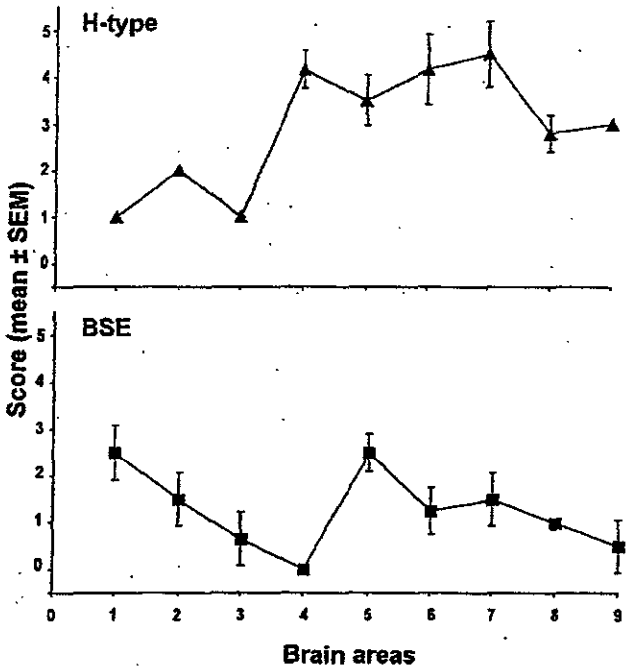

Figure 4. Lesion Profiles in tgBov Mice Infected with H-Type or BSE Agents

Mean scores (± SEM) reflecting the intensity of vacuolation are shown for H-type (no. 1 and no. 3, triangles) and BSE (no. 1 and no. 3, squares). The nine grey-matter areas used to construct the profile are as follows: dorsal medulla (1); cerebellar cortex (2); superior colliculus (3); hypothalamus (4); medial thalamus (5); hippocampus (6); septum (7); medial cerebral cortex at the level of the thalamus (8); and medial cerebral cortex at the level of the septum (9).

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(more than four brains analysed per isolate) was characterised by a significantly higher apparent molecular mass (difference of 0.7 ± 0.2 kDa) and a slightly higher proportion of diglycosylated PrP^{res} as compared with H-type–derived PrP^{res} (Figure 5A–5C). These features were conserved on secondary transmission (Figure 5A–5C). Another difference was again the pronounced accumulation of SSit-associated PrP^{res} in the spleen, while this was still impaired in H-type–infected mice, even after two passages (three to five spleens tested per combination; Figures 2B and 5A).

We then compared by histoblot distribution and nature of PrP^{res} deposits within the brains of tgOv mice infected with a second passage of H-type (no. 2) and SSit (no. 5) cases. Both markedly differed between the two agents as illustrated in Figure 5. Indeed, large plaques of SSit-associated PrP^{res} were present in the pretectal nuclei and in related structures of the limbic system such the fornix, the alveus, fimbria, and subiculum of the hippocampus. H-type–associated PrP^{res} was detected instead in the corpus callosum, cortex, and ventromedial thalamic nuclei (Figure 5D and unpublished data). The deposits seem rather thin or granular. SSit case no. 8, which gave the shortest incubation period in tgOv mice, was also inoculated by intracerebral route to tgBov mice. Of note, no disease has been observed yet in mice monitored up to 600 d after infection. In conclusion, these data suggest that the H-type agent is unrelated to the ovine TSE isolates transmitted so far to our transgenic lines.

Discussion

In this study we show that cattle brain samples positive for abnormal PrP with a distinct molecular pattern, called H-type, consistently produces a fatal, TSE-like disease upon inoculation to both bovine and ovine PrP transgenic mice. These results, corroborating the recent transmission to wild-type mice [18], formally establish that such cases involve an authentic TSE infectious agent. Importantly, we provide detailed evidence that this newly recognised agent differs from epizootic BSE agent derived from cattle or other species.

