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研究報告の概要

1．血液製剤のプリオンに対するリスク（ｖCJDの発生状況）
・BSE の発生は少なくとも英国では減少しつつある。BSE に感染したウシからのvCJD伝播の危険性は低下傾向にある。他方、輸血によるヒトーヒト伝播の危険性は依然として続いている。

2．血漿分画製剤の安全対策
・血液中のプリオン検出のための高感度なスクリーニング方法は現在のところまだない。従って、今のところ、血漿分画製剤のプリオンに対する安全対策は、主として地理的条件によるドナーの排除と製造工程でのプリオンの除去である。

3．プリオン除去試験の留意点と試験結果
・プリオン除去のための個々の製造工程は、実際の製造条件を実験室での条件にスケールダウンさせ、確立されているスクレイピー株をモデル系として用いて通常は評価されている。血液中のプリオン蛋白の存在形態が不明なので、評価実験のためのスパイク材料としてのプリオンの調製方法は注意深く考慮しなければならない。
・プリオンアッセイする方法としては2 つの異なる方法がある。その2 つとはウエスタンブロッティングなどの方法でプリオンを検出するin vitro 試験と、サンプルを動物に接種することによって感染性プリオンを検出するin vivo 試験である。ごく少量のプリオン、例えばウエスタンブロッティング法の限界（例えば、抗体の不適切な使用など）によって陰性の結果を示すような量のプリオンであっても、サンプル中に感染性を検出することができることもある。従って、製造工程評価には、試験方法の間の相異を注意深く考慮しなければならない。
・製造工程条件の如何によって、除去パターンが類似したものであっても、その製造工程のプリオン除去能は変わりうる。従って、評価試験のデザインはきわめて重要である。各製造工程は個別に評価しなければならない。
・現在のところ、血漿分画製剤の製造工程のうち、エタノール分画、PEG 分画、カラムクロマトグラフィー、ウイルス除去膜およびデプスろ過膜でのろ過、その他がプリオン除去に有効であると考えられている。
・製造工程で安定してプリオン除去を行うためには、プリオン除去に寄与する複数の製造工程を組み合わせることが必要である。
・これらのことから、プリオンのスクリーニングと除去のための新しい技法の開発と製造工程評価方法の改良が切望される。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
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2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
血漿分画製剤の製造工程におけるプリオン除去に関する総説論文であり、文献中の表に弊社が行なったプリオン除去試験結果を提示している。 これまで血漿分画製剤によってvCJDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	vCJD に関連する情報については、今後も注視することとする。

REVIEW

Possible removal of prion agents from blood products during the manufacturing process

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Blood products prepared from human blood theoretically risk contamination with infectious pathogens. Since recent reports now confirm the likely transmission of pathogenic prions through blood transfusion, effective measures to prevent transmission are required globally, although the prevalence of variant Creutzfeldt–Jakob disease outside of the UK is extremely low. Many studies evaluating the manufacturing process have been conducted for the potential removal of the prion protein from plasma derivatives. In this review, we discuss the possibility of removing prions via several processing steps, especially depth and virus-removal filtration. Through a discussion of the limitations and issues associated with such studies, we hope our review will be of help for better study design in the future.

Keywords: blood products, blood transfusion, bovine spongiform encephalopathy, clearance study, depth filter, prion, variant Creutzfeldt–Jakob disease, virus-removal filter

future medicine

The onset of illness in the first case of variant Creutzfeldt–Jakob disease (vCJD), which was published in 1996, occurred in early 1994. vCJD most probably results from the consumption of beef products contaminated by central nervous system tissue derived from bovines infected with bovine spongiform encephalopathy (BSE), which began in the UK sometime prior to 1986 [1]. The worldwide incidence of BSE and vCJD was approximately 190,000 animals and 185 patients (including 159 patients in the UK), respectively, in December 2005. In the UK, where the highest incidence of BSE and vCJD was reported, their peak incidences were observed in 1992 and 2000, respectively. Since these peaks, the incidence in the UK has decreased gradually [101]. There is also the possibility of continuing person-to-person transmission of vCJD through certain forms of healthcare (e.g., through surgery, blood transfusion or treatment with plasma products). Therefore, it is essential to maintain and promote active surveillance of vCJD and CJD (hereafter vCJD/CJD) to evaluate potential transmission by this route [2–4,102,103].

