20th WORLD STERILIZATION CONGRESS 2019
The Hague - The Netherlands 30 October - 2 November 2019

Session 3: Cleaning and disinfection

Elimination of prion protein in cleaning processes of medical devices in health care

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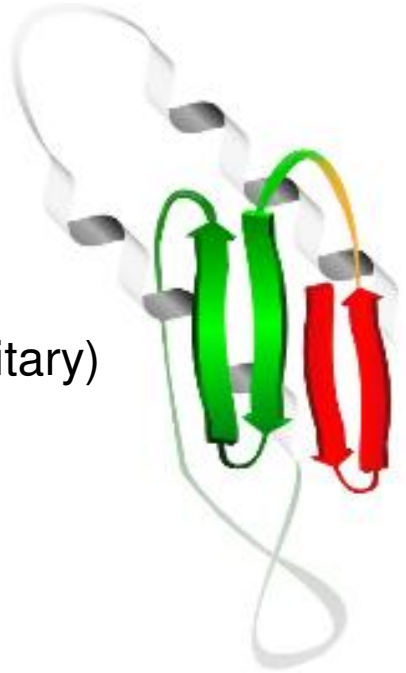
Inactivation and removal of pathogenic prion protein (PrP^{res}) in Cleaning Processes

- Essentials & Test methods
- Routine vCJK/CJK Prophylaxis by Protein removal
- Specific vCJK / CJK Prophylaxis by Prion-Inactivation

Prion Disease



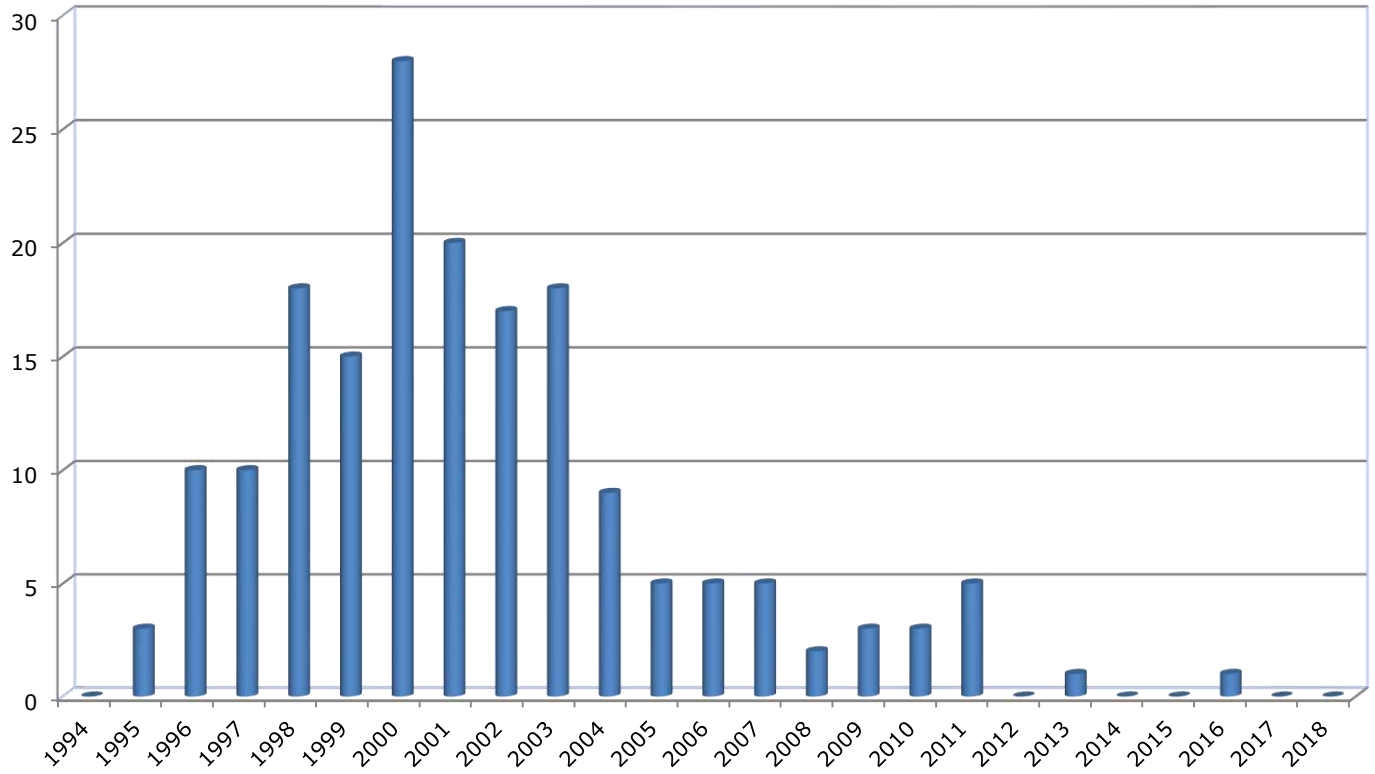
Humans

- Creutzfeldt–Jakob disease (CJD) (sporadic, iatrogenic, hereditary)
- variant Creutzfeldt–Jakob disease (vCJD)
- Gerstmann–Sträussler–Scheinker syndrome (GSS)
- Fatal familial insomnia (FFI)
- Kuru (transmitted via funerary cannibalism)