Both molecular and biological criteria support the conclusion that H-type and BSE agents are distinct prion strains. First, the incubation periods upon transmission to mice expressing either bovine or ovine PrP produced different patterns. Thus, while primary transmission to tgOv mice led to longer survival times for both agents, the increase relative to tgBov mice was significantly less for H-type than for BSE-type agents (Figure 1). Second, the molecular profiles of the PrP^{res} fragments detected in the brain of diseased mice were clearly distinguishable in either line. Strikingly, differences observed in terms of fragment size and glycoform ratio were essentially the same as in cattle brain. Third, unlike that for BSE agents, no PrP^{res} signal could be seen in the spleen of H-type–infected tgOv mice, indicative of a stronger neurotropism at least in this host. Fourth, histopathological examination of tgBov mice revealed a contrasting picture. Typically, severe spongiosis and diffuse PrP deposition were present in several areas of H-type–infected brains, while the same areas of BSE-infected brains showed limited spongiosis together with marked PrP deposition. Such discrepancies are unlikely to result from unequal survival times since they were also observed on secondary passage, where the two agents had comparable incubation duration (unpublished data).

The isolation from cattle of a prion strain distinct from the one implicated in the BSE epidemics raises several concerns. One is whether H-type isolates might result from an exposure to prions of small ruminants via alimentary or environmental sources, since cattle have been shown to be susceptible to experimental infection by sheep scrapie agent [19]. In this regards, the better compatibility between ovine PrP sequence and H-type as compared to BSE was intriguing (Figure 1). However, our investigations do not support this. Among the five groups of natural isolates we have identified so far in tgOv mice ([13,14] and our unpublished data), only one group, made up mostly of SSit isolates, proposed to be of iatrogenic origin [20], showed an incubation time as prolonged as for H-type cases. However, the PrP^{res} molecular profile, nature of deposits, and distribution within the brain as well as the differential accumulation in the spleen strongly distinguish H-type and SSit isolates. In addition, the latter failed so far to transmit to tgBov mice.

H-type and BSE agents might be related despite their distinguishable phenotypes. The isolation of an additional strain upon exposure of transgenic or wild-type mice to the epizootic BSE agent has been reported [21], thus questioning its strain homogeneity. Also, molecular typing studies have revealed the presence of a minor, non-BSE-type PrP^{res} component in BSE- and vCJD-infected brains [22]. Hence, H-type isolates could arise from the preferential amplification in certain individuals of a subcomponent present in BSE

