

## REVIEW ARTICLE

# Inactivation of Animal and Human Prions by Hydrogen Peroxide Gas Plasma Sterilization

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Prions cause various transmissible spongiform encephalopathies. They are highly resistant to the chemical and physical decontamination and sterilization procedures routinely used in healthcare facilities. The decontamination procedures recommended for the inactivation of prions are often incompatible with the materials used in medical devices. In this study, we evaluated the use of low-temperature hydrogen peroxide gas plasma sterilization systems and other instrument-processing procedures for inactivating human and animal prions. We provide new data concerning the efficacy of hydrogen peroxide against prions from *in vitro* or *in vivo* tests, focusing on the following: the efficiency of hydrogen peroxide sterilization and possible interactions with enzymatic or alkaline detergents, differences in the efficiency of this treatment against different prion strains, and the influence of contaminating lipids. We found that gaseous hydrogen peroxide decreased the infectivity of prions and/or the level of the protease-resistant form of the prion protein on different surface materials. However, the efficiency of this treatment depended strongly on the concentration of hydrogen peroxide and the delivery system used in medical devices, because these effects were more pronounced for the new generation of Sterrad technology. The Sterrad NX sterilizer is 100% efficient (0% transmission and no protease-resistant form of the prion protein signal detected on the surface of the material for the mouse-adapted bovine spongiform encephalopathy 6PB1 strain and a variant Creutzfeldt-Jakob disease strain). Thus, gaseous or vaporized hydrogen peroxide efficiently inactivates prions on the surfaces of medical devices.

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The physicochemical and biological properties of prions make them atypical transmissible pathogenic agents. Prions cause transmissible spongiform encephalopathies, also known as prion diseases. These diseases are associated with the accumulation in the brain of an abnormal isoform of a host protein, the prion protein (PrP). The precise nature of the infectious agent remains unclear. However, fibrils obtained from recombinant PrP have been shown to be infectious, and current evidence suggests that abnormally processed PrP may be an intrinsic component of the infectious agent.<sup>1</sup> During disease progression, the normal cellular PrP isoform is converted into the abnormal pathologic PrP<sup>Sc</sup> isoform. The mechanism by which this transconformational change takes place remains unclear. The pathologic PrP<sup>Sc</sup> isoform is partially resistant to proteases, including proteinase K in particular, and is therefore sometimes called PrPres.<sup>2,3</sup>

The transmissible spongiform encephalopathies affecting humans encompass various forms of Creutzfeldt-Jakob disease (CJD), including the variant form (vCJD). The pathogenesis of vCJD differs significantly from that of other forms of CJD. Prions (PrP<sup>Sc</sup> or infectivity) are systematically detected in the peripheral tissues (in the lymphoreticular system

and blood) of patients with vCJD, whereas they are not detected in the peripheral tissues of patients with classical CJD or inherited prion disease (see Wadsworth and Collinge<sup>4</sup> for review). Three cases of transfusion-associated vCJD prion infection have been reported.<sup>5-7</sup> This distinctive pathogenesis of vCJD and the possibility of silent infection have raised concerns that there may be a high risk of iatrogenic vCJD transmission via blood or blood derivatives and contaminated surgical and medical instruments, including endoscopes<sup>8-11</sup> and cerebral electrodes.

Prions are highly resistant to classical chemical and physical procedures for inactivating or eliminating conventional pathogens.<sup>12</sup> For example, the bovine spongiform encephalopathy (BSE) agent can remain in carcasses if an inappropriate and ineffective inactivation method is used during the preparation of meat and bone meals. Only severe treatments, such as sodium hydroxide, sodium hypochlorite, steam autoclaving (134°C for 18 minutes), or combinations of these procedures, are partly or completely effective against prions. Unfortunately, these procedures are not compatible with most medical devices, which are often made of materials sensitive to heat or moisture. Hydrogen peroxide is routinely used in hospital

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sterilization procedures, as it is an effective biocide in both liquid and gaseous forms. However, a lack of scientific results led to the misclassification of hydrogen peroxide in guidelines concerning prions, particularly French guideline 138.<sup>13</sup> Prions and PrP are nonetheless potentially interesting targets for hydrogen peroxide. Indeed, the PrP binds several metal ions in its N-terminal octapeptide repeat region.<sup>14</sup> As in the Fenton reaction (oxidation of metal ions by hydrogen peroxide to generate hydroxyl radicals and hydroxyl anions, and reduction of oxidized metal ions to generate peroxide radicals and protons by the same hydrogen peroxide), these metal ions generate a hydroxyl radical—a highly reactive oxygen species that directly damages nucleic acids and proteins—by reducing hydrogen peroxide. Recent studies, including preliminary experiments in our laboratory, have confirmed that prions may be susceptible to hydrogen peroxide, particularly in its gaseous form.<sup>15-17</sup> Thus, used alone or in combination with other mild methods, hydrogen peroxide may be a useful tool for eliminating the prion risk associated with medical materials.

