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Review

Prion disease and recommended procedures for flexible endoscope reprocessing – a review of policies worldwide and proposal for a simplified approach

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SUMMARY

Several guidelines recommend specific treatments for endoscopes, procedures of quarantine for endoscopes, or additional treatments for the endoscope washer disinfector (EWD) in suspected or confirmed cases of Creutzfeldt–Jakob disease (CJD) or variant CJD (vCJD) but vary in many details. This study therefore reviewed guidelines on reprocessing flexible endoscopes after use in patients with suspected or confirmed prion disease. In addition, a literature search was performed in Medline on prion, CJD, vCJD, chemical inactivation, transmission healthcare, epidemiology healthcare, concentration tissue human and endoscope. Thus far, no case of CJD or vCJD transmitted by flexible endoscope has been reported. In animals it has been shown that oral uptake of 0.1–5 g of bovine spongiform encephalopathy (BSE)-infected brain homogenate is necessary for transmission. The maximum prion concentration in other tissues (e.g., terminal ileum) is at least 100-fold lower. Automated cleaning of endoscopes alone results in very low total residual protein $\leq$5.6 mg per duodenoscopes. Recommendations vary between countries, sometimes with additional cleaning, use of alkaline cleaners, no use of cleaners with fixative properties, use of disinfectants without fixative properties or single-use disinfectants. Sodium hydroxide (1 M) and sodium hypochlorite (10,000 and 25,000 mg/L) are very effective in preventing transmission via contaminated wires implanted into animal brains, but their relevance for endoscopes is questionable. Based on circumstantial evidence, it is proposed to consider validated reprocessing as appropriate in the case of delayed suspected prion disease when immediate bedside cleaning, routine use of alkaline cleaners, no fixative agents anywhere prior to disinfection and single use brushes and cleaning solutions can be assured.

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Introduction

Prion diseases in humans comprise Creutzfeldt—Jakob disease (CJD), variably protease-sensitive prionopathy, Gerstmann—Sträussler—Scheinker disease, fatal familial insomnia, and kuru. Each is a uniformly fatal rare neurodegenerative disease in which conformational changes in the prion protein are thought to be the central pathophysiologic event. The majority of cases of human prion diseases occur worldwide in the form of sporadic CJD [1]. Up until 2016 there were 491 incidents of iatrogenic transmission of CJD, largely resulting from prion-contaminated growth hormone (238 cases) and dura mater grafts (238 cases). Four cases were reported after gonadotropin treatment, four were transmitted by surgical instruments in the 1950s (UK and France), three by blood transfusion, two by corneal transplant and two by electroencephalogram (EEG) depth electrode [2]. An analysis of 65 CJD blood donors along with 826 of their blood recipients showed that there is no evidence of CJD transfusion transmission in 3934 person years of follow-up; this risk remains theoretical to date [3].

Prion diseases occur in iatrogenic and zoonotic forms (iatrogenic CJD and variant CJD (vCJD), respectively), adding a public health dimension to their management [1,4]. A review published in 2017 showed that more than 20 years after identification of the first vCJD patients, only five cases were known that were probable consequences of iatrogenic vCJD transmission, all in the UK, and all associated with blood and blood products. These cases were caused by transfusion of non-leukocyte-depleted erythrocyte concentrates or by treatment involving large amounts of pooled plasma from the UK that were known to include donations from persons who later showed development of vCJD. None of the 220 other vCJD cases identified worldwide has been linked to any other medical or dental procedure [5].

From 1987 the number of bovine spongiform encephalopathy (BSE) cases increased dramatically in the UK, reaching approximately 37,000 new cases in 1992 [4]. With a delay of 8 years, human cases of vCJD were detected and also increased with 28 new cases in 2000 [4]. That is why a transmission by oral uptake of BSE prions was considered to be a possible cause of vCJD. Flexible endoscopes are typically classified as semi-critical items and reprocessed according to validated protocols [6]. In order to eliminate any possible risk of prion transmission via flexible endoscopes, several national and international guidelines have recommended specific treatments for endoscopes, procedures of quarantine for endoscopes or additional treatments for the endoscope washer disinfector (EWD) in suspected or confirmed cases of CJD or vCJD. They were based on the existing evidence and plausible assumptions at that time. Especially in suspected cases, it may be difficult to clearly distinguish between CJD and vCJD. In addition, some details of the recommendations appear to be questionable and vary largely between countries. This study therefore reviewed major national guidelines on endoscope reprocessing in relation to confirmed, probable or suspected prion disease, the type and impact of endoscopy procedure and its anatomical sites, the evidence of the efficacy of various treatments for prion inactivation including their test methods, data on the concentration of prion protein in various tissues, and finally evaluated their practical plausibility based on current evidence and experience.

Methods

Guidelines on reprocessing flexible endoscopes were searched on PubMed and Startpage and reviewed regarding all aspects of endoscopes used in patients with suspected or confirmed prion disease. Any information regarding reprocessing was extracted as well as any information on quarantine of endoscopes and on additional treatments of the EWD. In addition, a literature search was performed on Medline on 21 December 2017 and updated on 25 February 2019. The following search terms were used: prion chemical inactivation (53 hits), CJD chemical inactivation (nine hits), vCJD chemical inactivation (two hits), prion transmission healthcare (47 hits), CJD transmission healthcare (21 hits), vCJD transmission healthcare (15 hits), prion epidemiology healthcare (24 hits), CJD epidemiology healthcare (16 hits), vCJD epidemiology healthcare (15 hits), prion concentration tissue human (159 hits), CJD concentration tissue human (32 hits), vCJD concentration tissue human (18 hits), prion endoscope (32 hits), CJD endoscope (six hits), vCJD endoscope (10 hits), prion endoscopy (23 hits), CJD endoscopy (four hits) and vCJD endoscopy (six hits). Articles were reviewed for original data on the inactivation of prions by different procedures (cleaning agents, chemicals, autoclaving, automated cleaning and disinfection processes), the prion contamination level of human tissues, and the epidemiology and transmission of prion disease in healthcare settings with a focus on flexible endoscopes. Additional articles were considered when they were within the scope of the search, e.g., when referenced in the detected articles.

Evidence for prion transmission by flexible endoscopes

Thus far, no case of CJD or vCJD transmitted by a contaminated flexible endoscope has been reported. In addition, no studies were found measuring prion protein on flexible endoscopes, either after use in a patient with proven or suspected prion disease without processing or after use in a patient with proven or suspected prion disease but after reprocessing. Therefore, it is still unknown whether an endoscope harbours prion protein on its inner and outer surfaces after use in a patient with prion disease. Successful transmission with artificially contaminated flexible endoscopes has also never been described in animal experiments. It is therefore currently not possible to evaluate the real risk of prion protein transmission via flexible endoscopes.

