the existence of vacuoles only. Some other institutions use their own scoring system using several factors, such as spongiform vacuolization, gliosis or amyloid plaques in the lesion sites. The above judgments are sometimes problematic because these pathological observations must be performed by experienced investigators. To obtain more knowledge regarding the diagnosis of prion diseases, see the general review by Kretzschmar [27]. To avoid the above problems, rather than pathological evaluation, a recent trend is the *in vitro* detection of abnormal prion protein using BHs from inoculated animals by immunological procedures, such as WB.

Lee and colleagues published a report in 2004 regarding the relationship between *in vitro* and *in vivo* results [28]. Their data demonstrate that the partition of prion antigen in individual process samples detected by WB was consistent with that of infectious prions observed *in vivo*. These results suggest that it may be possible to evaluate prion partitioning during the manufacturing process by *in vitro* study using only WB. However, they also demonstrated that, in some cases, infectivity remains in a sample where the amount of abnormal prion protein is less than the limit of WB. One possibility for the phenomenon is the inappropriate use of antiprion antibodies for WB. Based on their results, the data obtained by *in vitro* study should be evaluated with the possibility that such study may have limitations for the detection of prion agents.

Cell culture to persistently maintain the infectious prion protein has been widely reported, and the development of a cell culture system for the quantitative detection of prion infectivity is now underway [21,29,30]. In the future, if a new assay system using a cell culture system demonstrates the same sensitivity as animal studies and good correlation, experiments to detect infectivity may be switched from animal systems to cell culture studies, as has occurred for some viruses used for virus validation studies.

Evaluation of the major manufacturing processes for prion removal

Concept for evaluation of manufacturing processes

Over the last 10 years, many reports have been published on prion removal during the manufacturing processes of plasma derivatives. To reassess these reports today, we must consider the technical background of the studies (discussed earlier). Before the European Medicines Agency published a statement in 2004, the strategy for establishing the study design was not as clear [105].

The log reduction factor of prion by a certain manufacturing process is often misunderstood as representing an unconditional absolute value; however, this factor is merely one of the indices for process evaluation. Therefore, based on the comprehensive grasp of all information, judgment should be made whether the process in question is effective, partially effective or ineffective for prion removal by individual manufacturing steps.

Several procedures are expected to remove abnormal prion protein. Fractionation with ethanol, PEG and glycine, and filtration with virus-removal and depth filter have been widely investigated, and many reports have been published on these steps (described later).

Fractionations during plasma protein purification steps

Many studies have already been performed on ethanol fractionation. Details of ethanol fractionation and prion partitioning during the manufacturing process have been described in several articles [31,32,104,105]. Among the ethanol fractionation processes, Fraction II + III, Fraction III and Fraction IV processes exhibited significant partitioning (Table 1). These are considered to be effective prion removal processes. For PEG and glycine fractionations, several studies have also been reported, as summarized in Table 2. PEG fractionation processes, including 8 and 11.5%, demonstrated good partition and are regarded as effective prion removal processes, such as ethanol fractionation processes, whereas glycine fractionation demonstrated less effective removal.

For column chromatography, various kinds of columns demonstrated a different tendency to partition prion protein (Table 3). All of the column chromatography processes reviewed here are not implemented specifically for the removal of prions, but for purification of the plasma protein of interest. In this sense, the removal of prion with these column chromatography processes is, if anything, a secondary effect. Therefore, the factors and/or parameters that are necessary to purify plasma proteins will differ from those for prion removal.

Virus-removal filters

Virus-removal filters were developed exclusively for effective removal of viruses during manufacturing processes, and had pore sizes of approximately

Table 1. Removal of prion by ethanol fractionation*.

