, 1%	30	4 hours	53, 54
⩾5.0	2	None	37	6 hours	52
⩾5.2	1	Tween 80, 1%	30	6 hours	52
⩾5.2	1	Triton X-45, 1%	30	6 hours	52
Sindbis Virus					
⩾6.9	1	Triton X-100, 1%	30	4 hours	53, 54
⩾5.2	2	None	37	6 hours	52
⩾5.5	1	Tween 80, 1%	30	6 hours	52
⩾5.5	1	Triton X-45, 1%	30	6 hours	52

Virus	LRV	TNBP%	Detergent, Concentration	Temperature (°C)	Time	Reference
Hepatitis Viruses						
HBV	⩾6.0	1	Triton X-100, 1%	30	4 hours	53, 54
	⩾6.0	2	None	37	1 hour	52
DHBV	⩾7.3	1	Triton X-100, 1%	30	4 hours	53
HCV	⩾5.0	1	Triton X-100, 1%	30	4 hours	53–55
	⩾5.0	2	None	37	1 hour	52
BVDV	⩾6.1	1	Triton X-100, 1%	30	4 hours	53
model	⩾5.0	0.4	Triton X-100, 0.85%	30	4 hours	53

LRV	TNBP%	Detergent, Concentration	Temperature (°C)	Time	Reference
Human Immunodeficiency Virus					
⩾7.2	1	Triton X-100, 1%	30	4 hours	53
⩾4.2	2	None	37	6 hours	52
⩾3.1	1	Tween 80, 1%	30	15 minutes	52
⩾6.2	1	Triton X-100, 1%	30	4 hours	54, 55

TNBP/Triton X-100 or TNBP/Tween 80 (0.3% TNBP and 1% Triton X-100 or 1% Tween 80 at 22°C or 25°C). Treatment with TNBP/Triton X-100 rapidly inactivated SIN, SFV, HSV-1, and VSV by 3.7–5.0 $\log_{10}$ within 15 seconds. Virus inactivation with TNBP/Tween 80 was slower, but effective inactivation of SIN, HSV-1, VSV, and HIV-1 was achieved (4.1 to >6.3 $\log_{10}$) within 30 minutes. To achieve a four $\log_{10}$ reduction of vaccinia required an incubation time of 10 minutes with TNBP/Triton X-100 and six to 24 hours with TNBP/Tween 80 (5).

The efficacy of S/D treatment for inactivation of HBV was evaluated by using SIN and HSV model viruses spiked into dissolved cryoprecipitate taken from the manufacturing process for FVIII and albumin. After S/D treatment (0.3% TNBP and 1% Triton X-100), SIN was reduced by a factor of 4.6 $\log_{10}$, and HSV by 7.2 $\log_{10}$ within five seconds (48).

S/D inactivation of VSV in an ultrapure FVIII was evaluated, and it was found that with 1% or 0.2% Triton X-100, inactivation was faster than with 0.2% sodium cholate or 1% Tween 80. Within three minutes, inactivation of the virus exceeded the assay sensitivity. In all cases, 0.3% TNBP was used (49).

After polyethylene glycol (PEG) precipitation and before chromatography, a FVIII intermediate was subjected to S/D using Tween 80. Log_{10} reduction values were ⩾10.0 for HIV-1, ⩾6.0 for HIV-2, ⩾6.8 for VSV, ⩾6.4 for SIN, ⩾4.5 for BVD, and ⩾8.0 for BHV (50).

S/D treatment (1% TNBP and 1% Triton X-100 at 30°C for four hours) was found to inactivate HIV-1, PRV, and SIN by more than five $\log_{10}$ (complete inactivation) in Octaplas (Octapharma AG, www. octapharma.com), a cell-free, blood group specific, human coagulation active ingredient for use in plasma for transfusion (51). A previous publication (in German) reported the same results with VSV and HSV-2, and chimpanzee studies have shown the same for HBV and HCV (52).

Inactivation kinetics using S/D (1% TNBP/ 1% Triton X-100 at 30°C) were evaluated in whole plasma. S/D treatment resulted in complete viral kill, typically within 15 minutes of the four-hour treatment. Inactivation rates were: ⩾7.5 $\log_{10}$ for VSV, ⩾6.9 $\log_{10}$ for SIN, ⩾6 $\log_{10}$ for HBV, ⩾6.1 $\log_{10}$ for BVDV, ⩾5 $\log_{10}$ for HCV, ⩾7.2 $\log_{10}$ for HIV, and ⩾7.3 $\log_{10}$ for DHBV. The effect of reduced levels of TNBP and Triton X-100 was evaluated for inactivation of BVDV. Viral kill was complete (⩾5 $\log_{10}$) at 15 minutes, even when the concentration of TNBP was reduced to 0.4% and Triton X-100 to 0.85% (53).