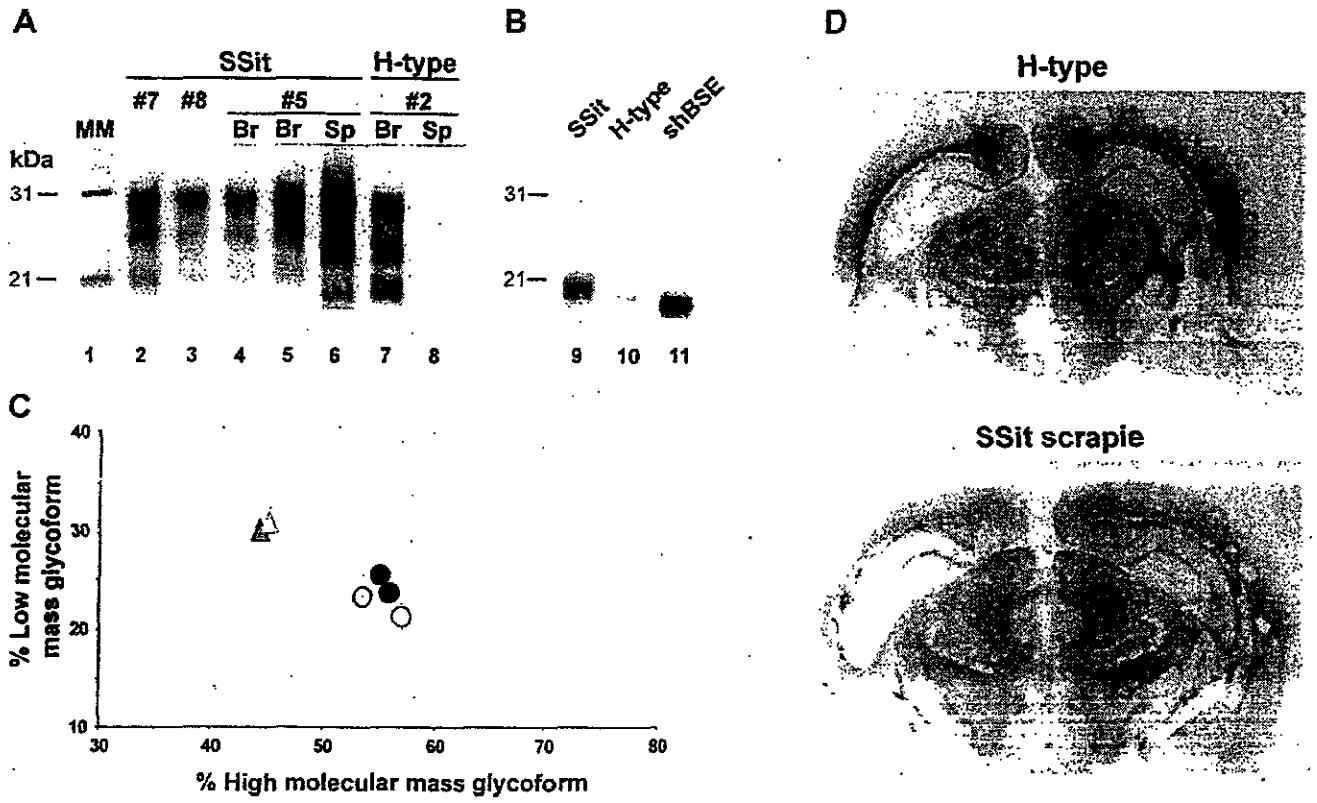

Figure 5. Comparison of H-Type and SSit Isolate Features upon Transmission to tgOv Mice

(A and B) Western blot analysis of PrP^{res} in the brains and spleens of tgOv mice infected with H-type or SSit isolates at first (lanes 2–4) and second passage (lanes 5–11). H-type PrP^{res} shows a distinct pattern in the brain (Br) compared to SSit. The apparent molecular mass of SSit PrP^{res} is higher than that of H-type or sheep BSE (shBSE), as shown after PNGase treatment (B). Note also that PrP^{res} is detected in the spleen (Sp) of SSit- but not of H-type-infected mice. Tissue equivalent loaded: 1.5 mg in lanes 2–4; 0.04 mg in lane 5; 0.5 mg in lanes 6–7; 2 mg in lane 8; 0.01 mg in lane 9; 0.1 mg in lanes 10–11. MM, molecular markers.

(C) Ratio of high- and low-molecular-mass PrP^{res} glycoforms in the brain of tgOv mice infected with H-type or SSit isolates (data plotted as mean ± SEM). One H-type isolate (no. 2) is represented as orange triangle. SSit isolates are represented as circles (SSit no. 5, red; no. 7, brown; no. 8, yellow). Secondary transmissions are represented by unfilled symbols of the same colour. Note the stably distinct glycoform ratios between H-type and SSit agents upon serial passage.

(D) Regional distribution of PrP^{res} in the brain of tgOv mice infected with H-type or SSit isolates. Histoblots of representative coronal sections of tgOv mouse brains at the levels of the hippocampus are shown. The distribution of H-type–associated PrP^{res} deposits was different from that of SSit in regions such as the alveus of the hippocampus, the corpus callosum, the pretectal nuclei, the cortex, and the ventromedial thalamus. Note that the size of PrP^{res} deposits markedly differed between the two types of isolates.

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infectious sources. Comparing H-type and BSE-derived variant prions identified in mice might be informative in that respect.

Alternatively, such unusual cases could reflect the existence of a natural, sporadic disease in cattle. Although it is unclear yet if such infections may lead to a clinical disease in the natural host, they seem to occur at a low frequency, which is reminiscent of the situation known for sporadic CJD in humans [23,24]. Of note, the disparities between intensity of PrP deposition and severity of vacuolation in the brains of H-type–inoculated tgBov mice have also been observed with sporadic CJD both in human or mouse infected brains [21,25]. These data, however, need to be consolidated through further investigations, including epidemiological analysis. Indeed, an implication of this latter scenario is that such bovine "atypical" cases could occur in countries free of BSE exposure. The acquisition of novel properties by an endogenous, sporadic cattle TSE agent, as occasioned on passage through an intermediary host or a physicochemical treatment such as that applied to carcass-derived products, has been invoked as one possible origin for the emergence of BSE epidemics [7]. With the isolation of such agents, we can now address this issue experimentally.

