

Comparison of Two Different Methods for Inactivation of Viruses in Serum

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In order to compare protocols for inactivation of viruses potentially present in biological specimens, three different model viruses were treated in bovine serum by two different inactivation methods: samples were subjected either to chemical inactivation with ethylenimine (EI) at concentrations of 5 and 10 mM at 37°C for periods up to 72 h or to electron-beam irradiation in frozen and liquid form with doses varying between 11 and 46 kGy. The chemical inactivation resulted in nonlinear tailing curves in a semilogarithmic plot of virus titer versus inactivation time showing non-first-order kinetics with respect to virus titer. The time for inactivation of 7 log₁₀ units of porcine parvovirus (PPV) was about 24 h for both EI concentrations, whereas 5 log₁₀ units of bovine viral diarrhea virus (BVDV) was inactivated in 2 h for both EI concentrations and 6 log₁₀ units of porcine enterovirus (PEV) was inactivated within 3 h. The inactivation with electron-beam irradiation resulted in almost linear curves in a semilogarithmic plot of virus titer versus irradiation dose, reflecting a first-order inactivation. The rate of inactivation was almost twice as fast in the liquid samples compared to the rate in frozen ones, giving values of the doses needed to reduce virus infectivity 1 log₁₀ unit for inactivation of PPV of 11.8 and 7.7 kGy for frozen and liquid samples, respectively, whereas the corresponding values for BVDV were 4.9 and 2.5 kGy, respectively, and those for PEV were 6.4 and 4.4 kGy, respectively. The nonlinear inactivation with EI makes it impossible to extrapolate the curves beyond the virus detection limit and thereby predict the necessary time for complete inactivation, i.e., to a level beyond the detection limit, of virus in a given sample. The first-order inactivation obtained with electron-beam irradiation makes such a prediction possible and justifiable. The two methods are discussed with respect to their different kinetics and applicability under different circumstances and criteria for inactivation, and considerations for choice of method are discussed.

The potential risk of introducing viruses not present in Denmark via biological materials, most notably serum, received from various countries, including developing countries in the tropics, constitutes an obstacle to scientific collaboration between scientists in these countries and in Denmark. In veterinary medicine this problem is especially relevant for countries with high zoonotic status, where strict import control is required to avoid accidental introduction of unwanted pathogens. Therefore, a standardized method for the inactivation of infectious agents, in particular viruses, is a prerequisite for joint research projects in which serum is exchanged between Denmark and various other countries. Alternatively, the material can only be handled in high-containment laboratories.

Because of the demand to inactivate any virus species potentially present in imported biological material, the method should be efficient for different types of viruses. We report here the testing of two different methods of virus inactivation: chemical inactivation with ethylenimine (EI) and physical inactivation by electron-beam irradiation. The viruses used were selected in order to represent different classes of viruses, e.g., naked or enveloped virus particles with a DNA or an RNA genome and with different degrees of resistance to physicochemical treatments. Because viruses in general are protected from inactivation by protein and since serum is a common

sample material in a research context, we used sterile bovine serum as the suspension medium. The serum was spiked with three model viruses: porcine parvovirus (PPV), a nonenveloped DNA virus; porcine enterovirus (PEV), a nonenveloped RNA virus; and bovine viral diarrhea virus (BVDV), an enveloped RNA virus. The samples were subjected to chemical inactivation with EI or to electron-beam irradiation in frozen and liquid form.

In this article we focus on the differences in inactivation kinetics of the two methods for different classes of viruses and use this information to highlight some of the issues to be considered before choosing an inactivation method for a given sample.

MATERIALS AND METHODS

EI for virus inactivation. Fresh EI (0.1 M) was prepared as described by Bahnemann (2). Briefly, 0.2 M stock solutions of bromoethylamine hydrobromide (FLUKA 6670) in deionized water, stored frozen at -20°C in small portions, were thawed and mixed with an equal amount of 0.4 M NaOH at 37°C for 1 h.

Serum. Sterile bovine serum was obtained from the donor herd at the Danish Veterinary Laboratory and was stored at -20°C until use. This is a controlled herd which is tested and declared to be free of BVDV and BVDV antibodies twice a year. The serum was tested for BVDV antibodies by a serum neutralization test and for BVDV by propagation on susceptible cells followed by peroxidase-linked BVDV antibody staining.