Since blood products are prepared from human blood, they may involve risks of contamination with infectious pathogens including pathogenic prions. Therefore, besides the measures for ordinary pathogens, effective measures implemented globally to prevent transmission of pathogenic prions (especially to prevent vCJD) are also required. Measures to prevent contamination by viruses/prions in plasma derivatives consist of donor plasma sourcing/screening and the elimination of viruses/prions during the manufacturing process. Currently, geographical deferral of blood donors before donation is the only method of identifying donors at higher risk for vCJD, since sensitive and rapid screening methods for prions in blood with the ability to handle many specimens have not currently been developed. The risks to a recipient from fractionated plasma products are probably less than from blood transfusion, not least owing to potential removal during the manufacturing process, as well as the volume of material to which an individual is exposed, which are likely to be important determinants of the level of risk [2]. However, the pooling of plasma donations and the large number of recipients from any given plasma pool complicates any calculations of residual risk.

Little is known regarding the native form of prion protein in blood, especially in plasma, although this information is essential for evaluating the safety of blood products. Under these circumstances, the regulatory agencies of several countries issued guidelines regarding measures to be taken to prevent or reduce the potential for prion transmission through pharmaceutical products. Manufacturers have implemented their measures according to these guidelines [5–7,104–107]. Recently, Brown reviewed prion infectivity in blood, prion removal by the manufacturing process and the current status of the development of prion-screening methods [8]. The removal of prions by partitioning during the manufacturing process is expected to be a practical and effective approach, particularly because effective methods for prion inactivation that are applicable to the manufacture of protein products, have not been developed to date. At present, the removal of prions by physical means is the main measure towards preventing prion contamination. There are numerous reports describing the partitioning and possible removal of prion

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during the manufacturing process. Processes that possibly remove prions include fractionation, using ethanol and/or polyethylene glycol (PEG), and filtration through virus-removal and/or depth filters. Many studies have been performed on the efficacy of ethanol fractionation processes to remove prion and demonstrated similar removal ability, regardless of differences in study conditions and the research institutions. By contrast, studies on depth filtration revealed that the efficacy of depth filters to remove prions is highly dependent on the composition of the solution and/or characteristics of the filters. In this review, we discuss the possibilities and limitations of several manufacturing steps to remove prions using evaluation data from several manufacturers. We hope our discussion will be of help to determine better study design in the future. Cleaning (inactivation) of equipment should also be considered in parallel to the removal of prions during the manufacturing process. However, we will not discuss this area, and the reader is referred to Lee and colleagues who have already discussed this matter in details [9,10,108].

Study design for prion removal ability

Procedures for safety evaluation of plasma derivatives for prions are basically similar to those used for viruses. Regarding the virus clearance study (also termed virus validation study), the first regulatory guidance was issued by the European Community in 1989. Since then, manufacturers have performed virus clearance studies in accordance with this guideline as well as other related guidelines. On the other hand, the European Medical Agency issued guidelines regarding prion clearance in 2004 [104]. Their guideline was largely based on the concept of the virus validation guidelines [109], although care was taken to refer to such studies as investigational, as opposed to validation.

The following should be considered when performing prion-clearance studies.

Model agents

The main purpose of the clearance study is to assess and identify the manufacturing process(es) that can be considered to be effective in eliminating prions using various model agents, such as scrapie. Based on the results with model agents, the partitioning of specific human pathogenic agents, such as vCJD, can be speculated. In this sense, the purpose of the study is to evaluate the risk of the pathogen itself. However, if the pathogen in question is significantly different from the model agent, partitioning of the specific human pathogen may yield incorrect data. Therefore, for clearance studies and related studies, it is indispensable to carefully consider the possible differences between the pathogens in question and model agents.