In conclusion, our findings support the view that at least two and potentially three [10] distinct prion strains may be present in cattle. The current uncertainties regarding the origin, prevalence, and potential risk for humans of a strain of TSE agent unrecognised until recently should support continued efforts to characterise it in vivo and uphold the surveillance exerted on cattle.

Materials and Methods

Isolates. The H-type [9], goat BSE (CH636 case [26]), and experimental sheep BSE samples [27] were provided by the French TSE Reference Laboratory (Agence Française de Sécurité Sanitaire des Aliments [AFSSA], Lyon, France). The samples from French BSE cases and from experimental sheep BSE (ARR [$Ala^{136}Arg^{154}Arg^{171}$] genotype [28]) were provided by the Institut National de la Recherche Agronomique (INRA; Toulouse, France) and the Institute for Animal

Health (IAH) Neuropathogenesis Unit (Edinburgh, United Kingdom), respectively. The vCJD isolate was a World Health Organization (WHO) reference sample from the National Institute for Biological Standards and Control (NIBSC; Potters Bar, United Kingdom). SSit isolates were provided by the Instituto Superiore di Sanita (ISS; Rome, Italy).

Transgenic mice and transmission assays. The tg540 line expresses the bovine PrP allele with 6 octarepeats under the control of the cytomegalovirus (CMV) promoter on a FVB mouse line with $PrP^{0/0}$ background (Protocol S1). The tg338 line expresses the $V_{136}R_{154}Q_{171}$ allele of ovine PrP at a homozygous state, on a mouse $PrP^{0/0}$ background [29]. The transgene construct (tg3) consists in a bacterial artificial chromosome (BAC) insert of 125 kb of sheep DNA [13]. All experiments were performed according to national guidelines. Each inoculum was prepared extemporaneously in a class II microbiological cabinet using disposable equipment. Individually identified 6- to 10-wk-old mice were inoculated intracerebrally with 20 μl of a 10% (wt/vol) brain homogenate in 5% glucose. Mice were monitored daily once ill and killed in extremis.

Analysis of PrP^{res} molecular pattern. All procedures regarding purification and detection of PrP^{res} from brains and spleens of infected mice were as described [14]. ICSM18 [30] or Sha31 [31] anti-PrP antibodies were used. Enzymatic deglycosylation was performed on denatured PrP^{res} with 1,000 U of recombinant PNGase (New England Biolabs, Beverly, Massachusetts, United States) for 2 h at 37 °C in 1% Nonidet P40 and the proprietary buffer as described [30]. Determination of glycoform ratio and apparent molecular mass was performed with the GeneTools software after acquisition of chemiluminescent signals with a GeneGnome digital imager (Syngene, Frederick, Maryland, United States).

Histopathology. For histoblot analysis [32], brains were rapidly removed from killed mice and frozen on dry ice. Thick 10-μm cryostat sections were cut, transferred onto Superfrost slides, and kept at −20 °C until use. The procedure was performed as described [14] using the 12F10 anti-PrP antibody [33]. All immunohistochemistry procedures regarding tissue processing have been described previously [34]. Samples were fixed in neutral-buffered 10% formalin (4% formaldehyde) before paraffin embedding. After deparaffinisation, 6-μm-thick tissue sections were stained with haematoxylin/eosin. Vacuolation profiles were established, following the standard method described by Fraser and Dickinson [15], by using two to three brains per isolate.

Supporting Information

Protocol S1. Description of the Bovine PrP Transgenic Mice (tg540 Line)

Found at DOI: 10.1371/journal.ppat.0020112.sd001 (92 KB DOC).

Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) accession numbers for the genes and gene products discussed in this paper are bovine PrP (NM181015) and sheep PrP (M31313).

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Author contributions. JLV and HL conceived and designed the experiments. VB, AB, ALD, FR, TLL, NC, GT, and JLV performed the experiments. VB, AB, and HL analyzed the data. AGB and TB contributed reagents/materials/analysis tools. VB and HL wrote the paper.