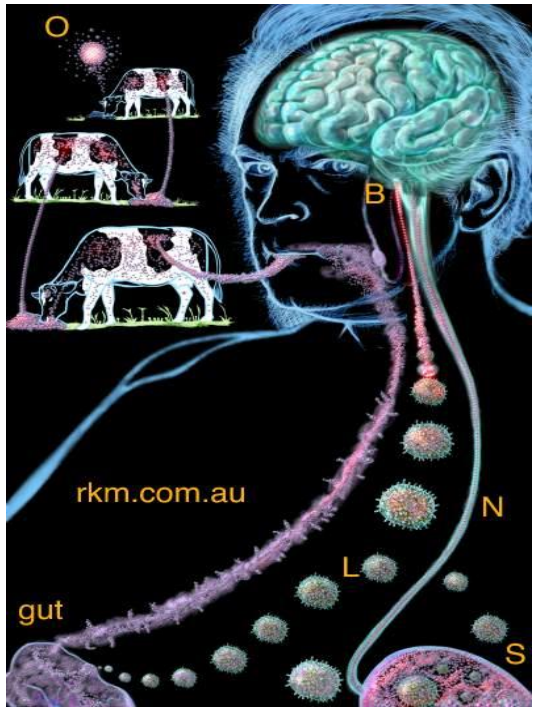
Animals

- Scrapie in sheep
- Chronic wasting disease (CWD) in deer
- Bovine spongiform encephalopathy (BSE) in cattle (mad cow disease)
- Feline spongiform encephalopathy (FSE) in cat
- Transmissible mink encephalopathy (TME) in mink

vCJD UK



BSE-prions
↓
Stomach
↓
Gut
↓
Lymphatic System
↓
Nervus splanchnicus and Nervus vagus
↓
Central nerve system → **vCJD**



http://cjd-goettingen.de/aktuell/aktuelle-zahlen/statistik-vcjk-weltweit-und-gb/

Based on investigations of surgically removed tonsils and appendixes in UK approximately 4000 cases can be expected.
In France: 25 vCJD cases

→ ?

Prions

Type of *proteinaceous infectious particle* (without nucleic acid) believed as cause of transmissible spongiform encephalopathies (TSE).

Alper T, Cramp WA, Haig DA, Clarke MC: Does the agent of scrapie replicate without nucleic acid? Nature. 1967 May 20; 214 (5090): 764-6.

Griffith JS: Self-replication and scrapie. Nature. 1967 Sep 2; 215 (5105): 1043-4.

Prusiner SB: Novel proteinaceous infectious particles cause scrapie. Science. 1982, April 9; 216 (4542): 136-44.

Gajdusek DC: The transmissible amyloidoses: genetical control of spontaneous generation of infectious amyloid proteins by nucleation of configurational change in host precursors: kuru-CJD-GSS-scrapie-BSE. Eur J Epidemiol. 1991 Sep;7(5):567-77.

1997 - Nobel Prize in Physiology or Medicine awarded to Stanley B. Prusiner "for his discovery of Prions - a new biological principle of infection."

Prions are believed to infect and to propagate by refolding normal molecules of the cellular protein into the abnormally structured form.

PrP^C „cellular“

- **Soluble in water**
- **Not agglomerative**
- **Digested by Protease**

Alpha helix structure

PrP^{Sc} „Scrapie-Isoform“

- **Not soluble in water**
- **Agglomerative**
- **Formation of so called amyloids**
- **Resistant against Protease**

Beta-folded sheet structure

Prions are misfolded structurs of a cellular protein.

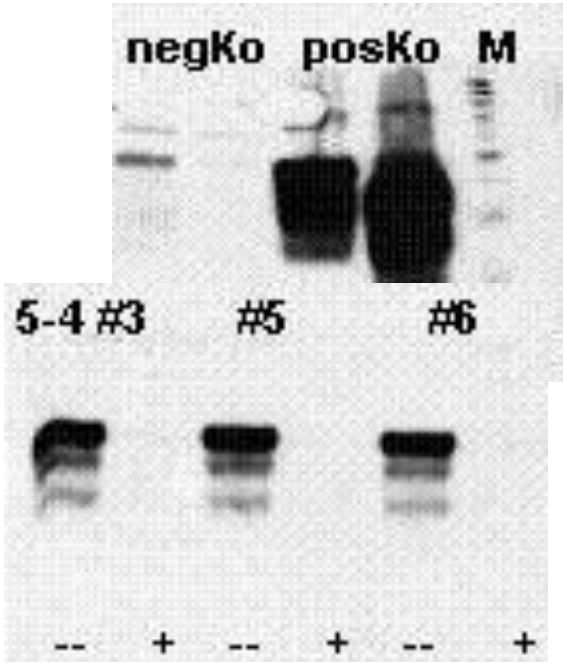
Identical amino acid sequence, but different conformation (tertiary structure) - One protein with two “different faces”

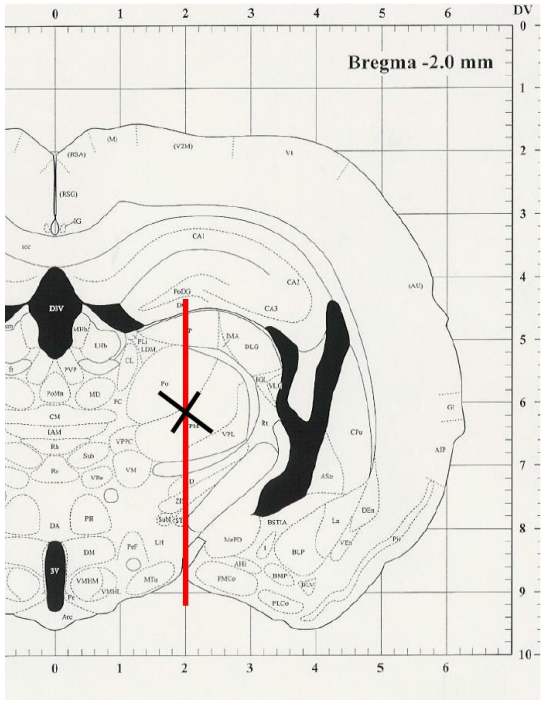
Pathogene Prions form aggregates (amyloids) and are higly resistant to protease, heat, radiation, and aldehydes a.o..