Probability for prion transmission by flexible endoscopes

Initially, it was assumed that more than a few thousand new cases were possible per year by any mode of transmission [7,8]. Transmission of vCJD by flexible endoscopes has also been assumed to be possible [9]. The epidemic curve of BSE and vCJD in the UK indicated that the rise, peak and decline of new probable or definite vCJD cases in humans can be observed in parallel to the BSE incidence with an 8-year delay [4]. Assuming that transmission of vCJD from an index patient to another patient is possible using a flexible reprocessed gastrointestinal

endoscope, it would have been most likely to see cases approximately 8 years after the vCJD peak in the UK in 2000 with 28 new cases. But even in 2008 and later, no transmissions of vCJD were reported via flexible endoscopes. Since 2008 the number of new vCJD cases in the UK has been very low with up to three cases per year, and is likely to remain at a very low level despite early assumptions that more than a few thousand new cases were possible per year [7,8]. Today the risk of new endoscope-associated vCJD cases is therefore low and more likely to remain at zero.

Prion protein concentration in human tissues

In 2017 it was shown that vCJD prion can be detected in various types of tissues in vCJD patients [5]. The highest level was found in the frontal cortex. Other tissues potentially relevant for flexible endoscopes also contained prion protein but at a concentration that was at least 100-fold (tonsils) or 1000-fold lower (distal ileum). An overview of tissues relevant in this context is provided in Table I.

Infective dose for oral BSE transmission

Experimental transmission studies have demonstrated that the volume of inoculum affects the likelihood of transmission. In 1995, oral transmission of BSE to sheep and goats was demonstrated with as little as 0.5 g of infective bovine brain [10]. The transmission rate in cattle was dependent on the inoculum with 100% transmission feeding 100 g BSE bovine brain, 70% transmission with 1 g, 20% transmission with 100 mg, and 7% transmission with 10 mg and 1 mg [11]. Based on these findings, the authors proposed an oral ID_{50} in cattle between 100 mg and 1 g [11]. Transmission in macaques has been successful in one of two animals with 5 g of orally introduced BSE-infected brain homogenate; lower inoculums were not investigated [11]. A 2002 study into the lethal challenge required to infect hamsters demonstrated a linear rate of transmission, which fell with increasing dilution of the oral inoculum [12]. This correlation is probably the same in humans, but the volume of tissue necessary for a transmission is unknown. Based on these cases, it appears very unlikely that a flexible endoscope used in the gastrointestinal tract of a vCJD or CJD patient and reprocessed with a validated protocol would transmit prion disease to another patient even if biopsies were taken.

Table I
Detection levels of prion protein in various types of tissues obtained from patients with proven variant Creutzfeldt–Jakob disease (vCJD)

Type of tissue/organ	Lowest dilution of prion protein detection*
Frontal cortex	10^{-8}
Tonsil	$10^{-3} - 10^{-6}$
Appendix	$10^{-3} - 10^{-4}$
Distal ileum	$10^{-2} - 10^{-5}$
Lung	10^{-2}
Pancreas	$10^{-2} - 10^{-4}$
Liver	$10^{-2} - 10^{-4}$
Salivary gland	$10^{-2} - 10^{-4}$

* Determined by protein misfolding cyclic amplification (PMCA) reactions seeded with postmortem tissue homogenate from symptomatic vCJD patients. This Table is an extract from Douet *et al. Emerg Infect Dis* 2017;**23**:946–56.

Probability of prion transmission with reprocessed flexible endoscopes

It is not known whether there is a genetic relationship between the species and the infective dose. Macaques need at least 5 g of BSE-infected brain homogenate for a 50% transmission rate, and for cattle it has been described to be between 100 mg and 1 g. Assuming only a 1000-fold lower prion concentration in the distal ileum compared to the frontal lobe, it would mean an equivalent infective dose of 5 kg (macaque data) or between 100 g and 1 kg (cattle and goats data) which would be necessary to be transmitted orally by a processed flexible endoscope. This amount of tissue would probably need to be present on the outer surface and inside the channels of the endoscope and be released during the endoscopic procedure in order to exhibit the same effect shown on the animals. This is a highly unlikely scenario. Automated cleaning of endoscopes alone results in very low residual protein with $\leq$9.4 $\mu g/cm^2$ on colonoscopes, $\leq$14 $\mu g/cm^2$ on duodenoscopes, 7 $\mu g/cm^2$ on gastroscopes, and 2.6 $\mu g/cm^2$ on bronchoscopes [13]. Taking into account the surface of each type of endoscope, the highest total residual protein on the surface would be 5.6 mg for duodenoscopes, 3.4 mg for colonoscopes, 1.8 mg for gastroscopes, and 0.15 mg for bronchoscopes [13]. The highest amount of protein after only cleaning of the endoscopes is at least 100,000 times below the amount of infective prion dose required to cause vCJD in animals by oral uptake. The total protein levels on endoscopes may be even lower after full automated reprocessing with additional rinsing steps.

Guidelines on handling flexible endoscopes in case of suspected or proven prion disease

Table II provides a summary of recommendations on endoscope quarantine and incineration. In most guidelines, quarantine or incineration of the endoscope is recommended after use in a patient with suspected or proven prion disease, partly only in procedures with nasal cavity contact or after invasive procedures (UK, Canada) or contact with high infectivity tissue (France, Australia). The tonsils or the distal ileum are not specifically mentioned probably because of the rather low risk of transmission by contact. Quarantine is recommended after reprocessing of the endoscope in the UK, Canada and Switzerland. In Germany only a pre-cleaning is recommended prior to quarantine. Re-use of the endoscope is considered possible in the same patient in the UK, Switzerland, Canada and Australia. Re-use in other vCJD patients is considered possible in the USA.

The recommended procedures of reprocessing in specific cases of suspected prion disease are summarized in Table III. In some countries, regular validated reprocessing is recommended, sometimes with additional requirements such as double cleaning (France), use of alkaline cleaners (Switzerland, European Society of Gastrointestinal Endoscopy (ESGE)), no use of cleaners with fixative properties (Germany, ESGE), use of disinfectants without fixative properties (UK, Canada, Germany) or single use disinfectants (UK, France). In Germany and Austria, a specific treatment with 4 M guanidine

Table II
Overview of recommended procedures such as quarantine or incineration of flexible endoscopes after use in specific cases of prion disease