Process (condition)	Spiking agent	Spike source	Before	Filtered (supernatant)	Paste (precipitate)	Clearance/reduction		Method	Ref.
						Filtered	*Paste*		
Cryoseparation	Human PrP^{vCJD}	BH	3.9	3.0	3.6	0.9	0.3	WB	[37]
	Human PrP^{sCJD}	BH	3.7	2.8	2.7	0.9	1.0	WB	
	Human PrP^{GSS}	BH	4.5	3.5	3.7	1.0	0.8	WB	
	Sheep PrP^{Sc}	BH	3.0	2.0	2.5	1.0	0.5	WB	
	Hamster PrP^{Sc} 263K	BH	5.9	4.7	5.3	1.2	0.6	WB	
	Hamster PrP^{Sc} Sc237	BH	2.3	2.0	2.1	0.3	0.2	CDI	[16]
		MF	3.4	3.2	2.9	0.2	0.5	CDI	
		CLDs	2.8	2.4	2.6	0.4	0.2	CDI	
		Purified	3.8	1.4	3.4	2.4	0.4	CDI	
	Hamster PrP^{Sc} 263K	TR	8.1	NA	6.0	NA	2.1	BA	[17]
	Mouse PrP^{GSS}	Blood	+ve	NA	+ve	NA	NA	BA	
	Hamster PrP^{Sc} 263K	BH	7.8	6.8	7.2	1.0	0.6	BA	[28]
			2.9	1.9	2.6	1.0	0.3	WB	
	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	<1.0	1.0	WB	[18]
Fraction I (8% ethanol)	Hamster PrP^{Sc} Sc237	BH	4.4	3.5	4.3	0.9	0.1	CDI	[16]
		MF	4.4	3.5	4.4	0.9	0.0	CDI	
		CLDs	3.7	3.0	3.7	0.7	0.0	CDI	
		Purified	4.1	1.0	3.9	3.1	0.2	CDI	
Fraction II + III (20% ethanol)	Hamster PrP^{Sc} 263K	BH	8.5	2.5	8.5	6.0	0.0	BA	[28]
			4.9	≤0.2	5.3	≥4.7	0.0	WB	
Fraction II + III (25% ethanol)	Hamster PrP^{Sc} Sc237	BH	4.1	0.5	4.1	3.6	0.0	CDI	[16]
		MF	4.9	1.8	4.9	3.1	0.0	CDI	
		CLDs	3.9	0.8	3.8	3.1	0.1	CDI	
		Purified	4.6	0.6	4.3	4.0	0.3	CDI	
Fraction I + II + III (19% ethanol), including filter aid	Hamster PrP^{Sc} 263K	BH	7.0	4.8	ND	2.2	NA	BA	[38]
			ND	ND	ND	3.8	NA	WB	

*Values given are expressed in log_{10} form. ‡Clearance was calculated by subtracting the effluent titers from the precipitate titers. §Including depth filtration. ¶Yunoki et al. Unpublished Data.
BA: Bioassay (in vivo study); BH: Brain homogenate; BSE: Bovine spongiform encephalopathy; CDI: Conformation-dependent immunoassay; CLD: Caveolae-like domain; GSS: Gerstmann–Sträussler–Scheinker syndrome; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; sCJD: Sporadic Creutzfeldt–Jakob disease; sMF: Sonicated MF; TR: Trypsin-treated minced brain; vCJD: Variant Creutzfeldt–Jakob disease; WB: Western blotting (in vitro study).

Table 1. Removal of prion by ethanol fractionation* (cont.).

Process (condition)	Spiking agent	Spike source	Before	Filtered (supernatant)	Paste (precipitate)	Clearance/reduction		Method	Ref.
						Filtered	*Paste*		
Fraction I + II + III (20% ethanol), evaluate from plasma	Hamster PrP^{Sc} 263K	TR	8.1	NA	6.1	NA	2.0	BA	[17]
	Mouse PrP^{GSS}	Blood	+ve	NA	+ve	NA	NA	BA	
Fraction I + II + III (21% ethanol)	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	1.3	<1.0	WB	[18]
Fraction III (17% ethanol)	Hamster PrP^{Sc} 263K	BH	ND	2.0	7.3	5.3[†]	0.0[†]	BA	[28]
			4.3	0.0	4.3	≥4.3	0.0	WB	
Fraction I + III (12% ethanol)	Hamster PrP^{Sc} 263K	BH	6.8	3.3	ND	3.5	NA	BA	[38]
			ND	ND	ND	4.5	NA	WB	
Fraction I + III (12% ethanol)	Mouse PrP^{BSE} 301V	MF	6.1	4.0	6.0	2.1	0.1	BA	[18,39]
	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥3.7	NA	WB	
Fraction IV (38% ethanol), high prion spiked	Hamster PrP^{Sc} Sc237	BH	4.1	0.9	3.4	3.2 (≥4.1)[§]	0.7	CDI	[16]
		MF	4.5	1.1	4.5	3.4 (≥4.5)[§]	0.0	CDI	
		CLDs	4.1	0.9	3.8	3.2 (≥4.1)[§]	0.3	CDI	
		Purified	4.6	2.4	4.4	2.2 (≥4.6)[§]	0.2	CDI	
Fraction IV (38% ethanol), low prion spiked	Hamster PrP^{Sc} Sc237	MF	3.7	0.8	3.5	2.9 (≥3.7)[§]	0.2	CDI	[16]
		CLDs	3.0	0.0	3.0	≥3.0 (≥3.0)[§]	0.0	CDI	
		Purified	3.2	0.0	2.8	≥3.2 (≥3.2)[§]	0.4	CDI	
Fraction IV (35% ethanol)	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥3.0	NA	WB	[18]
Fraction IV (40% ethanol)	Hamster PrP^{Sc} 263K	BH	7.0	4.0	ND	3.0	NA	BA	[38]
			ND	ND	ND	5.0	NA	WB	
Fraction IV_1	Hamster PrP^{Sc} 263K	BH	8.9	5.2	7.5	3.7	1.4	BA	[28]
			4.2	0.0	4.2	≥4.2	0.0	WB	
Fraction IV_4	Hamster PrP^{Sc} 263K	BH	7.6	3.0	7.2	4.6	0.4	BA	[¶]
			4.2	≤0.1	4.0	≥4.1	0.2	WB	
Fraction IV	Hamster PrP^{Sc} 263K	sMF	3.6	<0.6	3.8	≥3.0	0.0	WB	[¶]
Fraction IV_1 + IV_4 (40% ethanol) evaluate from plasma	Hamster PrP^{Sc} 263K	TR	8.1	NA	3.9	NA	4.2	BA	[17]
	Mouse PrP^{GSS}	Blood	+ve	NA	-ve	NA	NA	BA	