VSV and SIN spiked into plasma were inactivated by 1% TNBP and 1% Triton X-100 at 30°C within 15 minutes (VSV at ⩾7.5 $\log_{10}$ and SIN at ⩾6.9 $\log_{10}$). When 0.3% TNBP or a reaction temperature of 24°C were used, the rate of inactivation was slower. S/D (1% TNBP/1% Triton X-100 for four hours at 30°C) was able to inactivate ⩾6.0 $\log_{10}$ HBV, ⩾6.0 $\log_{10}$ HCV, and ⩾6.2 $\log_{10}$ HIV (54). S/D treatment of human plasma (1% TNBP/1% Triton X-100 at 30°C for four hours) inactivated ⩾6.0 $\log_{10}$ HBV, ⩾5.0 $\log_{10}$ HCV, and ⩾6.2 $\log_{10}$ HIV (55).

Table 9c. Variables in TNBP concentration, detergent type and concentration, temperature, and time affecting inactivation of SIN and VSV in coagulationh factors FVIII and FIX

Virus[a]	LRV	TNBP%	Temperature (°C)	Time	Reference
HIV-1	>5.0	0.3	Room temperature	Instantaneous	57
VSV	>4.0	0.3	Room temperature	ND	57
SIN	>5.2	0.3	Room temperature	Within minutes	57
HIV-1	>10.0	0.3	27	30 or 180 minutes	20
HIV-2	>6 (CR)	0.3	27	30 or 180 minutes	20
SIN	5.9 (CR)	0.3	27	30 or 180 minutes	20
VSV	5.5 (CR)	0.3	27	30 or 180 minutes	20
HCV	3 (CR)	0.3	27	30 or 180 minutes	20
Vaccinia	Incomplete	0.3	27	30 or 180 minutes	20
HIV-1[b]	4.7	3–4 mg/mL	30	6.5 hours	59
BVDV[b]	5.2	3–4 mg/mL	30	6.5 hours	59
PRV[b]	5.3	3–4 mg/mL	30	6.5 hours	59

[a]Tween 80, 1% was used as the detergent in all cases unless otherwise noted.
[b]Cholate at 2–3 mg/mL was used as the detergent for this virus.
[c]ND = not done [d]CR = complete removal [e]Removal by PCR

S/D treatment data from both laboratory and animal studies were summarized, showing that inactivation of enveloped viruses in all cases exceeded the virus challenge. Viruses that were inactivated included VSV, SIN, Sendai, HBV, HCV, HIV-1, HIV-2, CMV, HSV-1, and VEE (56). The $\log_{10}$ reductions of VSV, SIN, HIV, HCV, and HBV in plasma using various temperatures, TNBP alone, and TNBP with Tween-80 or Triton X-45 were summarized in a 1990 paper (52). Some of the data are included in Table 9.

Chang et al. reported UVU clearance of >5.7 $\log_{10}$ from a dissolved Cohn fraction II solution treated with S/D (0.3% TNBP and 1% Tween 80) for six hours at 25°C after anion-exchange chromatography (19). Clearance factors for other viruses were the same as those found by Eriksson in 1994 (57). S/D was used before the chromatography steps in the purification of anti-D immunoglobulin from plasma. The S/D treatment provided LRVs for HIV of ⩾6.0, for BVDV of ⩾5.4, and for PRV of ⩾5.6. Those viruses were inactivated to undetectable levels within two minutes (58).

After heating at 56°C for one hour, S/D treatment (1% TNBP and 1% Triton X-100 for four hours at 30°C) was applied to HIV-positive plasma used in the preparation of hyperimmune gamma globulins. HIV inactivation was ⩾6.2 $\log_{10}$ (43).

Eriksson et al. found that in the production of immunoglobulins from plasma, S/D treatment (0.3% TNBP and 1% Tween 80) inactivated >5.0 $\log_{10}$ of HIV-1, >4.0 $\log_{10}$ of VSV, and >5.2 $\log_{10}$ of SIN. They also compared the ability of S/D with pasteurization for inactivating HIV-1 and SIN. HIV-1 was instantaneously inactivated by S/D, whereas heating at 60°C was required for about 30 minutes to achieve the same level of inactivation through pasteurization. For SIN, total inactivation was achieved within minutes of exposure to S/D, but it took two hours at 60°C for pasteurization (57).

S/D treatment (1% Tween 80, 0.3% TNBP) used in the production of intravenous immunoglobulin completely inactivated HIV-1 (>10.0 $\log_{10}$), HIV-2 (>6.0 $\log_{10}$), SIN (5.9 $\log_{10}$), VSV (5.5 $\log_{10}$), and HCV (3 $\log_{10}$ by PCR). That study showed that complete inactivation for those viruses could be achieved in either 30 or 180 minutes. Vaccinia virus, however, was not completely inactivated by the S/D treatment (20).

Process intermediates of intramuscular immunoglobulins were treated with S/D using TNBP and cholate (pH 5.8, 3–4 mg/mL of TNBP and 2–3 mg/mL of cholate). Incubation was for 6.5 hours at 30°C. Viral clearance was ⩾4.7 $\log_{10}$ for HIV-1, 5.2 $\log_{10}$ for BVDV, and ⩾5.3 $\log_{10}$ for PRV (59).

Vegetable-derived Tween 80 was substituted for bovine-derived Tween 80, and inactivation by S/D treatment of SIN in two plasma products was studied. The results showed no difference in either rate or degree of virus inactivation in products treated with Tween 80 derived from corn oil and that derived from bovine tallow (60). Tables 9a–b summarize the variables studied using S/D treatment of plasma products.

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