Country: organization	Types of endoscopes	Case	Quarantine	Incineration	Reference
UK: Department of Health and Social Care	Neurological endoscopes, endoscopes with nasal cavity contact	• Definite or probable symptomatic CJD or vCJD • Possible sporadic symptomatic CJD or vCJD • Asymptomatic patients at increased risk of CJD • Asymptomatic patients 'at increased risk' through receipt of labile blood components (whole blood, red cells, white cells or platelets) from a donor who later developed vCJD • Asymptomatic patients 'at increased risk' of vCJD	Recommended *,†	Recommended	[39]
UK: British Society for Gastroenterology	Gastrointestinal endoscopes	If an 'invasive procedure' is undertaken in • Definite or probable vCJD • Diagnosis of vCJD is considered • Someone regarded as presumed infected having received labile blood products (such as whole blood, red or white cell concentrates) from a donor who subsequently developed vCJD	Recommended *,†	Recommended ‡	[34]
France: Ministry of Social Affairs and Health	All flexible endoscopes	Suspected or proven CJD/vCJD • Use on tissue with a high infectivity, e.g. the olfactory nerve • Rectal endoscopy • Aerodigestive endoscopy	Recommended until a diagnosis is confirmed	Recommended if diagnosis is confirmed	[45,46]
Germany: Robert Koch Institute	Flexible endoscopes in neurosurgery, oral surgery and otorhinolaryngology	Suspected or proven CJD/vCJD	Recommended	No recommendation	[40]
	Flexible endoscopes in gastroenterology, pulmonology, intensive care and urology	Suspected or proven CJD/vCJD	Recommended §	No recommendation	

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Table II (*continued*)

Country: organization	Types of endoscopes	Case	Quarantine	Incineration	Reference
Switzerland: SwissNoso	Gastrointestinal endoscopes	High and medium risk of CJD or vCJD	Recommended *,†	Recommended	[47]
Netherlands: SFERD	Any flexible endoscope	Prion disease	No specific recommendation	No specific recommendation	[48]
Austria: Department of Health/Medical Chamber	All flexible endoscopes	Definite or probable vCJD	No specific recommendation	Recommended	[44,49]
European Society for Gastrointestinal Endoscopy (ESGE)	Gastrointestinal endoscopes	vCJD	No specific recommendation	No specific recommendation	[42]
USA: American Society for Gastrointestinal Endoscopy	Gastrointestinal endoscopes	vCJD	No specific recommendation	Recommended ¶	[50]
Canada: Public Health Agency	Gastrointestinal endoscopes and bronchoscopes	After use for invasive procedures (e.g., biopsy) in individuals with definite, probable, or possible vCJD, or where the diagnosis of vCJD is unclear; also when found retrospectively ‡	Recommended *,†	Recommended when vCJD is confirmed	[35,36]
Australia: Department of Health	Any endoscope	CJD: high- or low-risk patient where higher-infectivity tissue has been exposed (e.g., ventriculoscope)	Recommended †	Recommended	[38]

CJD, Creutzfeldt–Jakob disease; vCJD, variant Creutzfeldt–Jakob disease.
* After reprocessing.
† re-use for the same patient is possible.
‡ confirmed contamination.
§ after pre-cleaning.
¶ re-use in other vCJD patients is possible.

thiocyanate for 2 × 30 min is recommended prior to regular reprocessing. The EWD should be treated with an empty cycle according to the guidelines from the UK, Canada and Switzerland. The major discrepancies in various aspects underline the lack of evidence supporting the recommendations and the different approaches to ensure best practice based on pragmatic assumptions.

Evidence for effective prion decontamination

In 2001 Rutala *et al.* published a review on all available data and described chlorine between 1000 and 10,000 mg/L, sodium hydroxide between 0.1 N and 2 N and guanidine thiocyanate at 4 M as effective against prions with >3 log reduction within 1 h [14]. Other agents were considered to be ineffective against prions with ≤3 log reduction within 1 h such as hydrogen peroxide (3%), peracetic acid (19%), potassium permanganate (0.1–2%), alcohol (50% or 100%), chlorine dioxide (50 mg/L), formaldehyde (3.7%), glutaraldehyde (5%) and hydrochloric acid (1 N) [14].

No animal data were found in the literature to show that an endoscope contaminated with prions but not treated in any way is able to transmit vCJD after contact with the gastrointestinal or pulmonary tracts. Many data on prion inactivation were published after 2001, all of them were obtained in animal models. In the majority of studies, wires of different diameters (0.15, 0.16 or 0.25 mm) and lengths (3, 5, 30 or 50 mm) were initially contaminated with prions in brain tissue followed by different types of treatment for prion decontamination. Wires were then permanently implanted into the brain of animals [15–22]. In a few studies infective brain tissue was directly transferred into the brain of test animals [23–26]. The incidence of prion diseases was regarded as a treatment failure.

Enzymatic cleaners and detergents

Various enzymatic cleaners and detergents have only poor efficacy for prevention of transmission in animal experiments. However, some of the available enzymes were quite effective after 60 min of immersion time (Table IV).

Alkaline cleaners

Alkaline cleaners, typically with pH 11.5–12.5, have in some test settings a good efficacy for prevention of transmission in animal experiments. The effect of mild alkaline cleaners with typical pH-values between 8.5 and 11, however, is poor (Table V). In addition, an alkaline cleaner at 0.5% and 1% was

Table III
Overview of recommended procedures of reprocessing flexible endoscopes after use in specific cases of prion disease

Country: organization	Types of endoscopes	Patient group	Type or reprocessing	Reprocessing steps			Treatment of EWD	Reference
				Bedside cleaning	Cleaning/type of cleaner	Disinfection/type of disinfectant		
UK: Department of Health and Social Care	Endoscopes other than those used in the CNS and nasal cavity	Asymptomatic patients "at increased risk" of vCJD	EWD, separately from other equipment, within 3 h after endoscopic procedure	Immediately after the procedure	No specific recommendation	No fixative disinfectants such as glutaraldehyde or OPA	Empty self-disinfection cycle	[39]
UK: British Society for Gastroenterology	Gastrointestinal endoscopes	Patient at risk of vCJD due to receipt of pooled plasma concentrates	Validated process in EWD, separately from other endoscopes, single quality-assured decontamination cycle	No specific recommendation	No specific recommendation	Single use disinfectant	Empty self-disinfection cycle (after invasive procedures)	[34]
France: Ministry of Social Affairs and Health	All flexible endoscopes	Suspicion of vCJD after the endoscopic procedure	Regular endoscope reprocessing	No specific recommendation	Double cleaning; no recycling of products in EWD	No recycling of products in EWD	No specific recommendation	[45]
Germany: Robert Koch Institute	Flexible endoscopes in gastroenterology, pulmonology, intensive care and urology	Suspected CJD	Validated reprocessing possible after the endoscope was soaked in 4 M guanidine thiocyanate for 2 × 30 min with a mechanical cleaning in between	No pre-treatment with fixatives such as aldehydes or alcohols	No specific recommendation	No use of disinfectants with fixative properties such as aldehydes	No specific recommendation	[40]
Canada: Public Health Agency	Gastrointestinal endoscopes and bronchoscopes	Definite, probable, or possible CJD/vCJD, or where the diagnosis of CJD/vCJD is unclear	No specific recommendation	No specific recommendation	No specific recommendation	None with fixative properties	Empty cycle	[35,36]
Switzerland: SwissNoso	Gastrointestinal endoscopes	Low risk of CJD or vCJD	Regular reprocessing	No specific recommendation	Alkaline cleaners	No specific recommendation	Empty cycle after reprocessing an endoscope which is going to be transferred to quarantine	[47]