Assay method

The detection of protease-resistant prion antigen (*in vitro* study) is performed as the first step (e.g., by western blotting [WB], and then, for certain process(es), an infectivity assay using animals (*in vivo* study) is also recommended. The *in vivo* assay remains the only possible option to confirm the quantitative infectivity titration of prions following inoculation of samples into animals. It should also be understood that, in some instances, there could be some discrepancy between *in vitro* and *in vivo* study results.

Simulation of manufacturing process

It may be impossible to exactly simulate all of the manufacturing process parameters on a laboratory scale. For experimental downscaling, it is impossible to use equipment and conditions that are identical to the actual manufacturing process. First, prion proteins are added intentionally, thereby changing the matrix. Second, the processes are downsized to laboratory scale. Therefore, in some instances, the best result that can be achieved is to approximate the behavior of the manufacturing process. Furthermore, it is important not to overestimate the prion removal ability of the manufacturing processes based on the data obtained under scaled-down conditions.

Native form of abnormal prion proteins

The native form of the abnormal prion proteins in blood is still unknown. Abnormal prion proteins in blood remain largely undetermined and may exist as various forms with different particle sizes or aggregation states. Therefore, the preparation method of the prion material used as a spiking agent for process evaluation studies would be an important factor.

Choice of spiking prion agent

Of the various prion diseases, vCJD is the primary concern for manufacturing plasma derivatives. It is difficult to use tissue samples taken from vCJD/CJD patients for the evaluation of manufacturing processes. Therefore, in general, laboratory strains of scrapie (e.g., 263K and ME7) and those of BSE (e.g., 301V) are used in place of vCJD materials [11–15].

Spiking materials are prepared from the brain of infected animals, and there are several methods of preparation. Brain homogenate (BH) has been widely used for a long time, because BH is easy to prepare and contains a high titer of infectivity. However, the uniformity of particle size is not ideal for evaluation purposes. The microsomal fraction (MF) is partially purified from BH, and the titer of MF may be slightly lower than BH. In addition, caveolae-like domains and semipurified scrapie prion protein (purified fibrils) may also be used in spiking studies. The partitioning of abnormal prion protein prepared by different preparations behaves in a similar manner, with the exception of purified fibrils [16–18]. Although the appropriateness of the materials used for spiking experiments was described in reported studies, there has been no discussion of their particle size.

In 2003, Yunoki reported that the particle size of MF used as spiking material was 800 nm on average, and that the particle size of MF fell to less than 220 nm through high-power sonication or detergent treatment [19]. At present, researchers tend to add steps such as sonication, detergent treatment and prefiltration in order to prepare MF or BH for spiking studies. However, discussion regarding the appropriateness of spiking materials is limited because the status of the abnormal prion protein in blood is not currently clear. Various preparation methods have been used in the above reports. Therefore, it is necessary to carefully consider such preparation methods used in individual reports in order to evaluate the removability of prion agents during the process.

As our knowledge regarding the form and characteristics of abnormal prion protein in blood accumulates, these problems are expected to be resolved. A new preparation method utilizing exosomes might be proposed because a recent report described that prions also exist in association with exosomes [20]. In addition, spiking materials derived from cultured cells producing abnormal prion protein may become one possible source for spiking prion material [21]. If strains of vCJD/CJD with a high titer are prepared in cultured cells, it would become possible to use such materials as spiking agents.

