Patient Safety
Challenges with Infectious Proteins
Standard Decontamination

Harmful Microorganisms

- HIV virus
- Cryptosporidium
- Mycobacterium tuberculosis
- SARS virus
- Yeast
- Flu-virus
- Malaria parasite
Challenges with Standard Decontamination
Infectious Proteins

Spaulding’s Classification of the resistance of various micro-organisms to sterilization & disinfection
## Infectious Proteins

### Prions

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>DNA (Y/N)</th>
<th>Volume (nm$^3$)</th>
<th>Mass (g)</th>
<th>Cost of claim validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria (E.coli)</td>
<td>Y</td>
<td>$10^9$</td>
<td>$10^{-12}$</td>
<td>~$400</td>
</tr>
<tr>
<td>Virus (Herpes simplex)</td>
<td>Y</td>
<td>$10^6$</td>
<td>$10^{-14}$</td>
<td>~$1,000</td>
</tr>
<tr>
<td>Prion</td>
<td>N</td>
<td>~10</td>
<td>~3×$10^{-18}$</td>
<td>&gt;$1,000,000</td>
</tr>
</tbody>
</table>

*Prions are ~10,000,000 times smaller than bacterial cells.*
BATON ROUGE, Louisiana (Reuters) -- A Louisiana man who may have been exposed to a rare, fatal brain-wasting disease during surgery is suing the university hospital where his operation was performed, he said on Wednesday.

A top-level committee will investigate sterilization standards at the Columbia University hospital in New York after 1056 patients were yesterday told they may have contracted a fatal brain disease after surgery with potentially contaminated instruments.

The scare follows brain surgery on a man with Creutzfeldt-Jakob disease.

The CJD surgeon tough

The mother of a boy who was not in surgery

More patients at risk

Two separate incidents have emerged in which patients have told they were put at risk of contracting Creutzfeldt-Jakob Disease (CJD).

In both cases the fatal brain-wasting disease could have been picked up during surgery.

At Queen's Hospital in Romford in Essex, 21 brain surgery patients have received letters.

A further 38 patients in Wales were told on
Infectious Protein
Mad Cow and Creutzfeldt-Jakob Disease (vCJD)
Infectious Proteins

Creutzfeldt-Jakob Disease

How Creutzfeldt-Jakob disease works

**CAUSE**
Creutzfeldt-Jakob disease is caused by abnormal proteins called prions that are not killed by standard methods for sterilizing surgical equipment.

**CONSEQUENCES**
Those affected lose the ability to think and to move properly and suffer from memory loss. It is always fatal, usually within one year of onset of illness.

As prions build up in cells, the brain slowly shrinks and the tissue fills with holes until it resembles a sponge.

up to 1 in 2000 adults born between 1947 and 1985 are CJD-positive*

Infectious Proteins
Transport and Replication
most likely through immune system/lymphocytes –\'vCJD is a prominently lymphotropic disease\'*

*M. Belondrade et al. Rapid and highly sensitive detection of CJD... doi:10.1371/journal.pone.0146833
# Infectious Proteins

## Prion Diseases

<table>
<thead>
<tr>
<th>Human Prion Diseases</th>
<th>Animal Prion Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>• CJD: Creutzfeld-Jacob Disease</td>
<td>• BSE (bovine spongiform encephalopathy): cows</td>
</tr>
<tr>
<td>• variant</td>
<td>• CWD (chronic wasting disease): muledeer, elk</td>
</tr>
<tr>
<td>• sporadic</td>
<td>• Scrapie: sheep</td>
</tr>
<tr>
<td>• familial</td>
<td>• TME (transmissible mink encephalopathy): mink</td>
</tr>
<tr>
<td>• iatrogenic</td>
<td></td>
</tr>
<tr>
<td>• GSS: Gerstmann-Straussler-Scheinker syndrome</td>
<td></td>
</tr>
<tr>
<td>• FFI: Fatal familial Insomnia</td>
<td></td>
</tr>
<tr>
<td>• Kuru</td>
<td></td>
</tr>
<tr>
<td>• Alpers Syndrome</td>
<td></td>
</tr>
</tbody>
</table>
Infectious Proteins

Why are prions so problematic?