Preparation of virus used for spiking. In order to study carefully the kinetics of inactivation, it is important that the inactivation be performed with high initial titers. Therefore, concentrated virus stocks were prepared as described below for each of the three viruses.

(i) **PPV.** Primary porcine kidney cells in 1-liter roller culture bottles were infected with PPV and incubated for 10 days at 37°C. They were then stored vertically at room temperature overnight, and the cells were shaken off into the growth medium and pooled. The virus was precipitated by adjusting the pH to 4.0 and was captured by filtration. The precipitate was extracted by stirring with 20

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TABLE 1. Inactivation of PPV, PEV, and BVDV with EI or irradiation

Virus	EI				D_{10} value (kGy) by irradiation	
	Initial inactivation rate (\log_{10} [TCID ₅₀ s/50 μ l]/h) ^a		Time for 4 \log_{10} inactivation		Frozen	Liquid
	5 mM	10 mM	5 mM	10 mM		
PPV	2.4 (1 h)	3.5 (1 h)	9 h	3 h	11.8	7.7
BVDV	2.3 (2 h)	2.3 (2 h)	1 h and 44 min	1 h and 44 min	4.9	2.5
PEV	5.5 (1 h)	5.8 (1 h)	24 min	17 min	6.4	4.4

^a The initial inactivation rates are based on the values measured within the first hour or the first 1 or 2 h, as indicated in parentheses.

mM glycine (pH 9.0; 5% of the original volume), and cell debris was removed by filtration. The extract was frozen and stored at -20°C until use as virus for spiking (spike virus). The titer of the spike virus was $9.2 \log_{10}$ (50% tissue culture infective doses [TCID₅₀s/50 μ l]).

(ii) **BVDV.** Secondary calf kidney cells in 1-liter roller culture bottles infected with BVDV and incubated at 37°C for 3 days were frozen, and the cells were shaken off during thawing and pooled. Virus was precipitated by the addition of 23 g of NaCl per liter and 7% (wt/vol) polyethylene glycol 6000 (PEG) and stirring on ice for 4 h. After centrifugation ($31,300 \times g$, 15 min, 5°C) the pellet was drained well and resuspended in growth medium that had been taken out before precipitation, equal to approximately 1% of the original volume of the pooled material, sonicated (three times for 15 s each time; 12- μm amplitude), and stirred overnight at 4°C . After stirring, the solution was centrifuged ($9,880 \times g$, 15 min, 5°C) to remove the cell debris. The supernatant was frozen and stored at -50°C until use as spike virus. The titer of the spike virus was $6.7 \log_{10}$ (TCID₅₀s/50 μ l).

(iii) **PEV.** Secondary porcine kidney cells in 1-liter roller culture bottles infected with a Talfan disease isolate of PEV serotype 1 and incubated for 2 days at 37°C were frozen, and the cells were shaken off during thawing and pooled. The pH was adjusted to 7.0 with 0.1 M NaOH. PEG was added to 7% (wt/vol) and stirred magnetically on ice for 2 h before centrifugation at $31,300 \times g$ for 15 min at 5°C . The pellet was drained well and resuspended in Tris-EDTA (0.05 M Tris, 0.01 M EDTA [pH 8.86]) at 1% of the original volume, sonicated (three times for 15 s each time; 12- μm amplitude), and stirred overnight at 4°C . After stirring, the solution was centrifuged ($9,880 \times g$, 15 min, 5°C) to remove the cell debris. The supernatant was frozen and stored at -50°C until use as spike virus. The titer of the spike virus was $7.6 \log_{10}$ (TCID₅₀s/50 μ l).

Inactivation with EI. The chemical inactivation of the viruses was carried out under constant stirring at 37°C . Mock-exposed (no EI) samples were included in all experiments. The 0-h value of the mock-exposed sample was used as the 0-h value for each EI concentration.

(i) **PPV.** The virus was diluted 1:10 in sterile bovine serum and filtered through a 0.2- μm -pore-size membrane filter before the addition of EI to final concentrations of 5 and 10 mM or mock exposure (all in duplicate). Aliquots were taken at 0.5, 1, 3, 5, 10, 14.75, and 24 h, frozen in a dry ice-ethanol bath, and subsequently freeze-dried to remove the EI. The dried samples were stored at 4°C , resuspended in sterile deionized water equal to the original volume, and titrated.