*Values given are expressed in log_{10} form. [†]Clearance was calculated by subtracting the effluent titers from the precipitate titers. [§]Including depth filtration. [¶]Yunoki et al. Unpublished Data.
BA: Bioassay (in vivo study); BH: Brain homogenate; BSE: Bovine spongiform encephalopathy; CDI: Conformation-dependent immunoassay; CLD: Caveolae-like domain; GSS: Gerstmann–Sträussler–Scheinker syndrome; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; sCJD: Sporadic Creutzfeldt–Jakob disease; sMF: Sonicated MF; TR: Trypsin-treated minced brain; vCJD: Variant Creutzfeldt–Jakob disease; WB: Western blotting (in vitro study).

Table 2. Removal of prion by polyethylene glycol, glycine and caprylate precipitation*.

Process (sample)	Spiking agent	Method	Before	Filtered (supernatant)	Paste (precipitate)	Clearance/reduction		Method	Ref.
						Filtered	*Paste*		
3% PEG (cryoprecipitate)	PrP^{vCJD}	BH	4.0	2.1	4.0	1.9	0.0	WB	[37]
	PrP^{sCJD}	BH	3.7	1.5	3.7	2.2	0.0	WB	
	PrP^{GSS}	BH	5.0	3.0	5.0	2.0	0.0	WB	
	Sheep PrP^{Sc}	BH	4.0	2.3	4.0	1.8	0.0	WB	
	Hamster PrP^{Sc} 263K	BH	6.3	4.1	6.1	2.2	0.2	WB	
3% PEG (cryoprecipitate)	Hamster PrP^{Sc} 263K	BH	7.2	5.0	7.2	2.2	0.0	BA	[28]
			5.2	2.2	4.9	3.0	0.3	WB	
8% PEG (IVIG)	Hamster PrP^{Sc} 263K	sMF	2.5	<0.1	3.2	≥2.4	0.0	WB	¶
		MF	2.5	<0.1	2.5	≥2.4	0.0	WB	
		sMF	Prob. +ve	Prob. +ve	ND	NA	NA	BA	
11.5% PEG (Fraction IV_1 precipitate)	PrP^{vCJD}	BH	4.0	0	4.2	≥4.0	0.0	WB	[37]
	PrP^{sCJD}	BH	3.0	0	2.9	≥3.0	0.1	WB	
	PrP^{GSS}	BH	4.0	0	4.0	≥4.0	0.0	WB	
	Sheep PrP^{Sc}	BH	3.5	0	3.5	≥3.5	0.0	WB	
	Hamster PrP^{Sc} 263K	BH	5.8	0	5.7	≥5.8	0.1	WB	
11.5 % PEG (Fraction IV_1 precipitate)	Hamster PrP^{Sc} 263K	BH	ND	≤1.1	6.5	≥5.4‡	0.0‡	BA	[28]
			4.9	0	4.6	≥4.9	0.3	WB	
Glycine (cryoprecipitate)§	Hamster PrP^{Sc} Sc237	MF	3.1	1.4	2.3	1.7	0.8	CDI	[16]
		Purified	3.8	0.5	3.1	3.3	0.7	CDI	
SD+8%Glycine (fibrinogen)	Hamster PrP^{Sc} 263K	sMF	3.0	2.7	3.5	0.3	0.0	WB	¶
SD+15%Glycine (Factor VIII)	Hamster PrP^{Sc} 263K	MF	2.5	2,2	1.5	0.3	1.0	WB	¶
		sMF	2.5	2.9	1.5	0.0	1.0	WB	
Caprylate precipitation/cloth filtration (Fraction II + III suspension/IVIG)	Hamster PrP^{Sc}	ND	ND	ND	ND	ND	2.9	WB	[40]
		ND	ND	ND	ND	ND	3.3	BA	