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Table III (*continued*)

Country: organization	Types of endoscopes	Patient group	Type or reprocessing	Reprocessing steps			Treatment of EWD	Reference
				Bedside cleaning	Cleaning/type of cleaner	Disinfection/type of disinfectant		
Australia: Department of Health	Any endoscope	No CJD high- or low-risk patient where higher-infectivity tissue has been exposed (e.g., ventriculoscope)	Regular reprocessing	No specific recommendation	No specific recommendation	No specific recommendation	No specific recommendation	[38]
Netherlands: SFERD	Any flexible endoscope	Prion disease	No specific recommendation; a loan endoscope should not have been used on a patient with prion disease	No specific recommendation	No specific recommendation	No specific recommendation	No specific recommendation	[48]
Austria: Department of Health/Medical Chamber	Any flexible endoscope	Suspected CJD/ vCJD	Validated reprocessing after the endoscope was soaked in 4 M guanidine thiocyanate for 2 x 30 min with a mechanical cleaning in-between	No specific recommendation	No specific recommendation	No specific recommendation	No specific recommendation	[44,49]
European Society for Gastrointestinal Endoscopy (ESGE)	Gastrointestinal endoscopes	vCJD	Regular reprocessing immediately after removal from the patient	Alkaline detergents or enzymatic type detergent solutions or non-coagulating disinfectants	No use of aldehyde-containing cleaning agents; no re-use of cleaning solutions	Use of aldehydes only after thorough cleaning, washing with detergent and rinsing with water	No specific recommendation	[42]
USA: American Society for Gastrointestinal Endoscopy	Gastrointestinal endoscopes	vCJD	Recommendation to follow ESGE guidelines	No specific recommendation	No specific recommendation	No specific recommendation	No specific recommendation	[50]

CJD, Creutzfeldt–Jakob disease; EWD, endoscope washer disinfector; vCJD, variant Creutzfeldt–Jakob disease; OPA, orthophthalaldehyde.

Table IV
Efficacy of enzymatic cleaners or detergents to prevent prion transmission in animal experiments

Type of product	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Enzymatic detergent 1:50 (wash)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	Unknown	Unknown	100% (10 of 10 hamsters)	[22]
Enzymatic detergent 1:1 (bath)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	30 min or 24 h	Unknown	100% (10 of 10 hamsters)	[22]
Hospital detergent (0.5%)	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	15 min at 20°C	Unknown	100% (24 of 24 mice)	[25]
Enzymatic cleaner (Prolystica 2× enzymatic, 0.4%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	15 min at 50°C	< 1 log	100% (8 of 8 hamsters)	[20]
Enzymatic cleaner (Prolystica 2× enzymatic, 0.4%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	30 min at 50°C	1 - 2 log	100% (8 of 8 hamsters)	[20]
Enzymatic cleaner (Klenzyme, 0.8%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	5 min at 43°C	3.5 log	100% (10 of 10 hamsters)	[17]
Various enzymes	Treated human vCJD brain homogenate followed by direct contamination of mouse brains (wild type CD-1 mice)	60 min at 40°C	Unknown	0% (0 of 10 mice)	[18]
Various enzymes	Treated human vCJD brain homogenate followed by direct contamination of mouse brains (TG 20 mice)	60 min at 40°C	Unknown	5.6% (1 of 18 mice) or 0% (0 of 3 mice)	[18]

CJD, Creutzfeldt–Jakob disease; vCJD, variant Creutzfeldt–Jakob disease.

largely effective in reducing prion protein PrP on contaminated wires within 5 min but left substantial amounts of residual PrP in the cleaning solution [27].

Peracetic acid

Peracetic acid has only little efficacy for prevention of prion protein transmission in animal experiments (Table VI). In addition it was shown that peracetic acid at 0.1% and 0.25% was not effective in reducing PrP on contaminated wires within 60 min [27]. Peracetic acid at 0.35% (30 min exposure at 20°C) has also been described to have a strong fixation potential of brain homogenate when tested on frosted glass carriers [28].

Hydrogen peroxide

Hydrogen peroxide in solution has only little efficacy for prevention of transmission in animal experiments. In combination with copper, the results are better but not entirely positive [19]. Data with vaporized hydrogen peroxide indicate sufficient efficacy when the concentration is at least 2 mg/L or a cleaning step precedes vaporization (Table VII). It has been described before that the effect on prion inactivation of vaporized hydrogen peroxide strongly depends on its concentration [29].

Sodium hydroxide

Sodium hydroxide has inconsistent efficacy for prevention of transmission in animal experiments in concentrations up to 0.5 M. At 1 M or higher, almost all data indicate complete prevention of transmission in animal experiments. Sodium hydroxide at 1 M was also effective in completely reducing PrP on contaminated wires within 5 min (Table VIII). No residual PrP was detected in the cleaning solution. Sodium hydroxide at 0.5 M has a similar effect on the wires but PrP was detectable in the cleaning solution. Sodium hydroxide at 0.1 M left residual PrP on contaminated wires even after 60 min of treatment [27].

Sodium hypochlorite

Sodium hypochlorite has inconsistent efficacy for prevention of transmission in animal experiments. Concentrations of 2000 and 5000 mg/L (or ppm) have poor prion efficacy whereas at 20,000 mg/L or higher the effect is very good (Table IX). Sodium hypochlorite at 10,000 and 25,000 mg/L was

Table V
Efficacy of alkaline cleaners to prevent prion transmission in animal experiments

Type of product	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Alkaline cleaner (1%) at pH 12 (neodisher FA forte)	Brain homogenates (5%), prepared from terminally ill scrapie-infected hamsters	30 min at 20°C or 55°C (in suspension)	Unknown	0% (0 of 35 hamsters)	[23]
Alkaline cleaner (Hamo 100, 0.2%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	5 min at 55°C	2.8 log	100% (8 of 8 hamsters)	[20]
Alkaline cleaner (Hamo 100, 0.4%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	5 min at 55°C	4.4 log	57% (4 of 7 hamsters)	[20]
Alkaline cleaner (Hamo 100, 0.8%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	7.5 min at 43°C	>4.9 log	0% (0 of 7 hamsters)	[20]
Alkaline cleaner (Hamo 100, 0.8%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	7.5 min at 43°C	≥5.6 log	0%*	[16]
Alkaline cleaner (Hamo 100, 1.6%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	15 min at 43°C	>5.6 log	0% (0 of 11 hamsters)	[17]
Alkaline cleaner (Hamo 100, 1.6%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	15 min at 43°C	≥5.6 log	0%*	[16]
Alkaline cleaner (Hamo 100, 1.6%)	Wires contaminated with the BSE strain 6PB1 adapted to mice implanted into the prefrontal subcortical region of mice	15 min at 43°C	≥5.5 log	0%*	[16]
Alkaline detergent (Anios)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	10 min at 20°C	≥ 5.25 log	0% (0 of 8 hamsters)	[19]
Alkaline cleaner (ProKlenz-One, 0.8%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	10 min at 25°C	> 5.1 log	0% (0 of 6 hamsters)	[20]
Mild alkaline detergent (Prolystica 2X alkaline, 0.4%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	5 min at 65°C	2.1 log	100% (8 of 8 hamsters)	[20]
Mild alkaline detergent (Prolystica 10X alkaline, 0.08%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	5 min at 65°C	2 log	100% (8 of 8 hamsters)	[20]
Mild alkaline detergent (Prolystica 10X alkaline, 0.16%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	2 min at 65°C	4.8 log	40% (2 of 5 hamsters)	[20]
Mild alkaline detergent (Valsure alkaline, 2.4%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	5 min at 65°C	Unknown	100% (6 of 6 hamsters)	[20]

BSE, bovine spongiform encephalopathy.
* No further information available.