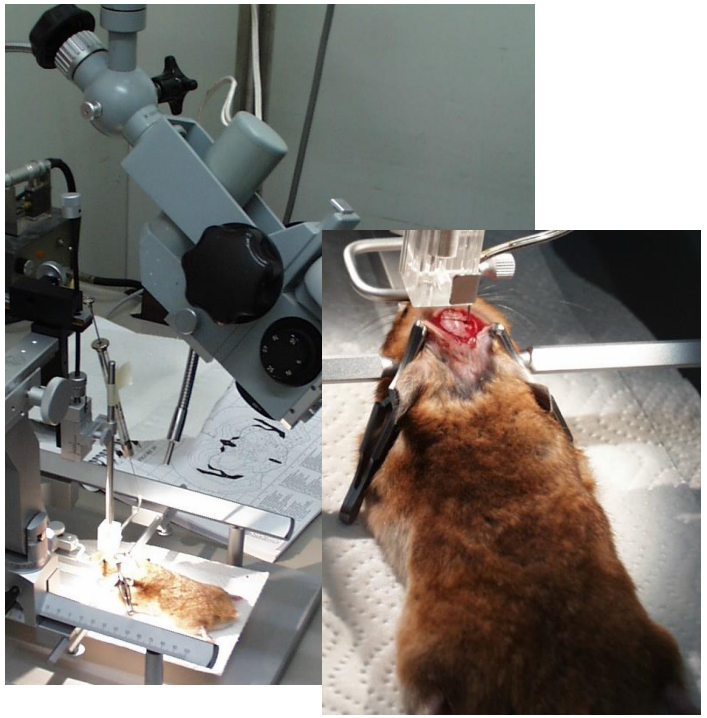
In vitro and In vivo tests for demonstrating Prion-activity

Test uses brain homogenates infected with pathogenic prion protein (PrP^{res})

In vitro	**In vivo**	**In vivo**
qualitative suspension test (e.g. western blot, amplification tests)	quantitative suspension test	quantitative carrier test







Source: Test Report to neodisher SeptoClean, SMP, Tübingen

Proof of removal and inactivation of pathogenic prion protein (PrP^{res})

In vitro and in vivo testing of the removal

Carrier test with specimens contaminated with infected (PrP^{res}) brain homogenate

1) In vitro detection of the residual protein PrP^{res} (western blot, amplification)

2) In-vivo titration of the reduction of infectivity (implantation)

In vitro and in vivo testing of inactivation

1. Suspension test with infected (PrP^{res}) brain homogenate
2. Carrier test with specimens contaminated with infected (PrPres) brain homogenate

1) In vitro detection of the residual protein PrP^{res} (western blot, amplification)

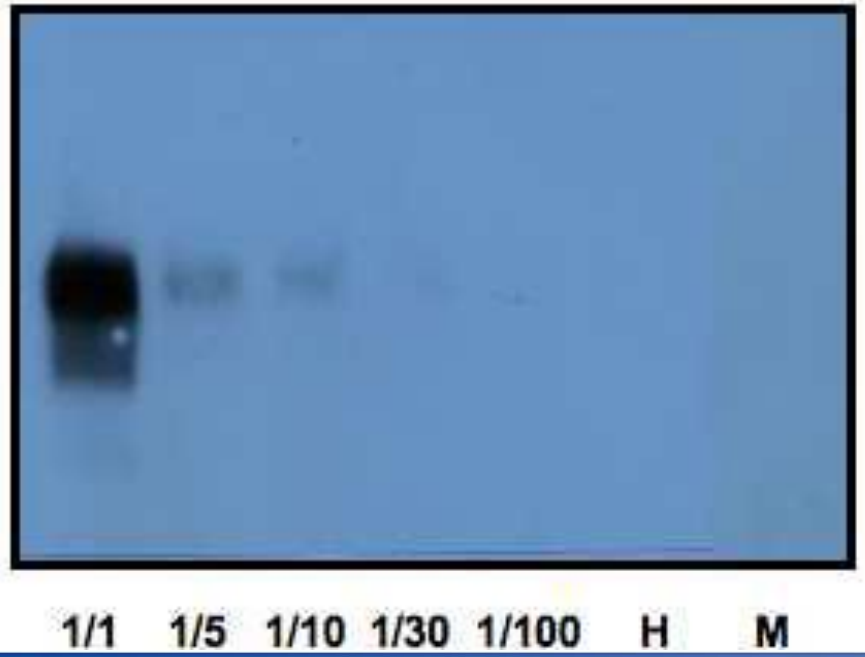
2) In-vivo titration of the reduction of infectivity (injection & implantation)

Removal vs. Inactivation

In vitro test for removal of pathogenic prion protein (PrP^{res}) from surfaces
mild alkaline cleaner with enzymes, 1%, pH 10.8, 55 °C, 10 min, without mechanics