Values given are expressed in log_{10} form. ‡Clearance was calculated by subtracting the effluent titers from the precipitate titers. §Cryoprecipitate after $Al(OH)_3$ adsorption. ¶Yunoki et al. Unpublished Data. BA: Bioassay (in vivo study); BH: Brain homogenate; CDI: Conformation-dependent immunoassay; GSS: Gerstmann-Sträussler–Scheinker syndrome; IVIG: Intravenous immunoglobulin; MF: Microsomal fraction; Prob. +ve: Probable positive; PrP: Prion protein; NA: Not applicable; ND: Not determined; Sc: Scrapie; sCJD: Sporadic Creutzfeldt–Jakob disease; sMF: Sonicated MF; vCJD: Variant Creutzfeldt-Jakob disease; WB: Western blotting (in vitro study).

Table 3. Removal of prion by column steps*.

Process	Sample	Spiking agent	Method	Before	Pass	Eluate	Retained	Reduction (clearance) for product fraction	Method	Ref.
DEAE Tyoperl 650M	SD contained Factor VIII	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	ND	≥3.5‡ 3.1§	WB	[18]
		Mouse PrP^{BSE} 301V	MF	8.7	ND	<5.9‡ 6.1§	7.6	≥2.9‡2.7§	BA	[41]
DEAE-sepharose	Factor IX	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	ND	3.0	WB	[18]
Heparin-sepharose	SD contained Factor IX	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	ND	1.4	WB	[18]
S-sepharose	SD contained thrombin	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	ND	2.9	WB	[18]
MoAb	Factor IX	Hamster PrP^{Sc} 263K	dMF¶	3.7	3.7	1.3	NA	2.4	WB	#

Values are expressed in log_{10} form. ‡Fibrinogen fraction. §Factor VIII fraction. ¶SD (0.3% TNBP and 1%Tween 80) treated. #Yunoki et al. Unpublished Data.

BA: Bioassay (in vivo study); BSE: Bovine spongiform encephalopathy; DEAE: Diethylaminoethyl; dMF: Detergent-treated MF; MF: Microsomal fraction; MoAb: Monoclonal antibody; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; SD: Solvent and detergent; WB: Western blotting (in vitro study)

15–35 nm depending on the filter type. For example, 15N (15±2 nm), 20N (19±2 nm) and 35N (35±2 nm) of Planova filters (Asahi Kasei Medical Co., Ltd., Tokyo, Japan); DV20 (>3-logs reduction of virus particles >20 nm in diameter, and >6-logs reduction of virus particles >50 nm in diameter); and DV50 (>6-logs reduction of virus particles >50 nm in diameter) of DV filters (Pall Co., NY, USA); and Viresolve70 (filtration of molecules with <70 kDa) (Millipore Co., Billerica, MA, USA). Although the filters were originally used for the removal of viruses, it is expected that they may also be applicable for the removal of prions. To date, only a few reports have been published on the prion removal capacity of virus-removal filters. In this review, we refer only to reports in which the name of the filter is specified (Table 4).

In 2005, Silveira and colleagues reported that prion protein particles with a particle size of 17–27 nm retain prion infectivity in an *in vivo* study [33]. However, prion particles can be much larger and 17–27 nm particles appear to be at the low end of size distribution [34]. Using this estimated size of the minimum infectious particle, we can infer useful information from the study results on parvovirus partitioning, because the particle size (20–26 nm) of the virus is similar to that of prions. Virus-removal filters with a nominal pore size of 15 nm can remove canine parvovirus and parvovirus B19 (B19) effectively [19,35,36]. Therefore, virus removal filters with 15-nm pore size should be useful for prion removal. We obtained evidence that scrapie prion could also be removed effectively by a 15-nm filter, at least when assayed using WB, although infectivity in the filtrate remained when we inoculated hamsters [Yunoki and colleagues, Unpublished Data].