(ii) **BVDV.** The virus was diluted 1:40 in sterile bovine serum and filtered through a 0.2- μm -pore-size membrane filter before the addition of EI to final concentrations of 5 and 10 mM or mock exposure (all in duplicate). Aliquots were taken at 2, 5, 24, 48, and 72 h. Immediately after removal of the samples from the inactivation mixture, aqueous sodium thiosulfate (2.0 M) was added to the samples to a final concentration of 0.02 M to quench the EI. The samples were chilled on ice, and in order to remove EI and sodium thiosulfate, they were dialyzed (1 liter/10-mL sample; dialysis was with Medicell Visking tubes; cutoff value, 12,000 to 14,000 Da) overnight at 4°C against Earle's balanced salt solution with the following supplements: 1 mg of streptomycin per ml, 0.05 mg of neomycin per ml, 5 mg of whey protein concentrate (WPH 800; Danmark Protein A/S, Nr. Vium, Denmark) per ml, 2 μg of choline chloride per ml, 2 μg of folic acid per ml, 2 μg of nicotinic amide (NAD) per ml, 2 μg of pantothenic acid, sodium salt per ml, 2 μg of pyridoxal hydrochloride per ml, 2 μg of thiamine hydrochloride per ml, and 0.2 μg of riboflavin per ml; the pH was adjusted to 7.5 to 7.8 with 1 M NaOH or HCl. After dialysis the samples were stored at 4°C for not more than 3 days until titration.

(iii) **PEV.** The virus was diluted 1:20 in sterile bovine serum and filtered through a 0.2- μm -pore-size membrane filter before the addition of EI to final concentrations of 5 and 10 mM or mock exposure (all in duplicate). Samples were taken at 0.25, 0.50, 1, 2, 3, 5, and 24 h. Immediately after removal of the samples from the inactivation mixture aqueous sodium thiosulfate (2.0 M) was added to the samples to a final concentration of 0.02 M to quench the EI. The samples were chilled on ice and dialyzed as described for BVDV. After dialysis the samples were stored at 4°C for not more than 3 days until titration.

Inactivation of virus by irradiation. The virus was diluted in sterile bovine serum (PPV, 1:10; BVDV and PEV, 1:40), and 3.5-ml aliquots were placed into polyethylene tubes (Minisorp; catalog no. 4-66982; Nunc, Roskilde, Denmark). The tubes were frozen in a vertical position by using dry ice-ethanol or at -50°C

and were then fixed horizontally for irradiation. Irradiation was carried out at the 10-MeV electron accelerator of Risø National Laboratory. The beam was directed vertically from above, the pulse rate was 200 Hz, the pulse length was 4 μs , and the pulse current was 1 A. Irradiation to 11 kGy takes approximately 30 s, and the irradiation was carried out in steps of approximately 11 kGy at 5- to 10-min intervals; the exact doses were measured with a dosimeter which followed the doses in the samples during irradiation. The irradiation was performed on frozen samples held on dry ice and on liquid samples on an ice-water bath. For each of the three viruses, frozen and liquid samples were irradiated with 11.9, 23.3, 34.8, or 45.9 kGy, each in duplicate. Mock-exposed samples, both frozen and liquid, were included in the experiment. All samples were covered with approximately 1.5 cm of ice or dry ice during irradiation. After irradiation all samples were frozen on dry ice and stored at -50°C until titration.