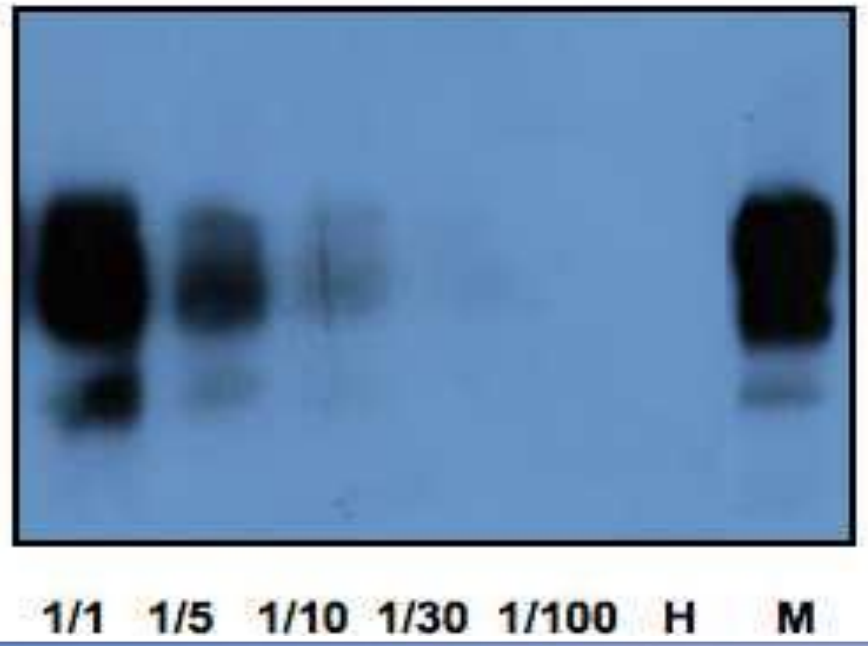
Removal - test

Cleaning of contaminated surfaces. Contamination with infected (263K PrP^{res}) brain homogenate (1/1 to 1/100), healthy brain homogenate (H), residual of PrP^{res} on specimen after cleaning by immersion (M).



Inactivation - test

Suspension Test. Suspension of infected (263K PrP^{res}) brain homogenate (1/1 to 1/100), healthy brain homogenate (H), with detergent solution treated suspension of PrP^{res} (M).



Activity of Reprocessing Procedures and Chemicals of Medical Device Reprocessing for Prion Inactivation

Partially Active Procedures / Chemicals	Inactive Procedures / Agents
Validated (alkaline) Cleaning (1.3.1.1)	Alcohol
1 M NaOH (40 g / l min. 1 h at 20 °C)	Aldehyde, Formaldehyde (vapor)
2,5 - 5 % NaOCl (min. 1 h at 20 °C; min. 20.000 ppm active Chlorine)	Ethylene oxid
≥ 4 M GdnSCN (min. 30 min at 20 °C)	Iodine
Steam Sterilization (min. 5 min at 134 °C)	HCl
H_2O_2 (certain procedures, 1.3.1.3)	Dry Heat
	UV-Radiation
	X-Ray, Gamma Irradiation
	Peracetic acid

Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz 10 · 2012 , S. 1278

Activity of Reprocessing Procedures for Prion Reduction

Parameter	Residual organic material	Reduction factor ID50	Residual Infectivity ID50
Initial contamination of Instruments	10 mg		10^{7}
Decontamination			
Cleaning /Disinfection			
1st Cleaning	100 bis 10 µg	$10^{-2 / -3}$	$10^{3 / 5}$
2nd Cleaning		$10^{0 / -2}$	
Inacktivation (Sterilization)			
1st Sterilization		$10^{-3 / -6}$	$0 - 10^{2}$
2nd Sterilization		$10^{0 / -3}$	$0 - 10^{1}$

Bundesgesundheitsbl - Gesundheitsforsch – Gesundheitsschutz, 2002 • 45:376–394

Some National Requirements for routinely instrument reprocessing in cases with no perceptible risk

Germany:

Instruments which were in contact with

- Background of the eye
- Central nerve system
- Opened lymphatic system

Reprocessing by a combination of „at least two partially effective methods with different mechanisms“

1. **Alkaline cleaning**
2. **Steam-sterilisation at 134°C with 5 min holding-time**

Recommendation for: mildly alkaline cleaners / alkaline cleaners

- ⇨ Alkaline cleaning is characterized by high efficiency in terms of the solution of protein and fat residues and microbial activity
- ⇨ may lead to adverse changes in material
- ⇨ Observe the manufacturer's instructions for material compatibility

Hygiene requirements for the reprocessing of medical devices
Recommendation of the KRINKO at the RKI and BfArM Bundesgesundheitsbl 2012 · 55:1244-1310

CDC
SAFER · HEALTHIER · PEOPLE™

- ⇨ **USA: CDC-Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008:**
- ⇨ "Alkaline-based cleaning agents are used for processing medical devices because they efficiently dissolve protein and fat residues."

results enzymatic cleaner 45 °C/10 min.

Cleaner	FlexiCheck	Simicon RI	WashCheck	Blood VR	Mean
OC	2	3	4	0	2,3
OED	2	3	4	4	3,3
ER	2	3	4	1	2,5
PEC	0	3	1	0	1,0
Mean	1,5	3	3,2	1,2	2,3

OC: Olympus Cleaner, ED: Olympus Enzymatic Detergent, ER Thermosept ER, PEC: Prolystica Enzymatic Cleaner

results alkaline cleaner 45 °C/10 min.

Cleaner	FlexiCheck	Simicon RI	WashCheck	Blood VR	Mean
MCF	0	0	0	0	0,0
ACF	1	2	3	2	2,0
PAD	0	0	4	0	1,0
Mean	0,3	0,7	2,3	0,7	1,0

MCF: Neodisher Mediclean forte, ACF: Alcaclean forte, PAD: Prolystika Alcaline Detergent

- ⇨ cleaning performance of various process chemicals on the basis of enzymes and alkali for WD-E
- ⇨ Different test samples
- ⇨ proof:
  cleaning in an alkaline environment leads to significantly better results!