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Competing interests. The authors have declared that no competing interests exist.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 3. 16	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		ProMED 20070302-0734, 2007 Mar 2. 情報源:NZFSA (New Zealand Food Safety Authority) official press release, 2007 Feb 28.	ニュージーランド

研究報告の概要

○ニュージーランド食品安全局はBSE輸入規制を改訂

ニュージーランド食品安全局(NZFSA)は、BSEを取り巻く最新の科学と実際の経験を踏まえて、ウシ及びウシ加工品の輸入規制を改訂する方針である。NZFSAの規制担当者は「1996年の旧規制は、不確実性の多い時期に実施された予防的取り組みが含まれているが、その後BSEとヒトの健康へのリスクについて多くのことが判明した」と話している。

新しい規制は、科学的証拠や最近の国際的な規制に合致したものとするため、ニュージーランドにウシやウシ加工品を輸出する国のBSEのリスクステータスの分類に、国際的に認められた3カテゴリーシステムを導入する。他の改訂内容は以下の通りである。

* 独自の方法ではなく、国際的なリスク分析を導入する。
* BSEの潜在的リスクのある国からの危険部位と認められた内臓肉の輸入を禁止する。
* この規制の対象となる商品由来のごく少量のウシ由来成分を含む加工食品は例外とする。
* 輸入品の受け入れ可否と何らかの証明の必要性を決定する強固な枠組みを構築する。
* 商品の原料については年齢制限を解除し、トレーサビリティの手段を特定しない。
* 原材料、リスクのある国からの輸入を問わず、全てのゼラチンの売買を自由化する。

最近の研究では、骨由来のゼラチンの製造工程で使用される科学的処理によってBSEの感染性を充分に不活化出来ることが確認された。

新しい規制は2007年6月29日から発効するが、ゼラチンの売買自由化はそれ以前から実施される。

報告企業の意見	今後の対応
ニュージーランド食品安全局がウシ及びウシ加工品の輸入規制を緩和したとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

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Subject PRO/AH/EDR> BSE, bovine - New Zealand: new import rules

BSE, BOVINE - NEW ZEALAND: NEW IMPORT RULES
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Date: Wed 28 Feb 2007
From: Stuart MacDiarmid <Stuart.MacDiarmid@maf.govt.nz>
Source: NZFSA (New Zealand Food Safety Authority) official press release [edited]
<http://www.nzfsa.govt.nz/publications/media-releases/2007-02-28.htm>

NZFSA updates BSE importing requirements

New Zealand Food Safety Authority (NZFSA) has moved to modernise the food safety importing requirements for beef and beef products in light of the new science and practical knowledge that now surrounds bovine spongiform encephalopathy (BSE).

The changes reflect recent findings from the growing body of science that more accurately identifies the risks and measures required to protect consumers from variant Creutzfeldt-Jakob disease (vCJD), the human disease that has been linked to eating beef offal containing the BSE agent.

"The old measures, in place since 1996 and adopted internationally as well as by New Zealand, reflected a precautionary approach which was taken during a time of uncertainty," explains Tim Knox, NZFSA's New Zealand standards director. "However, in the intervening years much has been learned about BSE and the risks to human health which has increased our understanding and virtually eliminated the risk of consumers contacting v[illegible]."

The new measures have undergone a comprehensive process of review and expert consideration to ensure they are consistent with scientific evidence and the emerging international standards. As a result New Zealand will move to an internationally agreed 3-category system for categorising the BSE risk status of those countries exporting beef and or beef-related products to New Zealand.

* adopt international risk assessments rather than conducting its own
* exclude offal that has been identified as BSE risk material from any country with a residual risk of BSE
* exempt processed foods that contain minimal bovine ingredients from those commodities that are covered by the measure
* adopt a consistent framework for determining the acceptability of imported products and the need for any certification
* remove age restrictions on the source of commodities and not specify measures to provide for traceability
* allow all gelatine to be traded freely, regardless of the source raw material and exporting country's BSE risk status.