Titration of viruses. Serial dilutions of the virus suspensions were made in Eagle's basal medium supplemented with 1 mg of streptomycin per ml and 50 μg of neomycin per ml. For each dilution 50 μl /well was applied to 7 or 10 wells in a microwell plate. Negative controls consisting of 50 μl of Eagle's basal medium per well were made. Each well was supplemented with 50 μl of Eagle's basal medium and 100 μl of cell suspension. The porcine testicle cell line ST117 (ATCC 1746-CRL) (approximately 230,000 cells/ml in Eagle's basal medium with 3% fetal calf serum [FCS]) was used for PPV titrations. Secondary calf kidney cells (approximately 200,000 cells/ml in Eagle's basal medium with 10% FCS) were used for BVDV titrations. Secondary porcine kidney cells (approximately 200,000 cells/ml in Eagle's basal medium with 10% FCS) were used for PEV titrations. After incubation for 4 days at 37°C in 5% CO_2 in air, the cells were fixed with 99% ethanol (45 min, 5°C). Virus-infected cells were stained with peroxidase-linked antibodies (PPV, peroxidase-linked monoclonal antibody against PPV; BVDV, polyclonal swine antibodies against BVDV, followed by peroxidase-linked guinea pig anti-swine antibodies [DAKO, Glostrup, Denmark]; and PEV, polyclonal guinea pig antibodies against PEV serotype 1, followed by peroxidase-linked rabbit anti-guinea pig antibodies [DAKO]) by using 0.04% ethylcarbazole and 1% hydrogen peroxide in ethanol as a chromogenic substrate. The results were visually read by using an inverted microscope.

RESULTS

The results of the experiments are presented in Table 1 and Fig. 1. In the following text all inactivation rates for EI inactivation are expressed as \log_{10} (TCID₅₀s/50 μ l)/h unless indicated otherwise. For comparison, inactivation is described in terms of the initial inactivation rate, the time for inactivation of 4 \log_{10} (TCID₅₀s/50 μ l), and the time before the detection limit (a titer of 0.5 \log_{10} [TCID₅₀s/50 μ l]) was reached.

Inactivation with EI. The results of inactivation with EI are presented in Fig. 1A to C. The inactivation followed a similar pattern for all viruses: a rapid initial phase followed by a gradually slower inactivation, resulting in tailing of the inactivation curves.

The inactivation of PPV was the slowest among the viruses investigated. The inactivation rates within the first hour of inactivation were 2.4 and 3.5 for 5 and 10 mM EI, respectively. The time for inactivation of 4 \log_{10} units was 9 h with 5 mM EI and 3 h with 10 mM EI (calculated by interpolation). The detection limit was reached after 24 h of inactivation.

During the first 2 h the inactivation rate for BVDV was 2.3 for both concentrations of EI. The time for inactivation of 4 \log_{10} units was 1 h and 44 min for both concentrations of EI (calculated from the initial inactivation rate). The detection limit was reached at between 2 and 5 h of inactivation.