The likelihood of prion inactivation depends on the prion strain and the nature of the surface concerned (eg, plastic or stainless steel) and of the inoculum (large lipid concentrations, as in brain homogenates, are known to protect PrPres from heat degradation,<sup>18</sup> for example).

This study provides new data on the efficacy of hydrogen peroxide for the elimination of prions. We considered, in particular, the efficiency of hydrogen peroxide treatment and its possible interactions with enzymatic or alkaline detergents, differences in efficacy between strains, and the influence of contaminating lipids, all of which were evaluated either *in vitro* or *in vivo*.

## METHODS

### Animals

Six- to eight-week-old female Syrian golden hamsters were housed in level 3 care facilities officially registered for experimental prion studies on rodents (agreements A92-032-02 for animal care facilities and 92-189 and for animal experimentation at CEA [Fontenay aux Roses, France] and registration numbers BFA 199, BFA 208, and FLI 217 for Service für Medizinprodukte [Tübingen, Germany]).

### Prion Strains and Inoculum Preparation

Three prion strains were evaluated during *in vitro* or *in vivo* experiments: the hamster-adapted scrapie 263K strain, the mouse-adapted BSE 6PB1 strain,<sup>19,20</sup> and a vCJD strain. The vCJD strain was extracted from the cerebral homogenate of a patient who died of CJD linked to BSE. The electrophoretic pattern of PrPres associated with this vCJD case was typical.

All brain extracts including the vCJD extract were homogenized in phosphate-buffered saline at 10% or 20% w/v. The sensitivity of Western blots was increased so that smaller differences could be observed between treatments, by

“overloading” 20% brain homogenate (by a factor of 10 or 50) with PrPres purified by a phosphotungstic acid precipitation method.<sup>11</sup> The vCJD extract was adjusted to a protein concentration of 3 mg/mL, and PrPres was also used as inoculum. All preparations for bioassays and *in vitro* experiments were carried out in accordance with safety recommendations for prions.

### Bioassays

Stainless steel wires (1.4301; diameter, 0.25 mm; Forestadent) were contaminated and implanted as described elsewhere.<sup>17</sup> Several dilutions of the 263K inoculum were also used to inoculate hamsters directly, for the comparison of incubation periods for contaminated wires with incubation periods for direct inoculation with brain homogenate.

Each group included 10 hamsters at the beginning of the study, housed in cages of up to 4 animals and initially examined twice weekly and then daily after 60 days postimplantation. Hamsters in the terminal stage of scrapie disease and at the end of the experiment (18 or 24 months) were euthanized. Whole brains were collected from all hamsters, and the presence of the abnormal form of PrP in the brain was evaluated by Western blotting. After PrPres purification, specific PrP<sup>Sc</sup> signals were visualized with the monoclonal 3F4 antibody (DAKO; performed by SMP) or with the directly HRP-conjugated monoclonal SAF83 antibody (ultrasensitive Western blot method<sup>21</sup>; performed by SPI-BIO) and chemiluminescence kits.

Bioassay data were analyzed by considering pathognomonic signs of transmissible spongiform encephalopathy and/or both Western blot methods, by means of the incubation time interval assay particularly for this hamster model that is described elsewhere.<sup>2,22</sup> Prion infectivity in each treatment group was calculated using the mean incubation period and the reduction factor (RF), determined as the ratio of prion infectivity before each treatment to the prion infectivity after each treatment. At the end of this bioassay, the minimum number of hamsters per group was 6 (mean, 8).

### In Vitro Experiments

Stainless steel, polypropylene, and polyethylene sheets (9 × 9 mm) were contaminated with various transmissible spongiform encephalopathy strains. Inoculum (20 μL) was adsorbed onto each sheet and dried for 16 hours before exposure (or the absence of exposure for controls) to various treatments. After treatment, the inoculum was efficiently desorbed, using the protocol described by Lemmer et al.<sup>23</sup> The various desorbed solutions were end point-diluted, and the amount of PrPres present was assessed by Western blotting with the PrP SAF83 antibody and the corresponding protocol, as described elsewhere.<sup>21</sup> A 50% PrPres titer was calculated for each desorbed solution by the Spearman-Kärber method.<sup>24</sup>

## Treatments

Wires or sheets were treated with various agents or processes, as described in Tables 1 and 2. Different hydrogen peroxide low-temperature gas plasma sterilizers (Sterrad 100S GMP [16 minutes, 50°C, 59%], 100S [20 minutes, 50°C, 59%], NX [7 minutes, 53°C, 90%], and 100NX [flex cycle: 16 minutes, 53°C, 80%; standard cycle: 16 minutes, 53°C, 90%] sterilizers; Advanced Sterilization Products) were tested, using routine cycles. Steam (134°C, 18 minutes) and the combination of steam (134°C, 18 minutes) after sodium hydroxide treatment (1 N, 1 hour) were used as controls. Cleaning agents were tested alone or in combination with steam or Sterrad systems (a 2% protease solution in enzymatic detergent was used in a washing machine at 20°C for 10 minutes and a 100% protease solution in a beaker was used as a bath at 20°C for 30 minutes; 2 alkaline KOH-based detergents were used, at either 55°C or 70°C for 10 minutes). After each detergent treatment, wires were rinsed once with 1X phosphate-buffered saline and then washed 3 times with double-distilled water.