Table VI
Efficacy of peracetic acid to prevent prion transmission in animal experiments

Type of product	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Peracetic acid bath (0.35%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	5 min	Unknown	100% (10 of 10 hamsters)	[22]
Peracetic acid, formulated (Steris 20 at use dilution of 0.25%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	12 min at 55°C	3.5 log	100% (12 of 12 hamsters)	[17]
Peracetic acid (600 ppm) plus copper sulfate (500 μmol/L) AFTER two cleaning steps of 10 min and 5 min at 40°C	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	10 min at 40°C	3.4 log	67% (probably 6 of 9 hamsters)	[19]
Peracetic acid (1500 ppm)	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	20 min at 20°C	Unknown	61% (14 of 23 mice)	[25]

also effective in completely reducing PrP on contaminated wires within 5 min. No residual PrP was detected in the cleaning solution [27]. Sodium hypochlorite at 20,000 mg/L (30 min exposure at 20°C) has been described to have a low fixation potential of brain homogenate when tested on frosted glass carriers [28].

Autoclaving

Specific autoclaving may have sufficient efficacy to prevent prion transmission in animal experiments. At 121°C some data indicate a good efficacy, other results describe a 33% transmission rate. Autoclaving at 134°C may reveal a good efficacy even in 4 min when pre-cleaning with alkaline agents is performed whereas data with 18 min indicate variable transmission rates between 0% and 70%. At ≥1 h transmission can be prevented at 134°C (Table X).

Other biocidal agents

Glutaraldehyde at 2% has insufficient efficacy to prevent prion transmission in animal experiments (Table XI). Glutaraldehyde at 2% (30 min exposure at 20°C) has also been described to have a moderate fixation potential of brain homogenate when tested on frosted glass carriers [28]. Some phenolic disinfectants have insufficient and some a better efficacy to prevent prion transmission in animal experiments. The effect seems to depend very much on the composition of the product (Table XI). Guanidine thiocyanate at 4 M has sufficient efficacy to prevent prion transmission in animal experiments but required 16 h in this study (Table XI).

Automated cleaning and disinfection in washer-disinfectors

Specific automated processes may have sufficient efficacy to prevent prion transmission in animal experiments (Table XII). In another study, surgical-grade stainless steel wires were inoculated with ME7 scrapie homogenate in order to assess residual contamination using simulated washer-disinfector cycles. Both total protein and prion-associated amyloid were measured. Immediate reprocessing following contamination was beneficial during the pre-treatment phase with either an enzymatic or pre-soak wetting agent. Cycles involving a pre-treatment with either an enzymatic cleaner or pre-soak, whether the soil was allowed to dry or not, showed complete removal of detectable prion amyloid. Based on these results, it was postulated that current decontamination procedures, combined with immediate processing of surgical instruments, have the potential to be highly effective alone at reducing the risk of surgical transmission of CJD [30].

Efficacy data and the risk of transmission in neurosurgery

In the research summarized above, the researchers have either used contaminated wires or brain homogenate itself that were treated with chemicals or processes. Afterwards, the wires or the tissue were directly implanted into animal brains. The experimental setting simulates the possible transmission in neurosurgery. This type of transmission of CJD has even been shown in humans. An accidental transmission of sporadic CJD into two persons via an intracerebral electrode was reported in 1974. The electrode was initially inserted for approximately 2 h into the cortex of an unrecognized CJD patient and decontaminated after each use with benzene, 70% ethanol and formaldehyde vapour. It was then used in succession on two additional patients who subsequently developed CJD. After these events the tip of the electrode was implanted into the brain of a chimpanzee where it again caused lethal spongiform encephalopathy [31,32]. The decontamination was certainly not appropriate based on current knowledge about validated reprocessing of neurosurgical instruments. Another report supports this view.

Table VII
Efficacy of hydrogen peroxide to prevent prion transmission in animal experiments

Type of product	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Hydrogen peroxide (6%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	1 h at 20°C	1 log	100% (11 of 11 hamsters)	[15]
Hydrogen peroxide (59%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	10 min	Unknown	30% (3 of 10 hamsters)	[22]
		20 min		40% (4 of 10 hamsters)	
Gas plasma sterilizer (Sterrad, standard cycle without cleaning)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	Unknown	Unknown	100% (9 of 9 hamsters)	[22]
Gas plasma sterilizer (Sterrad, standard cycle after enzymatic detergent wash 1:50)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	Unknown	Unknown	100% (10 of 10 hamsters)	[22]
Gas plasma sterilizer (Sterrad, 4 injections cycle after enzymatic detergent bath 1:1)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	Unknown	Unknown	87.5% (7 of 8 hamsters)	[22]
Gas plasma sterilizer (Sterrad, 4 injections cycle after alkaline detergent wash pH 11 at 70°C)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	Unknown	Unknown	0% (0 of 9 hamsters)	[22]
Hydrogen peroxide (7.5%) plus copper sulfate (500 μmol/L)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	30 min at 20°C	≥5.25 log	0% (0 of 8 hamsters)	[19]
Hydrogen peroxide (7.5%) plus copper sulfate (500 μmol/L) AFTER two cleaning steps of 10 min and 5 min (3 variations of cleaners)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	15 min at 20°C	4.55 to ≥5.25 log	0–43% (8 hamsters per group)	[19]
Hydrogen peroxide (vaporized at 1–1.5 mg/L)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	3 h at 25°C	4.5 log	33% (4 of 12 hamsters)	[17]
Hydrogen peroxide (vaporized at 2 mg/L)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	3 or 6 pulses of 5 min at 30°C	>5.5 log	0% (0 of 8 hamsters)	[15]
Hydrogen peroxide (vaporized at 2 mg/L)	Wires contaminated with the BSE strain 6PB1 adapted to mice implanted into the prefrontal subcortical region of mice	3 pulses of 5 min at 30°C	>5.5 log	0% (0 of 9 mice)	[15]
Hydrogen peroxide (vaporized at 2 mg/L)	Wires contaminated with the BSE strain TGB1 adapted to mice implanted into the prefrontal subcortical region of mice	3 pulses of 5 min at 30°C	>5.3 log	0% (0 of 9 mice)	[15]
Vaporized hydrogen peroxide at 1–1.5 mg/L AFTER cleaning with an enzymatic cleaner (5 min at 43°C)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	3 h at 25°C	5.6 log	0% (0 of 11 hamsters)	[17]

BSE, bovine spongiform encephalopathy.