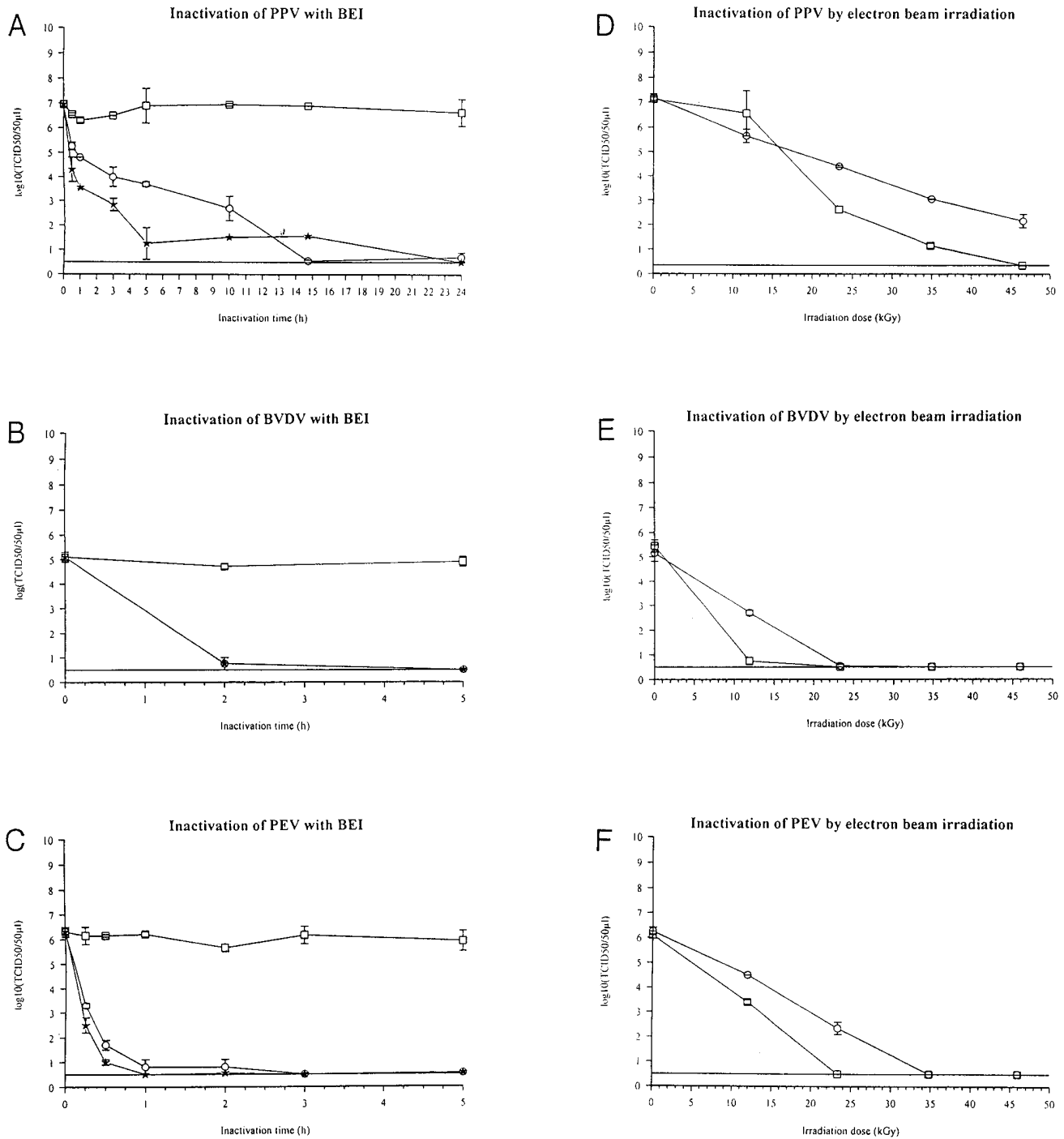


FIG. 1. (A) Inactivation of PPV with EI. □, 0 mM EI; ○, 5 mM EI; ★, 10 mM EI. (B) Inactivation of BVDV with EI. □, 0 mM EI; ○, 5 mM EI; ★, 10 mM EI. After 5 h no virus was detected in the inactivated samples. (C) Inactivation of PEV with EI. □, 0 mM EI; ○, 5 mM EI; ★, 10 mM EI. After 5 h no virus was detected in the inactivated samples. (D) Inactivation of PPV by electron-beam irradiation. ○, inactivation of frozen samples on dry ice; □, inactivation of liquid samples on ice-water. (E) Inactivation of BVDV by electron-beam irradiation. ○, inactivation of frozen samples on dry ice; □, inactivation of liquid samples on ice-water. (F) Inactivation of PEV by electron-beam irradiation. ○, inactivation of frozen samples on dry ice; □, inactivation of liquid samples on ice-water. The symbols and bars indicate the mean and range between two samples.

The highest inactivation rates were obtained for the inactivation of PEV. During the first hour the inactivation rates were 5.5 and 5.8 for 5 and 10 mM EI, respectively. The time for inactivation of 4 log₁₀ units was 24 min with 5 mM EI and 17

min with 10 mM EI (calculated by interpolation). The detection limit was reached after 2 h of inactivation.

Inactivation by electron-beam irradiation. The results of virus inactivation by electron-beam irradiation are presented in

Fig. 1D to F. The almost linear curves indicate a first-order inactivation of the virus. The apparent deviation from linearity of PPV in the liquid state is almost exclusively due to one of the two determinations of virus titer at 11 kGy. A similar effect was not seen for any of the other samples, and we ascribe this to experimental variation of this particular virus titration.

The D_{10} values (the irradiation dose necessary to inactivate 1 \log_{10} unit of virus) for the frozen samples were 11.8 kGy for PPV, 4.9 kGy for BVDV, and 6.4 kGy for PEV, calculated by linear regression (r^2 value for PPV was 0.93; the r^2 values were not calculated for PEV and BVDV due to the few datum points). The D_{10} values for the liquid samples with PPV, BVDV, and PEV were 7.7, 2.5, and 4.4 kGy, respectively, calculated by use of linear regression as above (r^2 for PPV = 0.99) (Table 1; Fig. 1D to F).