Treatments with an RF of 4 to 6 log or more were considered to be highly efficient, and the decontamination was considered to be complete when no positive animal (ie, transmission rate of 0% in bioassays) or PrPres signal (for in vitro Western blot analyses) was identified in the group. An RF of 2 to 3 log was considered to indicate a process that was only partially effective.

## RESULTS

### Bioassays

As described elsewhere,<sup>2,22</sup> stainless steel wires artificially contaminated with the 263K strain infected hamsters efficiently (Figure 1). We also confirmed that time intervals as short as 5 minutes were sufficient to contaminate animals

and that stainless steel wires were an excellent vector for prion transmission.

The efficiency of the various treatments tested was determined on the basis of these data, through a modification of the incubation-period assay described by Bolton et al<sup>2</sup> and by Müller et al.<sup>22</sup> In our experimental conditions, a reduction of infectivity of 1 log corresponds to a prolongation of the incubation period by 14.5 days. This prolongation is consistent with data reported by Bolton et al<sup>2</sup> and by Müller et al<sup>22</sup> for the hamster 263K model. For dilutions greater than 10<sup>-5</sup> (ie, hamsters surviving for more than 140 days), the residual infectivity of wires was assessed on the basis of transmission rates for each group of animals.

### Efficiency of Reference Treatments

The 2 reference methods tested in this study—steam (134°C, 18 minutes) and sodium hydroxide (1 N, 1 hour at room temperature) followed by steam—proved effective (at least 5–6 log) although residual infectivity persisted (eg, 50% for steam alone) (Table 1).

### Efficiency of Liquid and Plasma Hydrogen Peroxide Alone (In Vivo and In Vitro Data)

The immersion of contaminated stainless steel wires in 59% liquid hydrogen peroxide at room temperature for 10 or 20 minutes reduced infectivity significantly by 5–6 log; nevertheless, 50% of hamsters developed scrapie disease (Table 2).

By contrast, treatment with the Sterrad 100S regular long cycle (59% gaseous hydrogen peroxide) without precleaning resulted in only a 1.1-log reduction of infectivity (Table 2). Differences in cycle parameters between the Sterrad 100S long cycle (routine machine) and the Sterrad 100S GMP cycle (machine allowing more flexibility for scientific investigations) had no significant effect on efficacy against prions (Ta-

TABLE 1. Transmission Rates and Incubation Periods in the Control Group and Efficiency of Reference Methods in the Hamster-Adapted Scrapie 263K Strain Model

	Transmission rate, %	Incubation period, mean ± SD, days	RF, log
Negative control	0	606 ± 118 <sup>a</sup>	...
Positive control	100	83 ± 3	...
10% 263K-infected brain homogenate	100	78 ± 2	...
Wires implanted for 5 min	100	101 ± 5	1.5
Steam (134°C for 18 min)	50	428 ± 103	≥5 to 6
1 N sodium hydroxide for 1 h at room temperature followed by steam	28	554 ± 197	≥5 to 6

NOTE. Each group included 10 animals at the beginning of the study and a mean of 8 animals at the end of the study. Transmission rate and incubation period were calculated considering intercurrent animals (ie, death unrelated to transmissible spongiform encephalopathy). Moreover, bioassay data were analyzed by considering pathognomonic signs of transmissible spongiform encephalopathy and/or both Western blot methods (abnormal pathologic prion protein isoform signals were visualized with the monoclonal 3F4 antibody [DAKO; performed by Service für Medizinprodukte<sup>38</sup>] or by direct visualization with the HRP-conjugated monoclonal SAF83 antibody [ultrasensitive Western blot method; performed by SPI-BIO]). RF, reduction factor. SD, standard deviation.

<sup>a</sup> Some animals died of old age before being euthanized at approximately 740 days of age, accounting for the mean age (± SD) of 606 ± 118 days after inoculation, rather than the expected 740 days (no PrPres was detected in the brains of these hamsters).