Table VIII
Efficacy of sodium hydroxide to prevent prion transmission in animal experiments

Type of product	Organic material used	Exposure	Log reduction	Transmission rate	Reference
NaOH (0.1 N)	Brain homogenates (5%), prepared from terminally ill scrapie-infected hamsters	30 min at 20°C or 55°C (in suspension)	Unknown	0% (0 of 35 hamsters)	[23]
NaOH (0.1 M)	Scrapie ME7 brain homogenate used for intracerebral inoculation in mice	2 min at 60°C	4.0 log	25% (1 of 4 mice)	[24]
NaOH (0.15 M)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	1 h at 25°C	4.4 log	55% (6 of 11 hamsters)	[20]
NaOH (0.2 M)	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brain	1 h at 20°C	Unknown	50% (1 of 2 hamsters)	[26]
NaOH (0.25 M)	Scrapie ME7 brain homogenate used for intracerebral inoculation in mice	1 h at 30°C	3.9 log	40% (2 of 5 mice)	[24]
NaOH (0.5 M)	Scrapie ME7 brain homogenate used for intracerebral inoculation in mice	1 h at 30°C	≥4.2 log	0% (0 of 5 mice)	[24]
NaOH (0.5 M)	Scrapie ME7 brain homogenate used for intracerebral inoculation in mice	75 min at 30°C	4.2 log	20% (1 of 5 mice)	[24]
NaOH (0.5 N)	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	60 min at 20°C	Unknown	79% (19 of 24 mice)	[25]
NaOH (1 N)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	1 h at 20°C	>5.6 log	0% (0 of 12 hamsters)	[17]
NaOH (1 N)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	1 h at 20°C	>5.1 log	0% (0 of 12 hamsters)	[20]
NaOH (1 N)	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	15 min at 20°C	Unknown	0% (0 of 24 mice)	[25]
NaOH (1 M)	Treated scrapie brain homogenate followed by direct contamination of mouse brains	1 h at 25°C	Unknown	0% (0 of 6 mice)	[31]
NaOH (1 M)	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brain	1 h at 20°C	Unknown	17% (1 of 6 hamsters)	[26]
NaOH (2 M)	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brain	1 h at 20°C	Unknown	0% (0 of 6 hamsters)	[26]

In 2004 a patient underwent two brain surgeries and finally died from CJD. A total of 1056 neurosurgical patients were contacted who had their surgery within the 18 months following the index patient. Until 2013 the incident had not resulted in a reported iatrogenic case [33].

Facing a similar but larger-scale challenge in 2001 with the vCJD scare, the Economics and Operational Division of the Department of Health in the UK prepared a sequential operations model to estimate how many secondary infections could be expected to result from one operation on an infected patient by tracking infective material through the clinical system. It used a simple sequential algebraic approach to estimate the amount of infectious unit transfer after each cycle of cleaning. The model assumed an average of 10 mg of infected material on each instrument, as suggested by the Advisory Committee on Dangerous Pathogens and Spongiform Encephalopathy working group, with 20 instruments used per operation and each contacting the

Table IX
Efficacy of sodium hypochlorite to prevent prion transmission in animal experiments

Type of product	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Sodium hypochlorite (2000 mg/L)	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	15 min at 20°C	Unknown	88% (21 of 24 mice)	[25]
Sodium hypochlorite (2000 mg/L)	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	30 min at 20°C	Unknown	92% (22 of 24 mice)	[25]
Sodium hypochlorite (2000 mg/L)	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brain	1 h at 20°C	Unknown	100% (3 of 3 hamsters)	[26]
Sodium hypochlorite (5000 mg/L)	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	5 min at 20°C	Unknown	79% (19 of 24 mice)	[25]
Sodium hypochlorite (5000 mg/L)	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	15 min at 20°C	Unknown	83% (20 of 24 mice)	[25]
Sodium hypochlorite (5000 mg/L)	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brain	1 h at 20°C	Unknown	67% (2 of 3 hamsters)	[26]
Sodium hypochlorite (20,000 mg/L)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	1 h at 20°C	>5.6 log	0% (0 of 8 hamsters)	[17]
Sodium hypochlorite (20,000 mg/L)	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brain	1 h at 20°C	Unknown	0% (0 of 6 hamsters)	[26]
Sodium hypochlorite (25,000 mg/L)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	1 h at 20°C	>5.1 log	0% (0 of 8 hamsters)	[20]

same type of tissue as the index case. Each instrument may stay in the same set or may move to another set. The first decontamination cycle was assumed to result in a 10^5-fold infectious burden reduction, with each subsequent cycle resulting in a 10-fold reduction. These conservative estimates were based on studies of removal of protein soils from medical devices and autoclave inactivation of prions. Even neurosurgical instruments that have been used in patients with CJD or vCJD are therefore most likely safe after six cycles of regular processing based on a risk assessment in a patient with clinically suspected CJD [33]. The assumptions are supported by animal experiments showing that infective brain tissue diluted to 10^{-6} does not cause prion disease in mice even when inoculated permanently into the brain [25].

Invasive procedures during endoscopy: an additional risk?

The guideline of the British Society for Gastroenterology provides a definition of invasive procedures in endoscopy: any endoscopic procedure that breaches gut mucosa and is followed by the withdrawal of an unsheathed accessory through the working channel of an endoscope is deemed 'invasive'. Procedures that cause tissue vaporization (e.g., diathermy) are also deemed 'invasive" [34]. In Canada and the UK, quarantine of endoscopes is recommended after use in patients with definite or probable vCJD [34–36]. The risk of prion transmission by invasive procedures, however, is not clear. By the end of 2017, two healthcare workers and one laboratory worker had been reported in the UK with prion-disease exposures as a result of needle stick or sharp injuries. None subsequently developed prion disease, suggesting that the risk overall is small [37].

