New hospital Prion Disinfection Processes Compatible with Thermo-Sensitive Medical Equipment

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Transmissible Spongiform Encephalopathies (TSEs) or Prion diseases

Neurodegenerative disorders

In animals:

- Bovine Spongiform Encephalopathy (BSE)
- Scrapie (sheep)
- Chronic wasting disease (CWD)

In human:

- Creutzfeldt-Jakob disease (CJD)
- Gerstmann-Sträussler Syndrome (GSS)
- Fatal Familial Insomnia (FFI)

Sporadic (50-60 years), genetic or infectious origin

Clinical signs: Dementia, ataxia

No inflammatory signs, blood and CSF are normal in routine examination, 14-3-3 increased, EEG signs

The diagnosis is histopathological: spongiform degeneration of the brain, gliosis, detection of an abnormal protein (PrPSc), amyloid plaques
Prion diseases are transmissible
Infectious Prions are present in many organs.

**Creutzfeldt Jakob disease**

- Brain +++ (10⁹ infectious unit/g)
- Spinal cord, Retina
- Peripheral nerves / muscles
- Spleen
- Lymphoid system
- Blood (vCJD)
- Tonsil
- Appendix
- Peyer’s patches

Rare (1.5/M/yr) but worldwide distribution.

Additional carrier linked to the BSE crisis (UK, France...several thousand of persons).
Prions are extremely resistant to decontamination

- TSE agents exhibit an unusual resistance to conventional chemical and physical decontamination methods
- They are not adequately inactivated by most common disinfectants, or by most tissue fixative
- They are extremely resistant to high doses of ionizing and ultraviolet irradiation
- Some residual activity has been shown to survive for long period in the environment

→ concern for patient care and infection control
The "PRION" hypothesis

Peculiar properties of the infectious fractions isolated from the brain of affected animals (scrapie):
* not sensitive to agents denaturing nucleic acids
* not specific DNA or RNA molecules present

Prusiner (1982) PRION ("proteinacious infectious particles")

Prions:
* new type of infectious agent devoid of genetic material
* composed principally, even uniquely of a protein called:
  PrP\textsuperscript{Sc} for the scrapie isoform of the prion protein
Generation of *infectious prions*

**Normal protein**
- Normal protein of the neuronal surface
- Alpha helix structure
- Sensitive to protease
- Soluble in non-ionic detergents

**Prions**
- Present only in infected brains
- Beta structure
- Resistant to proteases
- Insoluble in detergents

PK - +

[www.cmfarm.ucsf.edu/cohen/prp](http://www.cmfarm.ucsf.edu/cohen/prp)
Aggregation of Prions

Amyloid fibers
Rapid method for the sensitive detection of protein contamination on surgical instruments
I.P. Lipscomb*, A.K. Sihota, M. Botham, K.L. Harris, C.W. Keevil

Tissue forceps tip

EF Image of protein contamination
(x 600)
The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on surgical instruments is by incineration. Determinate risk in healthcare environments...

Risk is dependent upon:

- Probability that an individual has or will develop TSE
- Level of infectivity in tissues or fluids of these individuals
- Nature or route of exposure to tissues

...in order to choose adequate procedures for decontamination.

Discard and destroy by incineration!

The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on surgical instruments according to the WHO Infection Control Guidelines for TSE (WHO/CDC/CSR/APH/2000.3), Geneva, Switzerland, 23-26 March 1999.
Prion decontamination procedures

Group I: ineffective products and procedures
Group II: variably or partially effective products and procedures
Group III: effective products and procedures, chemical or physical
Group IV: effective products and procedures, chemical and physical combined
Group V: disposal and incineration

Group I (ineffective)
- dry heat (<300 °C)(*),
- ethanol (*),
- gaseous formaldehyde (*),
- glutaraldehyde (*),
- formol (*),
- HCl,
- ammonia,
- propiolactone

Group II (partially effective)
- peracetic acid,
- autoclaving at 121 °C, 30 min,
- chlorine dioxide,
- NaOCl (0,5% for 15 min),
- iodophores,
- boiling in 3 % SDS for 3 min,
- Na metaperiodate,
- NaOH (0,5M for 30 min),
- urea (6 M for 4 heures),
- guanidium thiocyanate (4M)

(*) they fix infectivity
**Prion decontamination procedures**

Group III (effective products and procedures, chemical or physical)

1. Immersion into *Sodium hypochlorite* (NaOCl) 20 000 ppm, 1 h
2. Immersion into *Sodium hydroxide* NaOH 1M 1h
3. *Autoclave* at 134 °C for 18 min in porous load autoclave