TABLE 2. Transmission Rates and Incubation Periods for Groups of Animals Exposed to Sterrad Machines of Different Generations, Alone or in Combination With Enzymatic and Alkaline (A, B) Detergents, in the Hamster-Adapted Scrapie 263K Strain Model

Variable	Transmission rate, %	Incubation period, mean $\pm$ SD, days	RF, log
59% hydrogen peroxide 10 min room temperature	50	443 $\pm$ 140	$\geq 5$ to 6
59% hydrogen peroxide 20 min room temperature	50	428 $\pm$ 142	$\geq 5$ to 6
Sterrad 100S GMP 1 long cycle	100	96 $\pm$ 4	1.1
Sterrad 100S 1 long cycle	100	99 $\pm$ 6	1.3
Sterrad 100S 2 consecutive long cycles	100	104 $\pm$ 8	1.8
Sterrad NX 1 advanced cycle	0	570 $\pm$ 18 <sup>a</sup>	$\geq 5$ to 6
Sterrad NX 2 consecutive advanced cycles	0	574 $\pm$ 0	$\geq 5$ to 6
Enzymatic detergent (2%) 10 min 37°C	100	95 $\pm$ 0	1.1
Enzymatic detergent (100%) 30 min 37°C	100	94 $\pm$ 2	1.0
Enzymatic detergent (100%) 24 h 37°C	100	93 $\pm$ 1	0.9
Enzymatic detergent (2%) 10 min 37°C followed by steam (134°C, 18 min)	100	131 $\pm$ 17	4.0
Enzymatic detergent (2%) 10 min 37°C followed by Sterrad 100S 1 long cycle	100	111 $\pm$ 12	2.4
Enzymatic detergent (100%) 30 min 37°C followed by Sterrad 100S 2 consecutive long cycles	67	211 $\pm$ 125	$\geq 5$ to 6
Alkaline detergent A (1%) 10 min 55°C	11	446 $\pm$ 153	$\geq 5$ to 6
Alkaline detergent A (1%) 10 min 70°C followed by steam (134°C, 18 min)	0	525 $\pm$ 149	$\geq 5$ to 6
Alkaline detergent A (1%) 10 min 55°C followed by Sterrad 100S 1 long cycle	0	540 $\pm$ 14	$\geq 5$ to 6
Alkaline detergent A (1%) 10 min 55°C followed by Sterrad 100S 2 consecutive long cycles	0	540 $\pm$ 30	$\geq 5$ to 6
Alkaline detergent A (1%) 10 min 70°C followed by Sterrad 100S 2 consecutive long cycles	0 <sup>a</sup>	508 $\pm$ 57	$\geq 5$ to 6
Alkaline detergent A (1%) 10 min 55°C followed by Sterrad NX 1 advanced cycle	0	559 $\pm$ 22	$\geq 5$ to 6
Alkaline detergent B (1%) 10 min 55°C	0	524 $\pm$ 42	$\geq 5$ to 6
Alkaline detergent B (1%) 10 min 55°C followed by Sterrad 100S 1 long cycle	0	540 $\pm$ 13	$\geq 5$ to 6
Alkaline detergent B (1%) 10 min 55°C followed by Sterrad 100S 2 consecutive long cycles	0	552 $\pm$ 0 <sup>b</sup>	$\geq 5$ to 6
Alkaline detergent B (1%) 10 min 55°C followed by Sterrad NX 1 advanced cycle	0	562 $\pm$ 16	$\geq 5$ to 6

NOTE. Each group included 10 animals at the beginning of the study and a mean of 8 animals at the end of the study. Transmission rate and incubation period were calculated considering intercurrent animals (ie, death unrelated to transmissible spongiform encephalopathy). RF, reduction factor; SD, standard deviation.

<sup>a</sup> One hamster died of natural causes at 529 days, and Western blots of the brain for PrPres were negative.

<sup>b</sup> Animals euthanized at 552 days.

ble 2). When 2 consecutive Sterrad 100S long cycles were applied (exposure time of 40 minutes), infectivity was reduced by 1.8 log rather than 1.3 log, but transmission rates remained at 100%.

Unlike the Sterrad 100S system, the Sterrad NX system advanced cycle abolished infectivity on the surface of the segments (Table 2). The use of 2 consecutive Sterrad NX system advanced cycles, corresponding to exposure for 14 minutes, was at least as effective as 1 single cycle, but it was not possible to quantify an increase in efficiency, because 1 cycle was already fully effective (Table 2).

The efficacy of the Sterrad NX system was confirmed in vitro against the 263K strain (which was used to link in vitro and in vivo assays), the mouse-adapted BSE 6PB1 strain, and a vCJD strain. Stainless steel sheets used for in vitro assays were artificially contaminated, and PrPres was titrated by end point–dilutions of various desorbed solutions. PrPres signals were detected at dilutions of up to  $10^{-5}$  for untreated samples (50% PrPres titer,  $5.4 \pm 0.75$  log) (Figure 2A). PrPres was

detected on 3 of the 8 stainless steel sheets treated with the Sterrad 100S sterilizer (50% PrPres titer,  $3.5 \pm 0.7$  log for the 3 positive sheets) (Figure 2A). No PrPres was detected in the desorbed solutions obtained with the steam and Sterrad NX system, even without dilution. With respect to control values, RFs of only 4 log (only 1.9 log for positive sheets) were obtained for 100S, at least 5.4 log were obtained for steam, and at least 5.4 log were obtained for NX (Figure 2A). The in vitro data obtained with the 263K strain for treatments other than steam were consistent with those obtained in vivo. Indeed, the Sterrad NX system was found to be more efficient than the Sterrad 100S system. In addition to the PrP triplet bands corresponding to 3 levels of PrP glycosylation, larger amounts of aggregates of high molecular weight were detected, particularly after treatment with the Sterrad 100S system.