Study of the ÖGSV Source: www.oegsv.com/events

Buchrieser, N. & Miorini, T. (July 30 - August 01, 2010). Comparative Study upon Cleaning Indicators for Washer-disinfectors for Flexible Endoscopes and Routine Control after Maintenance and Repair. 11th World Sterilization Congress and the 7th International Symposium of Sterilization and Hospital Infection Control, (S. http://www.wfhss.com/html/conf/assets/wfhss_conf20100730_lecture_sp_s601_en.pdf). City of Sao Paulo – Brazil.

Proof for removal of pathogenic prion protein (PrP^{res}) from surfaces in Cleaning Processes

In vitro and in vivo test for the removal

Carrier test with PrP^{res} contaminated specimen

1) Contamination of specimen with PrP^{res} infected brain homogenate
2) Cleaning in defined process

3a) Collection of residual protein → 4a) In-vitro test on PrP^{res} (western blot)

3b) In-vivo titration of infectivity (implantation)

In vivo test for removal of pathogenic prion protein (PrP^{res}) from surfaces

BSE-6PB1 (C57Bl/6 mice)

Controls

Group	Dilution	% infected	Dead Animals	Survey (mean ± sd)
Normal wires				
SCWNC1	10^{-1}	100 %	6 / 6	208 ± 11
SCWNC2	10^{-2}	100 %	6 / 6	216 ± 9
SCWNC3	10^{-3}	100 %	6 / 6	245 ± 25
SCWNC4	10^{-4}	100 %	8 / 8	292 ± 43
SCWNC5	10^{-5}	67 %	4 / 6	360 ± 127
SCWNC6	10^{-6}	28 %	2 / 7	313 ± 51
SCWNC7	10^{-7}	0 %	0 / 8	-
SCWNC8	10^{-8}	0 %	0 / 5	-
SCWNCN	10^{-1} negative	0 %	0 / 4	-

In vivo test for removal of pathogenic prion protein (PrP^{res}) from surfaces

log-steps

mild alkaline cleaner with enzymes, 1%, pH 10.8, 55 °C, 10 min, without mechanics

BSE-6PB1 (C57Bl/6 mice)

	% infected	Dead animals	Survey (mean ± sd)	Reduction of infectivity (log estimation and 95 % confidence interval)
Normal wires SCWNT01 10^{-1}	29 %	2 / 7	448	4.6 [4.1 - 5.5]

In-vivo test for the removal of prions with new and corroded stainless steel surfaces in lg-steps

mild alkaline cleaner with enzymes, 1%, pH 10.8, 55 °C, 10 min
without mechanics

	new	corroded
Scrapie 263K	(> 2 in Vitro)	3.5
BSE-prions	4.6	3.3
vCJD-prions	2.5	2.5

Some National Requirements for routinely instrument reprocessing in cases with no perceptible risk

France:

Instruments which were in contact with

- Background of the eye
- Central nerve system
- Opened lymphatic system
- Nose mucous membrane

1. **Reprocessing with a cleaner which was successfully tested against prions**

Liste des produits inactivants totaux au regard du PSP (novembre 2011), utilisables dans le cadre des procédures prévues par l'instruction DGS/RI3/2011/449 du 1er décembre 2011

afssaps
Agence française de sécurité sanitaire des produits de santé

2. **Steam-sterilisation at 134°C with 18 min holding-time**

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World Health Organization (2006):

WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies

Decontamination: new procedures (JP Deslys):

The unique resistance of the vCJD agent to usual decontamination techniques poses a very difficult infection control challenge ….

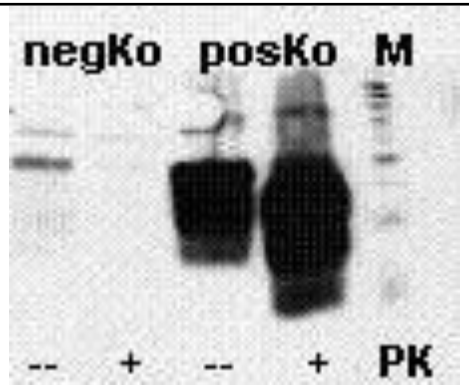
The drastic procedures usually recommended to decontaminate materials (1M sodium hydroxide or 2% sodium hypochlorite for 1 hour with 134°C autoclaving for at least 18 minutes) are not applicable to most fragile devices

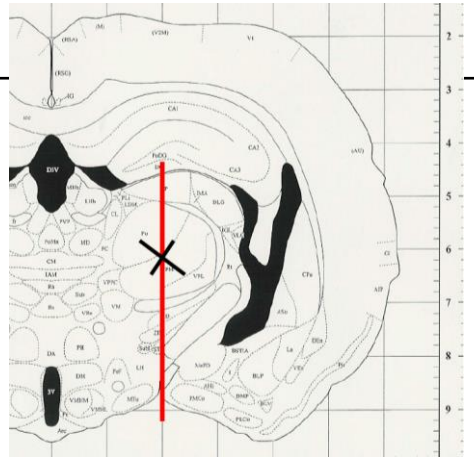
Reproducible results have been obtained with a 263K hamster model and a BSE mouse model using IC implantation of wires contaminated with infected brain, dried, and then exposed to different decontamination treatments.