The case of nasal contact

The relevance of the nasal cavity and the olfactory nerve is described inconsistently. Whereas in Australia it is stated that normal nasal endoscope procedures do not reach the olfactory epithelium [38], experts in other countries such as

Table X
Efficacy of automated processes to prevent prion transmission in animal experiments

Type of procedure	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Autoclaving	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brain	1 h at 100°C	Unknown	100% (3 of 3 hamsters)	[26]
Autoclaving	Treated scrapie brain homogenate followed by direct contamination of mouse brains	20 min at 121°C	Unknown	0% (0 of 6 mice)	[31]
Autoclaving with wires immersed in water AFTER cleaning with an enzymatic cleaner (5 min at 43°C)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	20 min at 121°C	5 log	10% (1 of 10 hamsters)	[17]
Autoclaving	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	30 min at 121°C	Unknown	0% (0 of 24 mice)	[25]
Autoclaving	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brains	1 h at 121°C	Unknown	33% (2 of 6 hamsters)	[26]
Autoclaving	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	4 min at 134°C	3.5–4.8 log	37–100% (3 of 8 and 8 of 8 hamsters)	[20]
Autoclaving AFTER cleaning with an enzymatic cleaner (5 min at 43°C)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	4 min at 134°C	4.4 log	57% (4 of 7 hamsters)	[20]
Autoclaving AFTER cleaning with a mild alkaline cleaner (Prolystica 2X alkaline 0.4%, 5 min at 65°C)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	4 min at 134°C	4.9 log	29% (2 of 7 hamsters)	[20]
Autoclaving AFTER cleaning with a mild alkaline cleaner (Prolystica 10X alkaline 0.08% for 5 min or 0.16% for 2 min at 65°C)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	4 min at 134°C	>5.1 log	0% (0 of 6 and 0 of 3 hamsters)	[20]
Autoclaving AFTER cleaning with a mild alkaline cleaner (Valsure alkaline 2.4% for 5 min at 65°C)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	4 min at 134°C	>5.1 log	0% (0 of 8 hamsters)	[20]
Autoclaving without cleaning	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	18 min at 134°C	Unknown	10% (1 of 10 hamsters)	[22]
Autoclaving in a porous load cycle	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	18 min at 134°C	4–4.5 log	60% (6 of 10 hamsters)	[17]
Autoclaving with wires immersed in water	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	18 min at 134°C	>5.6 log	0% (0 of 11 hamsters)	[17]

(*continued on next page*)

Table X (*continued*)

Type of procedure	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Autoclaving	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	18 min at 134°C	4–4.5 log	70%*	[16]
Autoclaving	Wires contaminated with the BSE strain 6PB1 adapted to mice implanted into the prefrontal subcortical region of mice	18 min at 134°C	≥5.5	0%*	[16]
Autoclaving	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	18 min at 134°C	4.1 log	57% (probably 4 of 7 hamsters)	[19]
Autoclaving	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	18 min at 134°C	3.8 to >5.1 log	0–70% transmission (0 of 11 and 7 of 10 hamsters)	[20]
Autoclaving	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	18 min at 134°C	Unknown	0% (0 of 24 mice)	[25]
Autoclaving after enzymatic detergent wash 1:50	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	18 min at 134°C	Unknown	100% (10 of 10 hamsters)	[22]
Autoclaving after alkaline detergent wash pH 11 at 70°C	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	18 min at 134°C	Unknown	22% (2 of 9 hamsters)	[22]
Autoclaving after a 24 h NaOH bath (1 M)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the thalamus region of hamsters	18 min at 134°C	Unknown	20% (2 of 10 hamsters)	[22]
Autoclaving	Treated human vCJD brain homogenate followed by direct contamination of mouse brains (wild type CD-1 mice)	20 min at 134°C	Unknown	0% (0 of 9 mice)	[18]
Autoclaving	Treated human vCJD brain homogenate followed by direct contamination of mouse brains (TG 20 mice)	20 min at 134°C	Unknown	100% (13 of 13 mice) or 25% (1 of 4 mice)	[18]
Autoclaving	Treated scrapie brain 263K homogenate followed by direct contamination of hamster brains	1 h at 134°C	Unknown	0% (0 of 6 hamsters)	[26]
Autoclaving	Wires contaminated with infective 22L brain homogenate implanted into the basal ganglia of mice	2 h at 134°	Unknown	0% (0 of 8 mice)	[21]

BSE, bovine spongiform encephalopathy; vCJD, variant Creutzfeldt–Jakob disease.

* No further information available.

Table XI
Efficacy of glutaraldehyde, phenolic disinfectants and guanidine thiocyanate to prevent prion transmission in animal experiments

Type of procedure	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Glutaraldehyde 2%	Treated scrapie strain C506M3 brain homogenate followed by direct contamination of mouse brains	20 min at 20°C	Unknown	54% (13 of 24 mice)	[25]
Phenolic disinfectants (Environ LpH or LpHse, both at 5%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	30 min at 20°C	>5.6 log	0% (0 of 11 hamsters)	[17]
Phenolic disinfectant (Environ LpH at 5%)	Wires contaminated with hamster-adapted scrapie strain 263K implanted into the prefrontal subcortical region of hamsters	30 min at 20°C	≥5.6 log	0%*	[16]
Phenolic disinfectant (Environ LpH at 5%)	Wires contaminated with the BSE strain 6PB1 adapted to mice implanted into the prefrontal subcortical region of mice	30 min at 20°C	≥5.5 log	0%*	[16]
Phenolic disinfectant (Environ LpH at 1%)	Treated scrapie strain 263K brain homogenate followed by direct contamination of hamster brain	16 h at 20°C	Unknown	0% (0 of 3 hamsters)	[51]
Phenolic disinfectant (LpH-SE at 1%)	Treated scrapie strain 263K brain homogenate followed by direct contamination of hamster brain	16 h at 20°C	Unknown	100% (12 of 12 hamsters)	[51]
Guanidine thiocyanate (4 M)	Treated scrapie brain homogenate followed by direct contamination of mouse brains	16 h at 25°C	Unknown	0% (0 of 6 mice)	[31]

the UK, Germany and Canada see a major risk in endoscopic procedures passing the nasal cavity [35,36,39,40]. The olfactory epithelium has a size of approximately 3 cm^2 on each side [41]. It is found in the superior nasal concha, but it may also spread into the middle nasal concha and contains numerous olfactory filaments. These filaments reach the mucosal surface and are located between supporting cells. The most common path to introduce a flexible bronchoscope is with contact to the middle nasal concha (M. Laudien, personal communication). It seems therefore possible that the endoscope may have direct contact with the olfactory filaments. The epithelium, however, is covered with mucus which acts as a mechanical barrier. That is why it appears unlikely that a direct contact can occur between the outside of the endoscope and the olfactory filaments, although some of the superficial tissue may be scratched away while introducing or removing the endoscope.

Flexible endoscopes after use in patients with known or probable prion disease

Although transmission of prion disease by flexible endoscopes has so far never been reported and a transmission appears to be very unlikely, it seems appropriate to establish a pool of endoscopes that have been used before in prion disease patients. The University Hospital Göttingen, Institute for Neuropathologie (Germany) is such an example where endoscopes are provided and also reprocessed after use. Large hospitals or manufacturers of endoscopes may also be an option to contact for providing quarantined endoscopes.