However, many plasma products cannot be filtered with this filter. In fact, even for the evaluation of virus-removal filters for prion removal, there are several technical issues to be noted. Most of the problems associated with the 15-nm filter occur due to clogging of the filter by spiking materials. Clogging often prolongs the filtration time and renders the filter unable to process the required loaded amount per unit surface area of the filter (termed deviation).

Table 4. Removal of prion by virus filters*.

Process	Sample	Spiking agent	Method	Before	Filtered	Retained	Clearance/reduction		Method	Ref.
							Filtered	*Retained*		
VireSolve180 (Millipore)	0.5% immunoglobulin	Hamster PrP^{Sc} 263K	dsBH**	6.4/6.9/6.9	<3.9/<3.9/<3.9	5.9/6.4/5.9	≥2.5/≥3.0/ ≥3.0	0.5/0.5/ 1.0	WB	[42]
Planova 75N (Asahi)	PBS	Hamster PrP^{Sc} 263K	MF	3.5/4.2	<1.0/<1.0	ND/ND	≥2.5/≥3.2	NA/NA	WB	[19]
			sMF	4.2/4.2	2.4/2.4	ND/ND	1.8/1.8	NA/NA	WB	
Planova 35N (Asahi)	2% albumin	Mouse PrP^{Sc} ME7	BH	8.13	3.20	ND	4.93	NA	BA	[43]
			dBH‡	7.32	5.71	ND	1.61	NA	BA	
	PBS	Hamster PrP^{Sc} 263K	MF	3.5/4.2	<1.0/<1.0	ND/ND	≥2.5/≥3.2	NA/NA	WB	[19]
			sMF	4.2/4.2	<1.0/<1.0	ND/ND	≥3.2/≥3.2	NA/NA	WB	
	IVIG	Hamster PrP^{Sc} 263K	sMF	3.2/2.5	0.8/0.8	ND/ND	2.4/1.7	NA/NA	WB	
	Haptoglobin	Hamster PrP^{Sc} 263K	sMF§§	2.4	<1.0	ND	≥1.4	NA	WB	##
Planova 20N (Asahi)	IVIG	Hamster PrP^{Sc} 263K	sMF	6.8/6.8	4.8/4.3	ND/ND	2.0/2.5	NA/NA	WB	##
	Haptoglobin		dsMF‡‡	6.7/6.1	4.8/4.7	ND/ND	1.9/1.4	NA/NA	WB	
Planova 15N (Asahi)	2% albumin	Mouse PrP^{Sc} ME7	BH	8.13	<2.26	ND	>5.87	NA	BA	[43]
			dBH‡	7.32	<3.11	ND	>4.21	NA	BA	
	PBS	Hamster PrP^{Sc} 263K	MF	3.5/4.2	<1.0/<1.0	ND/ND	≥2.5/≥3.2	NA/NA	WB	[19]
			sMF	4.2/4.2	<1.0/<1.0	ND/ND	≥3.2/≥3.2	NA/NA	WB	
	Antithrombin III	Hamster PrP^{Sc} 263K	dMF§	3.1/3.1	0.0/0.0	ND/ND	≥3.1/≥3.1	NA/NA	WB	
			sMF§§	3.6	<0.8	NA	≥2.8	NA	WB	##
				Prob. +ve	Prob. +ve	NA	NA	NA	BA	
	Thrombin	Hamster PrP^{Sc} 263K	dsMF†‡	3.7/3.7	<0.2/<0.2	ND/ND	≥3.5/≥3.5	NA	WB	##
Planova 10N (Asahi)	2% albumin	Mouse PrP^{Sc} ME7	dBH‡	7.32	<3.52	ND	>3.80	NA	BA	[43]
DVD + DV50 + DV20 (Pall)	Globulin	Human CJD^{Res}	BH	ND	ND	ND	3.0–3.3¶ >2.3¶¶ >1.6#	NA	WB	[44]

**Values given are expressed in log_{10} form. **Sonicated BH including 0.1% lysolecitin and followed by 0.45-0.22-0.1 µm serially filtered. ‡Including 0.5% sarcosyl. ‡‡SD (0.3% TNBP and 1% Tween 80) treated and followed by sonication. §Including 0.1% sarcosyl. §§0.22 µm filtered. ¶1:10 BH spiked. ¶¶1:100 BH spiked. #1:500 BH spiked. ##Yunoki et al. Unpublished Data.*