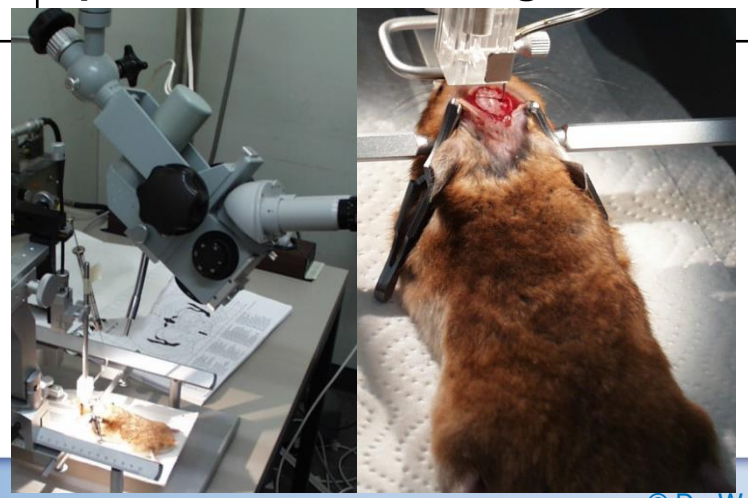
Proof of Effectiveness and Declaration suggested by the Robert Koch-Institute, Germany

The effectiveness against prions should be tested and declared in phases based on efficacy testing of bactericidal and virucidal substances; Use of **263K PrP^{res}** infected brain homogenates

Phase 1a	**Phase 1b**	**Phase 2**
Preliminary testing of procedures in vitro, Evaluation in the qualitative suspension test; eg with western blot	Quantitative suspension test Evaluation in animal experiments	Quantitative carrier test Evaluation in animal experiments
Basic test, no declaration	Declaration as being **"prion inactivating"**	In conjunction with successfully passed Phase 1b test, declaration as being **"prion decontaminating"**







Source: Test Report to neodisher SeptoClean, SMP, Tübingen

Quantitative Suspension Test in-vitro & in-vivo:

Robert Koch-Institute

M. Baier, A. Schwarz, M. Mielke, **„Activity of an alkaline 'cleaner' in the inactivation of the scrapie agent"**; *Journal of Hospital Infection (2004), **57**, 80-84*

Summary:
The capacity of a routinely available alkaline cleaner** for medical devices to inactivate the causative agent of a transmissible spongiform encephalopathy (TSE) was tested.

The co-incubation of brain homogenates, prepared from terminally ill scrapie-infected hamsters, with the cleaner led to the denaturation of misfolded protein as the proteinase K-resistant prion protein was no longer detectable after such treatment.
In addition, intra-cerebral inoculation of hamsters with the alkaline cleaner-treated and subsequently neutralized samples reduced the level of infectiousness of the material below the limit of detection.

This report shows the possibility that a routinely available alkaline cleaner could reduce the infectiousness of TSE agents and so minimize the risk of iatrogenic transmission of TSEs by asymptomatic carriers. This study is intended to encourage further investigations in this field.
** pH 12.2

Quantitative carrier test in-vivo:

Robert Koch-Institute:

Karin Lemmer, Martin Mielke, Christine Kraxel, Marion Joncic, Muhsin Oezel, Georg Pauli and Michael Beekes, **„Decontamination of surgical instruments from prion proteins II: in vivo findings with a model system for testing the removal of scrapie infectivity from steel surfaces“**
*Journal of General Virology (2008), **89**, 348 - 358*

Summary:

„Incubation of the wires in the commercially available alkaline cleaner* at a concentration of 1% (pH 12,2 as measured at room temperature) for 10 minutes at 55°C or for 60 minutes at 23°C resulted in complete removal of detectable infectivity and a titre reduction of 5,5 ≥ logs.“

* pH 12.2

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PROTOCOLE STANDARD PRION (Novembre 2011)

Pour la méthode *in vivo, comme pour la méthode in vitro, le protocole repose sur la contamination d'un* support-modèle par un homogénat de cerveau infecté par la souche 263K.

Méthode in vivo : modèle hamster infecté par la souche 263K

Méthode in vitro : Les souches à tester sont la souche 263K et au moins une autre souche d'origine bovine ou humaine.

PROTOCOLE STANDARD PRION (2018)

Méthode in vivo : une souche animale 263K, une souche de prion de type humain

Méthode in vitro : la souche 263K, la souche de prion de type humain utilisée dans l'étude in vivo, une souche d'origine bovine ou humaine.

In vivo test for removal and inactivation of pathogenic prion protein (PrP^{res}) from surfaces

BSE-6PB1 (C57Bl/6 mice)

Controls

Group	Dilution	% infected	Dead Animals	Survey (mean ± sd)
SCWNC1	10^{-1}	100 %	6 / 6	208 ± 11
SCWNC2	10^{-2}	100 %	6 / 6	216 ± 9
SCWNC3	10^{-3}	100 %	6 / 6	245 ± 25
SCWNC4	10^{-4}	100 %	8 / 8	292 ± 43
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SCWNC6	10^{-6}	28 %	2 / 7	313 ± 51
SCWNC7	10^{-7}	0 %	0 / 8	-
SCWNC8	10^{-8}	0 %	0 / 5	-
SCWNCN	10^{-1} negative	0 %	0 / 4	-

In vivo test for removal and inactivation of pathogenic prion protein (PrP^{res}) from surfaces

log-steps

BSE-6PB1 (C57Bl/6 mice)

Reference

	% infected	Dead animals	Survey (mean ± sd)	Reduction of infectivity (log estimation and 95 % confidence interval)
SCREF1 NaClO 20,000 ppm 1 hour	0 %	0 / 7	> 553	> 5.1
SCREF2 NaOH 1M 1 hour	0 %	0 / 8	> 553	> 5.1
SCREF3	13 %	1 / 8	289	5.1 [4.7 - 6.8]