Flexible endoscopes after use in patients with delayed suspected prion disease

A typical clinical situation is that an endoscopic procedure is performed in a patient not suspected for vCJD or CJD at that time (day 0). Thus, the endoscope will be treated as any other endoscope and reprocessed using a validated protocol. If sometime later, often 2–21 days, the patient is suspected as having a diagnosis of vCJD or CJD, the endoscope is often put into quarantine, and there is uncertainty on any additional steps regarding reprocessing of the endoscope and any additional treatment of the EWD. At this time it is an unconfirmed diagnosis, and it usually remains an unconfirmed diagnosis because in most cases an autopsy of a deceased patient with suspected prion disease will not be performed. What is the risk of transmission in this situation?

The index endoscope

Two days after use in an index patient and assuming an average use of the endoscope of at least two times per day, the index endoscopes has probably been used in four more patients in the meantime and the endoscope has also probably been reprocessed four times after use on the index patient. The potentially most dangerous time immediately after use at the index patient is already over on day 2. Still many guidelines recommend a strict protocol for the endoscope and the EWD as described below, probably because it is assumed that there may be some remaining risk of vCJD or CJD transmission by flexible endoscopy. Data obtained with

Table XII
Efficacy of automated processes to prevent prion transmission in animal experiments

Type of procedure	Organic material used	Exposure	Log reduction	Transmission rate	Reference
Washer disinfector G 7835; routine alkaline disinfection process	Wires contaminated with infective 22L brain homogenate implanted into the basal ganglia of mice	Normal alkaline wash with thermal disinfection	Unknown	37.5% (3 of 8 mice)	[21]
Washer disinfector G 7835; specially developed prion decontamination process	Wires contaminated with infective 22L brain homogenate implanted into the basal ganglia of mice	Normal alkaline wash with thermal disinfection PLUS additional intermediate oxidizing stage in combination with an alkaline detergent	Unknown	0% (0 of 8 mice)	[21]

neurosurgical instruments can help to assess the risk of transmission; it has been postulated that the risk of prion transmission is basically zero in used index instruments after four or six reprocessings although they had direct contact with brain tissue potentially containing a 100-fold or 1000-fold amount of prion protein compared to all tissue with relevance in flexible endoscopes. While the direct brain contact with reprocessed surgical instruments of a low technical complexity after use in known patients with prion disease was described to be of a low risk it is probably similar in flexible endoscopes of a high technical complexity but without direct brain contact.

Relevant elements for reprocessing flexible endoscopes

Some elements of reprocessing flexible endoscopes are considered to be of major relevance for further reducing the very low risk of transmission of prion disease via flexible endoscopes. They should be considered for routine implementation.

- Immediate bedside cleaning. Recommended in the UK and Germany [39,40]. This element may be particularly relevant in cases with nasal access. Besides prion disease, this is recommended anyway for routine reprocessing of flexible endoscopes and will help to immediately reduce as much bioburden as possible including prion protein.
- Routine use of at least mild alkaline cleaners. Recommended in Switzerland and by the ESGE [42,43]. Data obtained with contaminated wires placed into the brain of animals indicate that mild alkaline cleaners are less effective than strong alkaline cleaners (Table V). And yet they have an advantage over non-alkaline cleaning agents (Table IV). That is why it appears to be an additional safety step to use mild alkaline cleaners routinely. Double cleaning is recommended in France, but the routine use of alkaline cleaners is likely to be more effective due to the additional partial prion inactivation.
- No fixative agents anywhere prior to the disinfection step. Recommended in Germany and by the ESGE [40,42]. Brain homogenate can be fixed in 30 min with various chemicals. The strongest fixation was caused by 0.35% peracetic acid, it is moderate with 2% glutaraldehyde and low with 2% sodium hypochlorite [28].
- Validated protocol for reprocessing. Recommended in Austria [44]. This is recommended anyway as a standard for routine reprocessing of flexible endoscopes.
- Single use brushes and cleaning solutions per endoscope. Recommended in the UK and Australia [34,38,39]. Single-use cleaning solutions may be particularly relevant because even with alkaline cleaners substantial amounts of prion protein can be found in the cleaning solution indicating a successful removal from the contaminated wires [27].

Routine traceability is also advisable although it will have no preventive effect [34]. Quarantine would not be necessary any more in this setting.

Index EWD

The EWD used for reprocessing of the index endoscope may also be contaminated with prions, although no data have ever been published to demonstrate such a contamination. Assuming that an EWD is used throughout a working day it can be expected that approximately eight cycles were run per day. The potentially most contagious time in the EWD immediately after use at the index patient is already over on day 2 with approximately 17 cycles. Despite this, some guidelines recommend putting the EWD through an empty self-disinfecting cycle after use for an endoscope [34,39]. It remains difficult to understand how a very small amount of protein somewhere inside an EWD may yield during reprocessing a protein load on post-index flexible endoscopes which is considered to be of relevance for an oral transmission, especially after many runs of the EWD.

Relevant elements for the EWD

One element of reprocessing flexible endoscopes is considered to be of major relevance for further reducing the very low risk of transmission of prion disease via flexible endoscopes. It should be considered for routine implementation.

- Separate decontamination of each endoscope within the EWD. Recommended in the UK [39]. If there is any risk of transfer of prion protein from the index endoscope to another endoscope reprocessed at the same time in the same EWD it can only be controlled by separate reprocessing chambers or cycles.

Other endoscopes reprocessed in the index EWD

Assuming that two endoscopes can be reprocessed in an EWD at the same time it is likely that four or more other endoscopes have been reprocessed in the same EWD as the index endoscopes on day 0. Theoretically they may be contaminated with prion protein. Taking into account that the residual protein load on reprocessed endoscopes is already very low (as described above) it can only be assumed that the total residual protein load will not be higher on any other endoscopes reprocessed after the index endoscope. That is why it is not possible to see any plausible reason to put any of the post-index endoscopes in quarantine or to treat them with any special chemistry.

Limitations and future investigations

There is no direct evidence to support the proposal that a simplified processing is safe for all patients once a flexible endoscope is known to be used in a previous patient with delayed suspected prion disease. But based on the circumstantial evidence presented in this review, a number of good reasons can be seen to consider it appropriate for patient safety.

Manufacturers of flexible endoscopes and large healthcare providers may have flexible endoscopes in quarantine after use in patients with suspected or proven prion disease. In cases where there is no intention for further use, it is suggested that they should be donated to a research facility which is able to determine and quantify prion protein on inanimate surfaces. This type of evidence will further help to assess the potential risk of transmission whatever the result will be.

In conclusion, the risk of prion disease transmission by reprocessed flexible endoscopes including duodenoscopes used for endoscopic retrograde cholangiopancreatography is extremely low. Based on circumstantial evidence, it is proposed that careful routine performance of immediate bedside cleaning, use of mild alkaline cleaners, strict avoidance of any fixative agents anywhere before the disinfection step, using a validated protocol for reprocessing including using brushes and cleaning solutions only once per endoscope is very likely sufficient for patient safety to further reduce the risk in flexible endoscopes after use in patients with delayed suspected prion disease.

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Conflict of interest statement
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