Evaluation methods

To estimate the prion levels in samples, two different methods are used: one to detect abnormal prion protein in samples by WB, conformation-dependent immunoassay or enzyme-linked immunosorbent assay (*in vitro* study) [16,22–24]; and the other to detect pathogenic prion protein by inoculating animals with the samples (*in vivo* study). Although WB is widely used, assay conditions are different in every laboratory and there is no standard protocol for the assay. In general, samples taken from manufacturing processes contain plasma proteins at a high level, which sometimes disturb the specific detection of a small amount of prion by WB. To avoid these problems, optimization of assay conditions and/or adjustment of pretreatment conditions for each sample are necessary. Due to these assay variables, the sensitivity of the assay not only differs in every laboratory, but even from sample to sample. When an identical sample is used for comparison, WB generally gives a lower sensitivity than the *in vivo* method. To improve the sensitivity and specificity of WB, several methodologies have been performed; for example, the elimination of plasma proteins that disturb the assay, by heating at 80°C before proteinase K treatment, followed by ultracentrifugation to concentrate prion [25].

For viruses, it is required that clearance studies are performed following the detection of infectivity of process samples as an indicator. However, for prions, according to the guidelines [104,105,109], infectivity experiments *in vivo* are not always required for processes where the relationship between *in vivo* and *in vitro* results has been established. For processes where the relationship is unknown, such as new processes, it may be necessary to check the infectivity of samples *in vivo* following initial testing of samples *in vitro*.

In general, experimental conditions for the detection of infectivity using animals differ at every institution. Even if animals are the same species and age, the amount of inoculums given to the animals and/or incubation period of the animals after inoculation may be different. Symptoms of prion diseases are monitored by clinical signs during the incubation period in animals, although the monitoring procedure may also differ at each institution. Some institutions monitor abnormal behavior only, whereas other institutions use a scoring system for monitoring. However, such observation of clinical signs may not be regarded as a definitive indicator of disease. Classically, histopathology has been used to confirm disease lesions in brain samples taken from infected animals [26]. Similarly, different criteria in pathological examinations are used by institutions to determine prion lesions. Some institutions judge prion diseases by

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

Table 5. Removal of prion by depth filters*.

Step	Sample	Spiking agent	Method	Before	Filtered	Retained	Clearance/reduction		Method	Ref.
							Filtered	*Retained*		
Seitz Supra P80‡ (Pall)	Supernatant of 8% ethanol precipitation	Hamster PrP^{Sc} Sc237	BH	3.5	3.4	ND	0.1	NA	CDI	[16]
			MF	3.5	3.6	ND	0.0	NA	CDI	
			CLDs	3.0	3.0	ND	0.0	NA	CDI	
			Purified	1.0	1.0	ND	0.0	NA	CDI	
	Supernatant of 38% ethanol precipitation		BH	0.9	0.0	ND	≥0.9	NA	CDI	
			MF	1.1(H) 0.8(L)	0.0(H) 0.0(L)	ND	≥1.1(H) ≥0.8(L)	NA	CDI	
			CLDs	0.9	0.0	ND	≥0.9	NA	CDI	
			Purified	2.4	0.0	ND	≥2.4	NA	CDI	
AP 20 (Millipore)	Supernatant I + III	Mouse PrP^{BSE} 301V	MF	7.0(H) 4.0(L)	4.6(H) 3.4(L)	ND	2.4(H) 0.6(L)	NA	BA	[39]
		Hamster PrP^{Sc} 263K	MF	ND	ND	ND	<1.0(L)	NA	WB	
	Supernatant I + III	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	<1.0	NA	WB	[18]
Seitz KS 80 (Pall)	Supernatant I + III (AP20 filtered described as above)	Mouse PrP^{BSE} 301V	MF	6.3(H) 4.6(L)	≤3.2(H) ≤3.2(L)	ND	≥3.1(H) ≥1.4(L)	NA	BA	[39]
	Resuspended fraction V	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥4.9	NA	WB	[18]

Note: data are referred from indicated reports and partially altered.

**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. The spiking agent was added before precipitation and CDI was performed after precipitation and after depth filtration. §Salt strip 1 M NaCl followed by 2 M. ¶0.22 µm-filtered prior depth filter. #0.22 µm filtered. ##Yunoki et al. Unpublished Data.*

BA: Bioassay (in vivo study); BH: Brain homogenate; BSE: Bovine spongiform encephalopathy; CDI: Conformation-dependent immunoassay; CDLs: Caveolae-like domains; dsBH: Detergent treated and sonicated BH; E: Early filtrate; H: High titer of prion on assay of spiked feed stock; IVIG: Intravenous immunoglobulin; L: Low titer of prion on assay of spiked feed stock; La: Late filtrate; M: Middle filtrate; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; sMF: Sonicated MF; WB: Western blotting (in vitro study).