Many factors affect the inactivation of prions by physical and chemical procedures, including smearing, the prion strain, and the type of surface (eg, stainless steel or polypro-

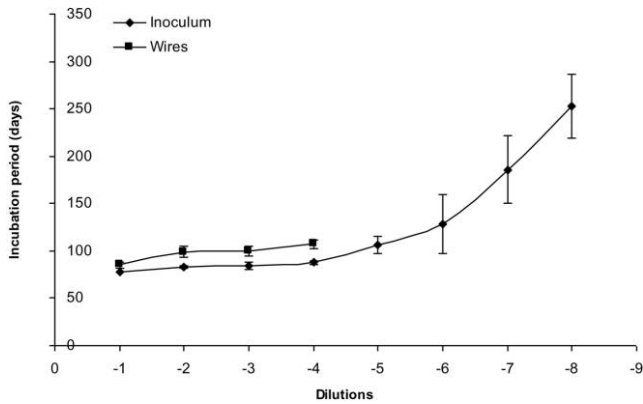


FIGURE 1. Relationship between the incubation period and dilutions of the hamster-adapted scrapie 263K strain inoculum (ie, prion infectivity) adsorbed onto wires (“wires”) or not adsorbed (“inoculum”): confirmation of the validity in our experimental conditions of the incubation time interval assay described elsewhere.<sup>2,22</sup> Results are representative of 2 independent experiments.

pylene). These factors were explored individually in experiments performed *in vitro*. Mammalian brain tissues are rich in fat, and lipids are known to protect PrPres against heat degradation.<sup>18,22</sup> We therefore carried out the same experiment described above but with stainless steel sheets contaminated with PrPres. The abnormal PrP was detected at dilutions of up to  $10^{-3}$  in untreated controls. No PrPres signal was detected (Figure 2B) for any of the treatments; this fact suggests that the Sterrad 100S system is more efficient in the absence than in the presence of lipids.

We assessed differences in sensitivity between prion strains by evaluating the efficiency of the Sterrad 100S and NX systems against the mouse-adapted BSE 6PB1 strain and a vCJD strain. No PrPres signal was detected for either strain after exposure to steam or to either of the 2 Sterrad systems (Figure 3); this fact suggests differences in sensitivity between the mouse-adapted BSE 6PB1, vCJD, and 263K strains.

As for stainless steel sheets, classic 263K PrPres electrophoretic profiles were observed in solutions desorbed from untreated polypropylene and polyethylene sheets (Figure 4). High-molecular weight signals corresponding to PrPres aggregates were also observed. PrPres signals were detected at dilutions of up to  $10^{-5}$  or  $10^{-6}$  for untreated samples (50% PrPres titers,  $5.1 \pm 0.55$  log for stainless steel,  $6.94 \pm 0.53$  log for polyethylene, and  $6.44 \pm 0.67$  log for polypropylene sheets), confirming that prions adsorbed well on plastic materials. No PrPres signal was detected on any of the surfaces tested with the Sterrad NX system (RF, at least 5.1 log for stainless steel, 6.94 log for polyethylene, and 6.44 log for polypropylene). These results demonstrate the efficacy of the Sterrad NX system in the absence of prior treatment or washing in detergent.

This efficiency was confirmed with the Sterrad 100NX system, which uses the same technology. Indeed, no PrPres signal

was found in desorbed solutions obtained from stainless steel sheets artificially contaminated with the 263K strain and treated with either standard or flex (for single-channel flexible endoscopes) cycles (RF, at least 5.1 log) (Figure 5).

### Efficiency of the Hydrogen Peroxide Gas Plasma and Detergent Combination

Theoretically, decontamination phases should be preceded by a cleaning phase. We therefore tested the effect of 3 detergents on the efficiency of the Sterrad 100S and NX systems.

Enzymatic detergent alone was not very effective, even at a concentration of 100% (Table 2). At this same concentration, incubation for 24 hours rather than 30 minutes did not improve its efficiency (Table 2). Enzymatic detergent treatment even reduced the efficiency of steam in our experimental conditions (a transmission rate of 100% and an RF of 4.0 log for the combination vs a transmission rate of 50% and an RF of at least 5–6 log for steam alone) (Table 2). By contrast, additive or synergistic effects were observed with the Sterrad 100S system (Table 2). Additive effects were observed for the combination of 2% enzymatic detergent for 10 minutes at 37°C and the Sterrad 100S regular long cycle (RF, 2.4 log for combination vs 1.1 log for the detergent alone and 1.3 log for the Sterrad 100S system alone). Synergistic effects were found for 100% enzymatic detergent for 30 minutes at 37°C followed by 2 consecutive Sterrad 100S long cycles. Nevertheless, transmission rates for both of these combinations remained high (100% for the first combination,