BA: Bioassay (in vivo study); BH: Brain homogenate; CJD: Creutzfeldt–Jakob disease; dBH: Detergent-treated BH; dMF: Detergent-treated MF; dsBH: Detergent treated and sonicated BH; dsMF: Detergent-treated and sonicated MF; IVIG: Intravenous immunoglobulin; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PBS: Phosphate buffered saline; Prob. +ve: Probable positive; PrP: Prion protein; Res: Protease resistant; Sc: Scrapie; sMF: Sonicated MF: WB: Western blotting (in vitro study).

Such deviations from standard manufacturing conditions should be carefully considered, without overestimation, for the acceptability of such studies. In such instances, the smaller pores tend to clog first, which diverts more of the flow through the larger pore sizes, thereby changing the effective pore size of the filter. For the purposes of risk assessment, it appear to be appropriate to assume that, even with a 15-nm filter, leakage of only a small amount of prion (less than the limit of *in vitro* detection methods) may occur, as often found in parvovirus studies.

Process evaluation of virus-removal filters must be performed considering the above points. Since the published study design for process evaluation of prion removal is often unclear, reports must be reassessed carefully to exclude the possibility that prion clearance has been adversely affected. It is generally accepted that the basic principle of virus-removal filtration is size exclusion. Depending on the filtration conditions, the performance of the filters may vary. At present, the 15-nm filter is the most effective for prion removal, although filters with a pore size of 20 nm or more can also remove prions to some extent. However, it should be understood that, in theory, all filters may leak infectious prions into the filtrate. Owing to clogging and other problems, the percentage spiking may need to be reduced in many cases. Consequently, the removal factor tends to be lower, which should also be considered carefully.

In the future, it may be necessary to develop virus-removal filters with smaller pore sizes. However, smaller pore size may also be more problematic because not only contaminants but also the desired plasma protein may be captured by the filter. Therefore, some other measures, such as improvement of filtration efficiency with 15-nm pore size filters, or identifying suitable filtration conditions for larger pore filters (e.g., by inducing prion aggregates at low pH prior to filtration) may become important.

Depth filters

The basic principle of depth filtration is to remove and/or capture impurities by filtration through a multilayered matrix structure. Pore sizes of depth filters usually range from 0.1 to 5.0 µm. Some improved filters are electrically charged to capture impurities more efficiently. Depth filtration was originally introduced for the clarification of protein solutions and, thus, was not intended specifically for prion removal. Therefore, contaminating prion agents are removed as a secondary effect during purification of the desired protein. Considering the pore size of depth filters, the filtration mechanism for prion removal cannot be simply explained only by size exclusion, because the charge of the depth filter could also be involved in prion removal. However, certain conditions may result in significant prion aggregation (e.g., low pH), and under such conditions, removal by size exclusion may be the primary mechanism of removal. To date, only a small number of reports have been published on prion removal by depth filtration. The results of the studies are summarized in Table 5 (reports where the name of the filter was not specified are not included).

According to a report that even prion particles of 17–27 nm in diameter still remain infective [33], such infectious prions should theoretically pass through depth filters. However, a number of reports highlight that, in some cases, abnormal prion was actually removed by depth filtration. Even with an identical filter, filtration efficiency varies significantly depending on filtrating conditions. Therefore, depth filtration cannot guarantee consistent prion removal in each instance, but rather conditions may need to be optimized for each product. Thus, in actual manufacturing, conditions for depth filtration must be defined with strict process controls in order to ensure effective prion removal. Furthermore, any evaluation study of the process should be designed very carefully while considering the processing conditions. The correlation of prion partitioning via depth filtration in model systems and vCJD/CJD systems remains to be confirmed.

There are several technical problems to be noted when we evaluate depth filtration. The biggest problem is that the mechanism of prion removal has not currently been clarified. Size exclusion alone cannot explain the mechanism to remove prions by depth filters. Electrically charged matrices may adsorb prions, but this has not been investigated in detail. Therefore, process evaluation for depth filtration may require very careful design, since there is a possibility of behavioral differences between model and vCJD/CJD systems during filtration. In addition, it is difficult to obtain depth-filter materials of uniform quality. This particular problem must be improved for the usefulness of the depth filtration process to remove prions at the manufacturing level.

In general, from the results of previous studies, depth filtration may be effective, to some extent, for prion removal, as Foster and coworkers