DISCUSSION

Inactivation with EI. EI is known to be an alkylating agent, reacting with, e.g., free sulfhydryl and amino groups, and has been used as an agent for inactivating viruses. It has been found to be more gentle to proteins in the samples than, e.g., formaldehyde or glutaraldehyde inactivation (4). The inactivation of viruses with EI and similar aziridines has been used extensively during the past 20 years and longer (1–3, 8, 16). However, the experimental conditions have varied, and therefore, the results are seldom directly comparable. Our initial inactivation rates (within the first hour of inactivation) for PEV of 5.5 and 5.8 \log_{10}/h for 5 and 10 mM EI, respectively, compare well to those in other studies of the family *Picornaviridae*, in which inactivation rates between 2.8 and 6.0 \log_{10}/h have been found by others using 4.6 mM EI (1, 6). Two different serotypes of foot-and-mouth disease virus inactivated with 10 mM EI showed inactivation rates of 10.2 and 5.4 \log_{10}/h , respectively (2). The experimental conditions in those studies were similar to the conditions in the present study, i.e., they were performed at 37°C in either serum or tissue culture medium with serum supplement.

The kinetics of EI inactivation was similar for all viruses: a rapid drop in infectivity, followed by a gradual decline in the inactivation rate. The cause of this tailing effect remains unknown, but it could be due to inhomogeneity of the virus preparation, with a fraction of virus being protected against the reaction with EI. Such an effect might be exerted by the masking of virus particles by proteins or lipids from the sample, preventing the diffusion of EI into the virus particle and thereby possibly the inactivating effect of EI on the nucleic acid. Such inhomogeneity of the samples is unpredictable and could very well be dependent on the exact virus species involved, as well as on the material in which it is present. The present experiments were conducted with serum spiked with cell culture-derived virus. Masking could be more pronounced if the virus has multiplied in the animal and possibly secreted into the bloodstream together with, e.g., cell debris. Such masking would influence the time necessary for inactivation by EI. Another reason for the tailing effect when using EI for inactivation could be a decrease in the concentration of EI during the inactivation process. Using the assay of May et al. (14) for determination of the EI concentration, we have been able to exclude this possibility (data not shown).

In previous studies the tailing of the inactivation curve has only been seen by Hassanain (8) during inactivation of African horse sickness virus. The lack of reported tailing might be explained by the fact that most of the experiments with EI inactivation of viruses have been limited to 1 to 5 h (1, 2, 8). In two studies in which the inactivation was carried out for 6 h,

TABLE 2. Some previously published data on virus inactivation by ^{60}Co irradiation

Reference	Medium	Virus family	D_{10} value (kGy)	
			Liquid samples	Frozen samples
Elliott et al. (7)	Human serum	<i>Arenaviridae</i>	1.89	3.12
		<i>Filoviridae</i>	1.47	2.17
			1.19	2.13
House et al. (9)	Bovine serum	<i>Bunyaviridae</i>		2.5
				3.5
		<i>Rhabdoviridae</i>		2.9
		<i>Picornaviridae</i>		5.0
				5.3
		<i>Togaviridae</i>		5.5
		<i>Reoviridae</i>		8.3
		<i>Paroviridae</i>	10.7	
Mahnel et al. (13)	Water	<i>Flaviviridae</i>	0.25	
		<i>Picornaviridae</i>	0.30	
			0.25	
		<i>Reoviridae</i>	0.35	
		<i>Parvoviridae</i>	0.40	
		<i>Poxviridae</i>	0.20	
		<i>Herpesviridae</i>	0.17	
	Water + 25% FCS	<i>Flaviviridae</i>	1.80	
		<i>Picornaviridae</i>	1.90	
			1.80	
		<i>Reoviridae</i>	3.00	
		<i>Parvoviridae</i>	4.50	
		<i>Poxviridae</i>	1.00	
		<i>Herpesviridae</i>	0.40	

one of them (16) had only four points on the curve and assumed linearity. The other study (3) showed a few more datum points, but although linearity was assumed, the data suggest a slight tailing of the curve within the last part of the inactivation. Such tailing has a very important impact on the estimation of the time needed for complete inactivation (i.e., no infectious virus in a batch of, e.g., serum).

Despite this difficulty of estimating the endpoint for complete inactivation, it is possible from our results to specify conditions for treatment of serum samples with EI leading to material which can be handled in an open laboratory with a minimal risk of spreading unwanted viruses.