Gelatine derived from bones was originally considered a risk because of fears that it could contain the BSE prion. However, recent studies have confirmed that chemical processes used in its manufacture are sufficient to inactivate any BSE infectivity that may have been present in the raw material, even under worst-case conditions. "Gelatine produced by modern

industrial processes does not pose a BSE risk to consumers, regardless of the raw material from which it is produced and the source country from which it is derived," explains Mr Knox.

The new requirements will come into effect on 29 Jun 2007 although gelatine will be freely traded before then.

Strict controls have been in place for beef products around the world since 1996 when vCJD was linked to eating beef products contaminated with offal that had come from cattle with BSE in the 1980s. Since that time, however, changes to production rules (such as not feeding ruminant material to ruminants and removing the parts of the animal from the food chain that could contain the BSE agent) have dramatically reduced the incidence of BSE.

The new requirements are not related to New Zealand's domestic animal health measures regarding BSE and will not override current animal health requirements, such as the ruminant-to-ruminant feed ban.

New Zealand has been classified as BSE-free by the World Organisation for Animal Health (OIE) and is regarded by the European Food Safety Authority as a country in which BSE is "highly unlikely" to be present.

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All information on the NZFSA website is subject to a disclaimer:
<http://www.nzfsa.govt.nz/site/disclaimer/standard.htm>.

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Stuart MacDiarmid
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[The new rules reflect much of the same rules as other countries. - Mod.TG]

[see also:
BSE & Scrapie - New Zealand: Free 19960725.1327]

.....tg/mj/sh

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 9 月 29 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Guideline on validation of immunoassay for the detection of hepatitis B virus surface antigen (HBs Ag) in plasma pools. London, 21 September 2006 EMEA/CHMP/BWP/298390/2009	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
調査書類の評価中に見受けられた，プール血漿のヒト B 型肝炎抗原 (HBsAg) 検査におけるバリデーションの不備のある点を改善するためにこのガイドラインが作成された。HBsAg 測定法は血漿分画製剤の原料となるプール血漿内の HBsAg を検出する定性試験である。しかし，市販の HBsAg 検出キットは個人供血サンプルに対してのみバリデーションされている。したがって，血漿分画製剤用プール血漿における検査・バリデーションが必要となる。しかし，プール血漿の血清学的検査は，ウイルス感染の安全性の保証を意味するのではなく，GMP ガイドラインに対する重大な不備を検出する方法とみなされるべきである。市販キットのバリデーションとは，頑健性 (信頼性) はもちろん，特異性と検出感度 (抗原の検出限界値やカットオフ値) の決定を含む多段階プロセスである。加えて，分析法内・分析法間での変動も多数の方法を用いて行うべきである。試験方法は，準備，保管管理，試験，再試験，及び判定の手順に関する情報を含む標準作業手順書 (SOPs) で文書化されなければならない。また，弱い HBsAg 陽性コントロール (個人供血のカットオフ値の 2-3 倍) を試験ごとに組み入れるべきである。陽性サンプルは複数の異なる抗体を用いてバリデーションされた血清学的検査で陰性と実証されない限り，陽性と見なすべきである。核酸増幅検査 (NAT) は陰性を実証する方法としは使用不可能である。	BYL-2007-0243

報告企業の意見	今後の対応
EMEA から発行された本ガイダンスは，血漿分画製剤に使用されるプール血漿の HBsAg スクリーニングの改善と標準化を目的としている。EMEA は，個人供血の試験用に開発された市販の HBsAg 検出キットをプール血漿に適応する場合，バリデーションを完全に行うよう勧告している。バリデーションされた確認法は陽性サンプルの再試験に対しても実施すべきであると考える。また，医薬品市販承認取得者と血漿マスターファイル所持者はこのガイドラインに照らし合わせてプール血漿の試験法のバリデーションを検討する必要がある。	本ガイドラインに記載された重要事項に対して，弊社の血漿分画製剤に使用されるプール血漿は，既存のバリデーションで適用できていることが確認されていることから，現時点では新たな対応は必要ない。

European Medicines Agency
Pre-authorisation Evaluation of Medicines for Human Use

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COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF HEPATITIS B VIRUS SURFACE ANTIGEN (HBSAG) IN PLASMA POOLS

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