Group IV (effective products and procedures, chemical and physical combined)

Group V: incineration

Use of all disposable instruments, materials, and wastes

Preferred method for all instruments exposed to high infectivity tissues

Main concern arises for heat-sensitive instruments such as *endoscopes*
Basic research on the normal protein

Metal ions binding sites

Modified from Mangé and Lehmann
Relation Prion / metal ions

Metal ions

- **Cu** $^{2+}$
  - Structure
    - Add structure to the N-terminus
    - Trafficking
    - Cleavage
  - Function
    - SOD-like activity
    - Transport and chaperon activity
    - Oxidative stress
  - Pathology
    - Conformational changes
    - Proteinase K resistance
    - Toxicity

- **Zn** $^{2+}$
- **Mn** $^{2+}$
Effect on normal protein
(basic research on prion protein function)

Normal protein

Copper (Cu) / hydrogen peroxide (H$_2$O$_2$) mix

5 min  15 min  30 min  1 h
**Effect on Prion proteins**

**Infectious Prions**

PrPSc → Cu / H₂O₂ mix → Mouse, CJD, vCJD

Human
Importance of Cu in the degradation of Prions

\[ \text{Fe}^{2+}, \text{Mn}^{2+}, \text{Cu}^{2+}, \text{Al}^{3+}, \text{Mn}^{2+}, \text{Fe}^{2+} \]

→ CNRS international patent on the use of Copper and Hydrogen Peroxide for Prion decontamination
Comparison with other chemicals

Cu/H₂O₂ mix

15 min, 30 min

vMCJ

Alcaline

PAA ↔ Acetic acid + H₂O₂

Peracetic acid (PAA)

Cu/H₂O₂ mix

PAA

PAA + Cu

vMCJ

vMCJ
Validation on steel wires
Validation on steel wires

20% Brain Homogenates
22L, CJDs, hamster 263K...

Steel wires
L = 5mm, Ø = 0.25 mm

Dried 16h in laminar flow

2h under agitation

In vitro

Ex vivo

In vivo

Dr. A. Perret-Liaudet - M. Richard
: Hôpital Neurologique de Lyon -
LDMP - H.C.Lyon

Dr. P. Clayette - Dr C. Rogez-Kreuz : SPI-BIO
In vitro validation on steel wires


Test decontamination procedures

Elution of remaining PrP<sup>Sc</sup>

Western blot

Ctrl (dH<sub>2</sub>O)
Ctrl -2Log
Ctrl -3Log
Cu<sup>2+</sup> / H<sub>2</sub>O<sub>2</sub>

vCJD
sCJD
In vivo validation on steel wires

Hamster 263K

Test decontamination procedures

One year follow up, titration by comparison to wires contaminated with diluted homogenate

<table>
<thead>
<tr>
<th>Groups</th>
<th>Titer reduction Log$_{10}$</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (untreated)</td>
<td></td>
<td>100 %</td>
</tr>
<tr>
<td>dH$_2$O control</td>
<td>0,89</td>
<td>100 %</td>
</tr>
<tr>
<td>Alkaline control</td>
<td>&gt;5,25</td>
<td>0 %</td>
</tr>
<tr>
<td>Cu H$_2$O$_2$</td>
<td>&gt;5,25</td>
<td>0 %</td>
</tr>
<tr>
<td>Autoclave</td>
<td>3,40</td>
<td>57 %</td>
</tr>
</tbody>
</table>
Validation on classical pathogens

European norms for

**Bactericidal and mycobactericidal activities**

- NF EN 13727, NF EN 14561
  - *(Enterococcus hirae, Pseudomonas aeruginosa, Staphylococcus aureus)*
- NF EN 14348, pr EN 14563 *(M. terrae & M. avium)*

**Fungicidal**,  
- NF EN 13624, NF EN 14562
  - *(Candida albicans, Aspergillus niger)*

**Virucidal**,  
- NF EN 14476 + A1
  - *(Adenovirus type 1, Poliovirus type 1)*

**Sporicidal activities**  
- AFNOR NF T 72 230
  - *(Bacillus cereus & subtilis, Clostridium sporogenes)*
Peroxide + Copper

→ Elimination of Prion proteins and prion infectivity
→ Validated on steel wire, both in vitro and in vivo
→ On different strains and on genuine prions (human, BSE...)
→ Efficacy similar or superior to reference methods
Application

Hospital desinfection

→ For endoscopes...
  → Control of chemical compatibility, stability, reduced chemical risks...
→ Full integration in hospital desinfection procedures
Desinfection of other equipments and surface...