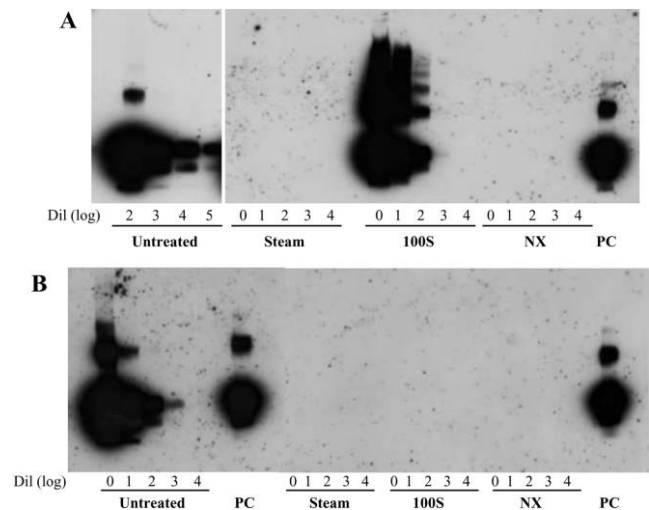


FIGURE 2. Western blot analysis of the hamster-adapted scrapie 263K strain (A, for brain homogenate inoculum; B, for protease-resistant form of the prion protein) adsorbed onto stainless steel sheets and treated with steam, Sterrad 100S, and Sterrad NX. Each experiment was performed using 6 sheets for each set of experimental conditions (eg, for the untreated positive control [PC]). Results are representative of 2 independent experiments. Dil, dilution.

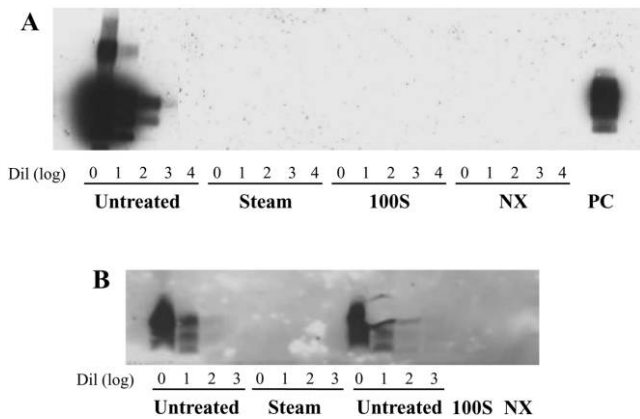


FIGURE 3. Western blot analysis of the mouse-adapted bovine spongiform encephalopathy (BSE) 6PB1 strain (A) and the variant Creutzfeldt-Jakob disease strain (vCJD) (B) adsorbed onto stainless steel sheets and treated with steam, Sterrad 100S, and Sterrad NX. Each experiment was performed using 3 sheets (for vCJD) or 6 sheets (for mouse-adapted BSE 6PB1) for each set of experimental conditions (eg, for the untreated positive control [PC]). Only the mouse-adapted BSE 6PB1 experiment was repeated to obtain 2 independent experiments. Dil, dilution.

and 67% for the second); this fact shows that the overall effects against prions were weak.

The alkaline detergent A alone was more effective than the enzymatic detergent and significantly decreased the transmission rate (Table 2). Increasing the temperature from 55°C to 70°C did not seem to improve efficacy (data not shown). Stronger effects were observed if this detergent was combined with steam (Table 2). The combination of this detergent with 1 or 2 consecutive Sterrad 100S regular long cycles also decreased the transmission rate to 0% (Table 2). The alkaline detergent B was highly efficient when used alone, with a transmission rate of 0%. No deleterious effects were observed when this detergent was combined with 1 or 2 consecutive Sterrad 100S regular long cycles (Table 2). Due to these beneficial effects of alkaline detergents and “double hydrogen peroxide gas plasma” treatments, we evaluated the effects of these alkaline detergents on the Sterrad NX system in both regular and advanced cycles. As stated above, the Sterrad NX system was highly efficient, even with 1 advanced cycle (Table 2).

## DISCUSSION

Much remains unknown about prions, including the precise nature of the pathogenic agent, its extreme resistance to some chemical and physical decontamination methods, the possible differences between prion strains, and the effect of the experimental conditions. We analyzed the efficiency of the Sterrad systems with consideration of these factors.

### Methodological Aspects

Bioassays were performed to determine prion infectivity. In these assays, female Syrian golden hamsters were inoculated

with the hamster-adapted 263K scrapie strain. This well-validated animal model, with its short incubation times and high titers in the brain, is widely accepted for the evaluation of new methods or processes for the removal or inactivation of prions. Several studies have shown that the high infectious titer results in a shorter incubation period.<sup>2,25</sup> This is also the case for this model with hamsters infected with the scrapie 263K strain.<sup>22</sup> This approach has been used in various studies and appears to be reliable for calculating the reduction of infectious titer, as shown by Müller et al<sup>22</sup> in the 263K-infected hamster model. We confirmed that this model and this mode of calculation (calculation of the time interval from wire implantation or inoculation to onset of clinical symptoms and/or positive Western blot) can be used to quantify a decrease in the infectious titer of prions. Our results further demonstrate the efficiency of new physicochemical processes against prions and confirm the relative efficiency of steam.<sup>15,16,26,27</sup> The incubation period was used to calculate the infectious titer and the RF. However, beyond 150 to 200 days, corresponding to the inflection point of the standard curve, efficiency is defined purely by the transmission rate.