In vivo test for removal and inactivation of pathogenic prion protein (PrP^{res}) from surfaces

log-steps

BSE-6PB1 (C57Bl/6 mice)

alkaline cleaner with surfactants, 1%, pH 12.2, 55 °C, 10 min
without mechanics

		% infected	Dead animals	Survey (mean ± sd)	Reduction of infectivity (log estimation and 95 % confidence interval)
SCWNT01	10^{-1}	11 %	1 / 9	342	5.2 [4.7 - 6.8]
SCWNT02	10^{-2}	0 %	0 / 7	> 573	> 5.1

In-vivo test for the removal and inactivation of prions on new and corroded stainless steel surfaces

alkaline cleaner with surfactants, 1%, pH 12.2, 55 °C, 10 min without mechanics

Was successfully tested in accordance with all recommended methods in vivo with

- Scrapie 263 K (syrien hamster modell)
- *BSE (6 PB 1-infected C 57 B1/6 mouse model)*
- *vCJD (vCJD-infected Swiss mouse model)*

using intracerebral implantation of stainless steel wires contaminated with infected brain.

Tested with new wires but also successfully with corroded wires

Was also successfully tested in vitro with all mentioned prion strains

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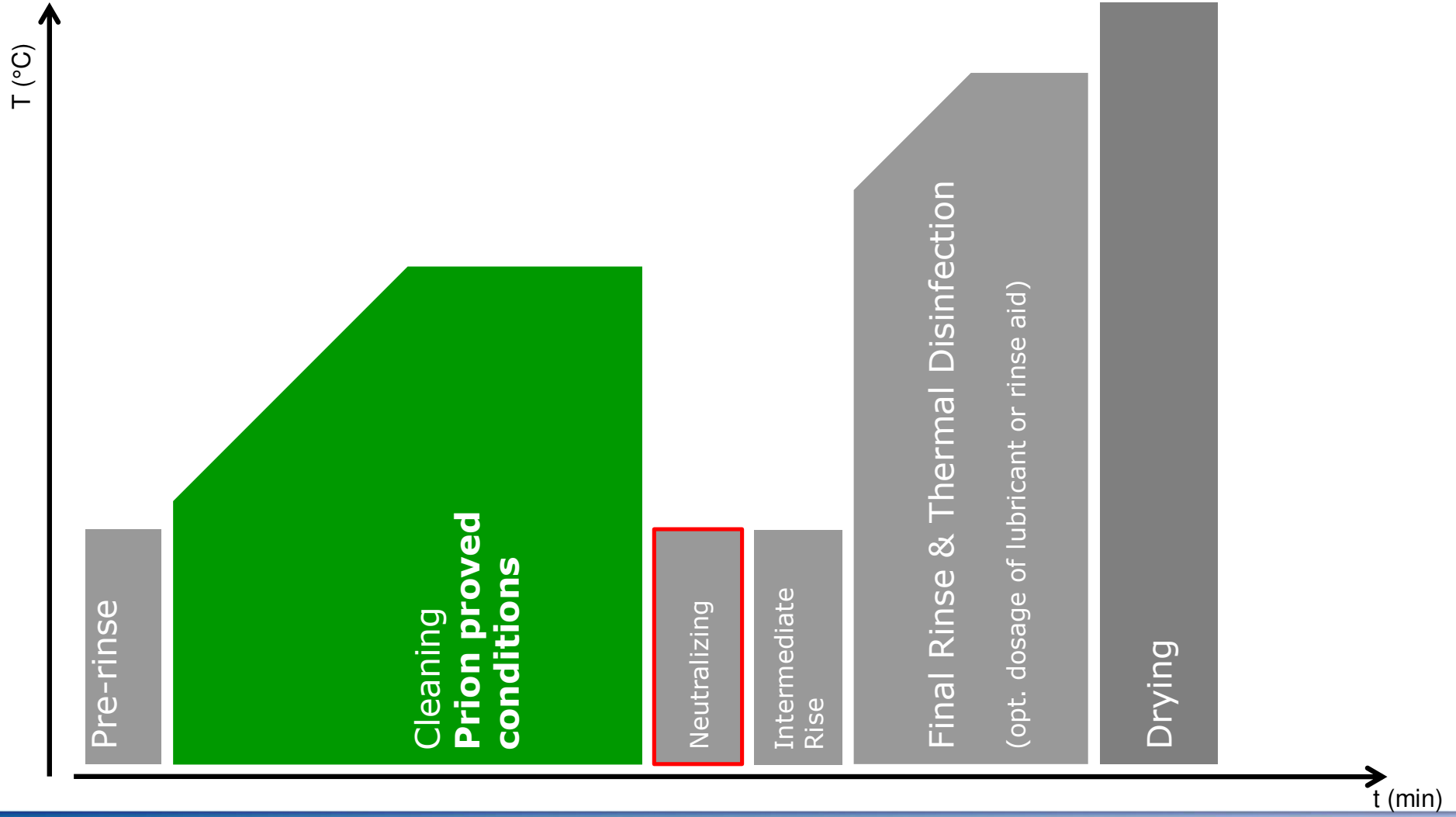
Liste des produits et procédés inactivants totaux au regard du PSP (novembre 2011), utilisables dans le cadre des procédures prévues par l'instruction DGS/RI3/2011/449 du 1er décembre 2011