Inactivation by electron-beam irradiation. In this study the D_{10} values found for samples irradiated in the liquid state were between 51 and 69% of the values for the corresponding frozen samples; i.e., a higher dose was needed to inactivate the same amount of virus when the samples were frozen. The same phenomenon was observed by Elliott et al. (7) when inactivating viruses of the families *Arenaviridae* and *Filoviridae*. This is to be expected since the mobility of reactive (virus-inactivating) free radicals generated by irradiation is higher in liquid material than in solid material (12, 17). Some previous studies on inactivation by irradiation are listed in Table 2 for comparison. Most of the previous experiments with virus inactivation by irradiation were made with γ -irradiation from ^{60}Co sources (7, 9, 13, 16, 17), but inactivation with electron-beam irradiation has been described as well (10). Because of different experimental conditions, these results are not directly comparable to the results achieved here or in the other experiments mentioned earlier. We do not expect the nature of irradiation

(γ or electron) to be the cause of any significant differences in D_{10} values.

The results of Mahnel et al. (13) indicate that a higher protein content results in higher D_{10} values. It is also seen that the D_{10} values for viruses of the families *Picornaviridae* and *Togaviridae* in the same experiment (9) were about the same, whereas members of the family *Parvoviridae* have D_{10} values approximately twice as high (9, 13), which is in agreement with our results.

Comparative aspects. The kinetics of the inactivation process with EI differs significantly from that of inactivation by irradiation. A first-order inactivation (with respect to virus concentration) will give a straight line in the semilogarithmic plots in Fig. 1. Inactivation by irradiation shows an almost perfect first-order inactivation for all three model viruses, whereas the chemical inactivation shows a pronounced decrease in the rate of inactivation with time, resulting in tailing of the curve. In contrast, the penetrating capacity of the electron-beam irradiation through biological material should ensure a safe inactivation, irrespective of the possible masking of virus particles by serum components and, furthermore, should also be applicable for inactivation of virus in other types of biological samples. However, large samples are problematic with regard to electron-beam irradiation, because the penetration is limited. The D_{10} values that we obtained are only valid when using similar penetration depths. Chemical inactivation requires addition of the inactivating reagent to each sample and subsequent incubation at 37°C. Therefore, this process might cause greater variation between samples than irradiation. Furthermore, chemical inactivation will presumably be problematic in clumpy or inhomogeneous samples, and in such cases irradiation might be preferable.

Considering the level of infectious virus possibly present in blood from animals with no signs of clinical disease, a reduction factor of 10^6 represents a margin of safety sufficient to ensure safe handling of possibly infected biological material in open laboratories. This reduction factor is also the criterion used in tests of the virucidal activities of disinfectants in suspension experiments according to the CPMP Notes for Guidance on Virus Validation Studies (5).

Furthermore, PPV belongs to the family *Parvoviridae*, the members of which exhibit extraordinary resistance to physicochemical reagents compared to the resistance of other viruses (15) (Table 2), which also has been confirmed in the present study. Therefore, parvovirus can be considered a suitable worst-case agent in inactivation experiments.

By using the criterion of a $6 \log_{10}$ reduction in the infectivity of PPV, the conditions of treatment of serum samples to obtain an adequately safe product will be inactivation with 5 mM EI at 37°C for 24 h. This period of time is about fivefold longer than the time necessary for inactivation of BVDV and PEV present at concentrations of 5.1 and $6.3 \log_{10}$ (TCID₅₀/50 μ l), respectively. Reduction of PPV infectivity as a reference thus ensures an even more efficient reduction of viruses with lower levels of resistance to physicochemical treatments.

For electron-beam irradiation a $6 \log_{10}$ reduction in PPV infectivity could be obtained by a dose of 35 kGy for liquid samples or a dose of approximately 55 kGy (extrapolated) for frozen samples. The choice of method should depend on the influence of the process on the parameters to be studied, e.g., antibody content, enzymes, or hormones. Data regarding an-

tibody activity have been published separately, showing different influences of inactivation in various assays (11).

In conclusion, all the issues mentioned above regarding sample type, state, size, possible contamination level, and influence on the analysis to be performed after inactivation should be taken into consideration when designing an inactivation protocol for a specific sample.

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