In vitro assays are often criticized for several reasons, including lack of sensitivity and effects on PrP that do not reflect infectivity. However, they do provide a rapid preliminary indication of the efficiency of new processes. They can

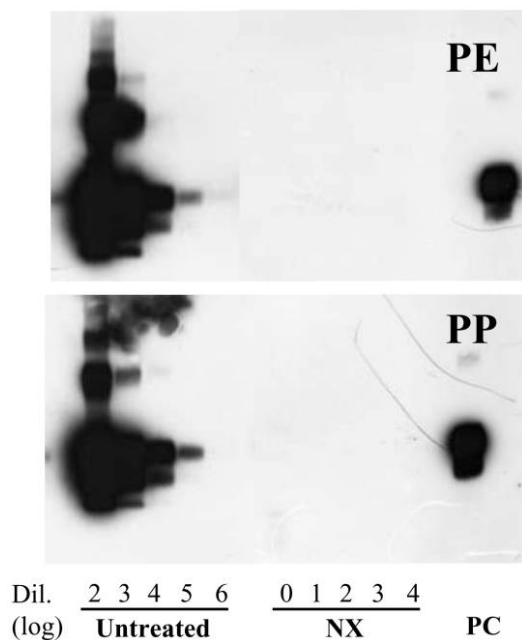


FIGURE 4. Western blot showing the efficiency of Sterrad NX treatment on heat-sensitive surfaces artificially contaminated with the hamster-adapted scrapie 263K strain. Each experiment was performed using 6 sheets for each set of experimental conditions (eg, for the untreated positive control [PC]). Results are representative of 2 independent experiments. Dil, dilution; PE, polyethylene; PP, polypropylene.

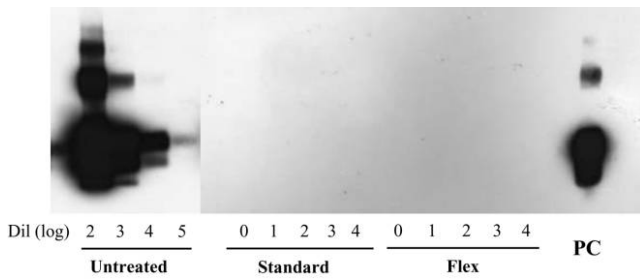


FIGURE 5. Western blot showing the efficiency of flex and standard cycles of the Sterrad 100NX system, by means of sheets artificially contaminated with the hamster-adapted scrapie 263K strain. Each experiment was performed using 6 stainless steel sheets for each set of experimental conditions (eg, for the untreated positive control [PC]). Results are representative of 2 independent experiments. Dil, dilution.

also add to the data obtained *in vivo* with different materials and inocula (eg, PrPres vs homogenate). In this study, our *in vitro* data complemented the data obtained in hamsters, and PrPres titers (not infectious titers) were high enough (ie, 5–7 log) to provide evidence of a significant effect of various treatments.

#### Efficiency of Reference Treatments

Two reference treatments (steam and sodium hydroxide followed by steam) were tested in this study, and for both, infectivity persisted even if infectivity decreased by more than 5 to 6 log. Such incomplete efficacy has previously been reported for steam. Jackson et al<sup>27</sup> reported a transmission rate of 25% with steam in the Tg20 animal model inoculated with the RML strain. In the 263K-infected hamster model, Fichet et al<sup>16</sup> reported transmission rates of 60% to 70%, and a similar level of residual infectivity was observed by Taylor<sup>12</sup> after steam treatment at 134°C, 136°C, and 138°C. Prions are more resistant to inactivation by autoclaving if they have been allowed to dry on a surface, and this is particularly true for metal surfaces.<sup>28,29</sup> Fichet et al<sup>16</sup> observed complete inactivation only if wires were immersed in water during the steam cycles. We obtained similar results with a shorter drying time: 1 hour for our study compared with 16 hours for Fichet et al.<sup>16</sup> Humidity plays a role, and 1 hour of drying is sufficient to reduce the efficacy of steam considerably. Surprisingly, the sodium hydroxide and steam combination was not entirely effective. Complete inactivation was described by Caughey et al<sup>30</sup> with the 263K strain, after exposure to 1 N sodium hydroxide followed by autoclaving at 121°C for 30 minutes. However, the experimental conditions in their study differed from those used here, with treatment with 1 N sodium hydroxide for 16 hours compared with such treatment for only 1 hour in our study. These data confirm the extreme resistance of prions but cannot necessarily be extrapolated to all strains and all processing conditions encountered in hospitals. Moreover, although there is a substantial body of evidence to sug-

gest that this combination is one of the most effective methods of prion decontamination, risks to patient safety and of damage to devices remain a matter of concern, as pointed out by McDonnell and Burke.<sup>31</sup> All these limitations require further research to develop more effective processes.