Table 5. Removal of prion by depth filters* (cont.).

Step	Sample	Spiking agent	Method	Before	Filtered	Retained	Clearance/reduction		Method	Ref.
							Filtered	*Retained*		
Seitz K200P (Pall)	Resuspended fraction II	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥2.8	NA	WB	[18]
Delipid 1 (Cuno)	Clarified fraction V suspension	Hamster PrP^{Sc} 263K	MF	ND	ND	ND	≥3	NA	WB	[18]
Zetaplus Delipid Plus (Cuno)	IVIG	Hamster PrP^{Sc} 263K	sMF	2.8	2.5	ND	0.3	NA	WB	##
			sMF#	4.1	2.7	ND	1.4	NA	WB	
			MF	2.8	1.1	ND	1.7	NA	WB	
Zetaplus 30LA (Cuno)	IVIG	Hamster PrP^{Sc} 263K	sMF	2.8	<0.4	ND	≥2.4	NA	WB	##
			MF	3.5	<0.4	ND	≥3.1	NA	WB	
Zetaplus 90SP (Cuno)	Supernatant III	Hamster PrP^{Sc} 263K	dsBH‡	7.9	<2.7(E) 4.2(M) 4.3(La)	7.4§	>3.3	0.5	WB	[45]
				7.1 <4.1¶	<2.7(E) <2.1(M) <2.0(La)	<3.6§	NA	NA	WB	
	Tris-buffered saline			7.0	6.2(E) 6.7(M) 6.0(La)	4.8§	0.1	2.2	WB	
Zetaplus 90LA (Cuno)	Clarified fraction V suspension	Hamster PrP^{Sc} 263K	sMF	3.5	<0.4	ND	≥3.1	NA	WB	##
			sMF#	4.5	<0.9	ND	≥3.6	NA	WB	
			MF	3.5	<0.4	ND	≥3.1	NA	WB	

Note: data are referred from indicated reports and partially altered.

**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. The spiking agent was added before precipitation and CDI was performed after precipitation and after depth filtration. §Salt strip 1 M NaCl followed by 2 M. ¶0.22 µm-filtered prior depth filter. #0.22 µm filtered. ##Yunoki et al. Unpublished Data.*

BA: Bioassay (in vivo study); BH: Brain homogenate; BSE: Bovine spongiform encephalopathy; CDI: Conformation-dependent immunoassay; CDLs: Caveolae-like domains; dsBH: Detergent treated and sonicated BH; E: Early filtrate; H: High titer of prion on assay of spiked feed stock; IVIG: Intravenous immunoglobulin; L: Low titer of prion on assay of spiked feed stock; La: Late filtrate; M: Middle filtrate; MF: Microsomal fraction; NA: Not applicable; ND: Not determined; PrP: Prion protein; Sc: Scrapie; sMF: Sonicated MF; WB: Western blotting (in vitro study).

summarized study results on the depth filtration of prions in 2004 [31]. However, since the mechanism and consistency of the filtration system is not clear, an evaluation study should be performed using individual manufacturing processes. Thus, it is clear that further knowledge is required regarding the depth filtration systems.

Conclusion

The current status of process evaluation methods for prion removal during manufacturing processes of plasma derivatives was introduced and discussed in this review. Problems to be stressed are:

- The form of pathogenic prions in blood is not clear, which in turn raises questions about the appropriateness of prion materials (spiking materials) used for evaluation studies;
- Preparation methods of prion materials for studies are very important to consider;
- Although some data are already available, the equivalency between model systems and vCJD/CJD must be strengthened.