Produits	Fabricant	Conditions d'utilisation
ACTANIOS HLD	ANIOS	Immersion/solution prête à l'emploi/température ambiante/ 30 min
ACTANIOS HLD + ACTANIOS P1 +ACTANIOS P2	ANIOS	-P1 : immersion/ 0,5%/température ambiante/ 10 min -Rinçage -P2 : immersion/ 0,5%/température ambiante/ 5 min -Rinçage -HLD : immersion/solution prête à l'emploi/température ambiante/15 min
ACTANIOS WD	ANIOS	Immersion (bain thermostaté), laveur-désinfecteur, tunnel de lavage/ 0,8 43°C/ 7,5 min
ALKA 100	SODEL (ex ALKAPHARM)	Immersion/1%/température ambiante/15 min
HMTS-30E	HUMAN MEDITEK	Cycle Advanced
HMTS-80E	HUMAN MEDITEK	Cycle AUTO
HMTS-142	HUMAN MEDITEK	Cycle Advanced
NEODISHER SEPTOCLEAN	DR WEIGERT	-Immersion / 1%/ température ambiante/ 60 min -Laveur-désinfecteur, tu de lavage / 1%/ 55°C/ 10 min
PROLYSTICA 100 PID Prion Inactivating Detergent	STERIS	Immersion (bain thermostaté), laveur-désinfecteur, tunnel de lavage/ 0,8%/ 43°C/ 7,5 min
PROLYSTICA 2X + cycle non lumen du VPRO 1	STERIS	-PROLYSTICA 2X : immersion/0,4%/65°C/5 min -VPRO 1 : cycle non lumen
PROLYSTICA 2X + cycle non lumen du VPRO Max	STERIS	-PROLYSTICA 2X : immersion/0,4%/65°C/5 min -VPRO Max : cycle non lumen
SEKUMATIK FR + SEKUMATIC Oxivario dans un laveur – désinfecteur MIELE, séquence inactivation du programme OXIVARIO PLUS	MIELE	Laveur-désinfecteur MIELE/Programme Oxivario Plus - SEKUMATIC FR : 0,5%/55°C/5min - SEKUMATIC FR 0,8% + Oxivario 0,7%/ 55°C/ 10 min
STERIZONE VP4	TSO_3	Cycle 1
STERRAD® NX™	Advanced Sterilization Products (ASP)	-Cycle Avancé
STERRAD® 100NX™	Advanced Sterilization Products (ASP)	-Cycle Standard -Cycle Flex

https://www.ansm.sante.fr/var/ansm_site/storage/original/application/8c2a007bb67cb38dd98d0012aa60ff28.pdf (27.10.2019)

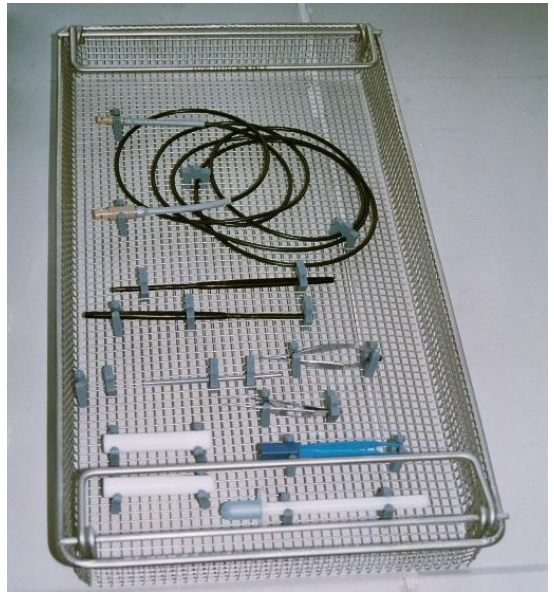
08 mars 2019

Decontamination cycle with prion inactivation and prion decontamination to prevent a transmission of prions via surgical instruments: also suitable for anodized aluminum

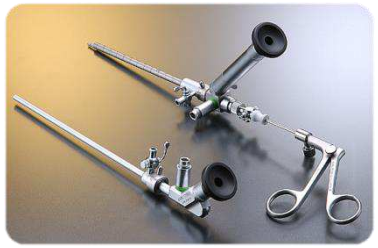


Reliability

Formulations based on potassium hydroxide, silicates, phosphates and different surfactants are:



- Suitable for washer disinfectors
- Suitable for reprocessing of surgical instruments including MIS-Instruments and rigid endoscopes
- Suitable for ophthalmological instruments
- Tested and approved by instrument manufacturers



Inactivation and Removal of Prion Protein in Cleaning Processes – Résumé I

- In-vitro test methods for proving the removal of pathogenic prion protein (PrP^{res}) are established
- In-vivo test methods for proving the inactivation of pathogenic prion protein (PrP^{res}) are established but resource demanding – and costly
- General alternatives for in-vivo test methods for proving the inactivation of pathogenic prion protein (PrP^{res}) has to be established (PMCA)

Inactivation and Removal of Prion Protein in Cleaning Processes – Résumé II

- For routine prophylaxis of transmission of vCJD/CJD by medical devices a cleaning with proven protein removal is essential

- The effectiveness of cleaning depends on the used procedure and has to be proven by performance qualification

- For routine prophylaxis of transmission of vCJD/CJD by medical devices cleaning procedures with a proven reduction of Prion Protein PrP^{res} by 2-3 Log are established

Inactivation and Removal of Prion Protein in Cleaning Processes – Résumé III

- For specific prophylaxis of transmission of vCJD/CJD by total inactivation of Prion Protein PrP^{res} a procedure with proven reduction (in-vitro and in-vivo) is necessary

- For specific prophylaxis of transmission of vCJD/CJD by total inactivation of Prion Protein PrP^{res} procedures with proven reduction of infectivity by > 5 log are available

Thank you for your attention!