#### Efficiency of Liquid and Plasma Hydrogen Peroxide Alone (In Vivo and In Vitro Data)

Hydrogen peroxide, in its liquid and gaseous forms, is routinely used as a biocidal chemical agent. Hydrogen peroxide is relatively stable, has broad-spectrum efficacy, and is readily degraded into water and oxygen. The precise mode of action of hydrogen peroxide against prions remains to be clarified. The emergence of PrPres multimers suggests that interchain disulfide bonds form after the reduction of the intrachain disulfide bond of each PrP molecule (between Cys179 and Cys214; reduction related to an attack by hydrogen peroxide or 1 oxygen radical generated from hydrogen peroxide). Nevertheless, there is currently no evidence that this mechanism applies to high concentrations of hydrogen peroxide, which are highly effective against prions.

Fichet et al<sup>15</sup> suggested that prion inactivation might be altered by condensation or use of the liquid form of hydrogen peroxide and proposed this explanation for the limited efficacy of the Sterrad 100S sterilizer. However, our findings suggest that exposure to 59% liquid hydrogen peroxide for 20 minutes gave better results than the Sterrad 100S with a similar concentration and time of exposure to the active agent. Thus, although we agree that gaseous hydrogen peroxide may be more effective than the liquid form in certain conditions, as shown by the results of the Sterrad NX system, deleterious effects of condensation or of the liquid form remain to be demonstrated. It also remains to be demonstrated that less condensation occurs in the process used by Fichet et al<sup>15</sup> than in the Sterrad 100S system. Moreover, other factors, such as controlled condensation, may play a key role in inactivation and have not yet been explored in detail.

It is often thought that higher concentrations of gaseous hydrogen peroxide, decreasing the time required for a cycle of the sterilization process and increasing penetration into the lumen, might help to overcome possible deleterious effects of other phenomena that we cannot control completely. We agree with Fichet et al<sup>15</sup> that gaseous hydrogen peroxide sterilization is indeed an interesting tool for dealing with the risk of prion contamination and that its mode of action merits additional investigations. While awaiting the results of such investigations, we recommend the use of caution, with claims of inactivation based solely on experimental data obtained in accurately defined and controlled conditions. Fichet et al<sup>15</sup> described multimers on Western blots that were also observed with the Sterrad 100S system. We did not observe these multimers with the Sterrad NX sterilizer. We detected no signal for PrPres multimers or PrPres itself or of infectivity with this Sterrad NX machine. For the mouse-adapted BSE 6PB1

strain and for the vCJD strain, no such aggregates were observed with any of the Sterrad systems tested. Indeed, for these "human" strains, no PrPres signal was observed, even with the Sterrad 100S sterilizer. This suggests that the 263K scrapie strain was the strain most resistant to the effects of hydrogen peroxide. This has already been suggested by other authors, for both hydrogen peroxide<sup>15</sup> and for other processes, such as steam.<sup>12</sup>

The penetration of biocides to their molecular targets was also explored by comparing isolated PrPres (ie, PrP27–30) and brain homogenate. PrP27–30 is the most stable fraction of infectious PrP<sup>Sc</sup> (see Müller et al<sup>22</sup> for review), and its presence is therefore the worst case scenario in the absence of lipids, sugars, nucleic acids, or other protein contaminants that might react with an oxidizing agent such as hydrogen peroxide. In particular, PrP27–30 isolated from Syrian golden hamsters is thought to be highly resistant to decontamination methods. However, no differences in the efficacy of the Sterrad NX system were observed between isolated PrP27–30 and brain homogenate, because this system was already efficient against the homogenate. Such differences are difficult to demonstrate in the absence of lipid contamination. With the Sterrad 100S sterilizer, efficacy is higher in the absence of lipids or other constituents of a brain homogenate. These results confirm the deleterious effect of these constituents, particularly lipids, which are better able to absorb reactive oxygen species. The use of a detergent might therefore be beneficial. For the Sterrad 100S system, prior treatment with detergent is necessary, as shown by the synergistic or additive effects observed with the 3 detergents (2 alkaline and 1 enzymatic) tested in our bioassay. This aspect was not the primary focus of this work and will therefore not be discussed in detail, but we, like many others, observed that alkaline detergents were more effective than enzymatic detergent. However, this effect may not be general, and it is necessary to demonstrate the efficacy of each detergent, whether enzymatic or alkaline.

In conclusion, the results of this study confirm that gaseous or vaporized hydrogen peroxide efficiently inactivates prions on the surfaces of medical devices. However, the efficacy of this method depends on the conditions used, including the concentration of hydrogen peroxide in particular.

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