The current status of the problems and the limitations of measures taken to overcome the problems are described. In addition, the difficulties in establishing conditions for down-scaled experiments are also discussed. More research on spiking materials of model systems for vCJD/CJD is necessary to know whether the currently used materials are appropriate for conducting process evaluation studies. Based on the outcome of such research, it should be carefully judged whether the spiking materials used by model systems are appropriate. At present, ethanol and PEG fractionations, filtration with virus-removal filters, depth filters, protein purification columns and so forth are thought to be effective for prion removal (to some extent).

For virus-removal filters, the partition mechanism is based on size exclusion. The performance of filters in different studies is consistent, and the pore size correlates well with prion removal. However, as aforementioned, variability in the performance is observed depending on the filtration conditions. In contrast to virus-removal filters, depth filters may remove prions more efficiently if process conditions can be optimized. Consequently, virus-removal and depth filters may have great potential for prion removal, although we do not know whether these filter steps (such as depth filters that are adventitiously effective, rather than effective by design) are perfect or not. Therefore, more work is required to establish the filtration conditions that are optimal for prion removal.

Future perspective

In the future, detection methods for pathogenic prions applicable for blood screening are likely to be introduced, and it is expected that the risks of vCJD/CJD transmission through blood will be further clarified. With the introduction of screening tests, it is expected that safety measures for plasma derivatives for prion contamination will be composed of two procedures, screening of source materials and removal during manufacturing processes (the same as for viruses). With progress in the status of pathogenic prions in blood, preparation methods for spiking materials will also likely be optimized. Equivalency between model systems and vCJD/CJD will probably be determined more precisely, although from a safety perspective such studies are challenging. Furthermore, if the usefulness of a quantitative infectivity assay method using cultured cells is confirmed for prion-clearance study, process evaluation could be performed using such an assay. Several techniques to effectively remove prions during the manufacturing process are now under development [46,47]. Once the processes are validated or effective removal has been demonstrated under a variety of process conditions, they may be introduced in actual manufacturing scenarios. The removal ability of processes and the accuracy of process evaluation will be highly improved by combining these new observations and techniques. These improvements will significantly contribute to the safety of plasma derivatives with respect to prion contamination; however, for safety assurance, there is no limit to improvement.

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Executive summary

- Incidence of bovine spongiform encephalopathy (BSE) is falling, at least in the UK. Risk of variant Creutzfeldt–Jakob disease transmission derived from BSE-infected bovines is tending to decrease. On the other hand, the risk of human-to-human transmission by blood transfusion persists.

Executive summary

- Sensitive screening methods for the detection of prions in blood are not currently available. Therefore, for plasma derivatives, safety measures against prions are mainly geographical donor deferral and the removal of prions during manufacturing processes.
- Individual manufacturing processes for prion removal are usually evaluated by scaling down the actual manufacturing conditions to laboratory conditions, and using established scrapie strains as model systems. Since the status of the prion protein in blood is not known, the preparation method of prions as spiking materials for these experiments must be considered carefully.
- There are two different procedures to assay prions: *in vitro* study to detect prions by western blotting (WB) and so forth; and *in vivo* study to detect infectious prions by inoculating samples into animals. In some cases for only a slight amount of prion, even when WB demonstrates a negative result, due to the limitation of this technique, for example inappropriate use of antibody, infectivity may be detected in samples. Therefore, for process evaluation, differences between test methods must be considered carefully.
- Depending on process conditions, the prion-removal ability of the process may vary, even if the removal pattern is similar. Thus, the design of the evaluation study is very important. Each manufacturing process must be evaluated independently.
- At present, among the manufacturing processes of plasma derivatives, ethanol fractionation, polyethylene glycol fractionation, column chromatography and filtration with virus-removal filters and depth filters are considered to be effective for prion removal.
- The combination of processes that contribute to prion removal is necessary in order to improve the consistency of the manufacturing processes for prion removal.
- Consequently, the development of new techniques for screening and prion removal, and improvement of process evaluation methods is highly desirable.

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