- Prion disease (PrP<sup>sc</sup>) infectivity is buried within one of the most tightly folded, water insoluble protein structures known.

- Within this structure, the infectivity is inaccessible to immune system defenses as well as to common disinfection protocols.

- Beta-sheet folding creates an even more hydrophobic core within already hydrophobic structure. With large glyco-groups protecting the infectious core.

- Immune system does not recognize PrP<sup>sc</sup> as a foreign particle.

- Traditional disinfectants are water-based and barely penetrate to the tightly packed core.

- Heat does not affect the protein folding within the core.

10 ‘traditional’ CSSD cycles are required to clear CJD infectivity. Roger Magnusson. DISPUTES & DILEMMAS IN HEALTH LAW, 2006
Infectious Proteins

Misfolding Protein Diseases

<table>
<thead>
<tr>
<th>Function</th>
<th>Protein</th>
<th>Associated disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport molecules</td>
<td>Serum amyloid protein A</td>
<td>Secondary systemic amyloidosis</td>
</tr>
<tr>
<td></td>
<td>Apolipoprotein A-I</td>
<td>Familial amyloid polyneuropathy Type II</td>
</tr>
<tr>
<td></td>
<td>Apolipoprotein A-II</td>
<td>Familial amyloid polyneuropathy Type III</td>
</tr>
<tr>
<td></td>
<td>Transthyretin</td>
<td>Familial amyloid polyneuropathy Type I</td>
</tr>
<tr>
<td></td>
<td>Lactoferrin</td>
<td>Corneal amyloidosis</td>
</tr>
<tr>
<td>Coagulation factors</td>
<td>Fibrinogen</td>
<td>Fibrinogen amyloidosis</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>Lysozyme amyloidosis</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Keratin</td>
<td>Cutaneous amyloidosis</td>
</tr>
<tr>
<td></td>
<td>Tau</td>
<td>Alzheimer’s disease, frontotemporal dementia</td>
</tr>
<tr>
<td>Cytoskeletal proteins</td>
<td>Amylin</td>
<td>Type II diabetes</td>
</tr>
<tr>
<td></td>
<td>Calcitonin</td>
<td>Medullary carcinoma of the thyroid</td>
</tr>
<tr>
<td></td>
<td>Prolactin</td>
<td>Aging pituitary prolactinomas</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td>Insulin-related amyloid</td>
</tr>
<tr>
<td></td>
<td>Atrial natriuretic factor</td>
<td>Atrial amyloidosis</td>
</tr>
<tr>
<td>Hormones</td>
<td>Gelsolin</td>
<td>Finnish hereditary amyloidiosis</td>
</tr>
<tr>
<td>Regulatory proteins</td>
<td>Cystatin C</td>
<td>Icelandic hereditary cerebral amyloid angiopathy</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Immunoglobulin light chains (κ and λ)</td>
<td>Primary systemic amyloidosis, amyloidosis associated with multiple myeloma</td>
</tr>
<tr>
<td>Immune system-related</td>
<td>Immunoglobulin heavy chain β2-Microglobulin</td>
<td>Primary systemic amyloidiosis</td>
</tr>
<tr>
<td>Cell-adhesion molecules</td>
<td>Kerato-epithelin</td>
<td>Hemodialysis-related amyloidosis</td>
</tr>
<tr>
<td>Unknown function</td>
<td>Lactadherin (Medlin)</td>
<td>Corneal dystrophy</td>
</tr>
<tr>
<td></td>
<td>Amyloid-β</td>
<td>Aortic medial amyloidosis</td>
</tr>
<tr>
<td></td>
<td>Prion protein</td>
<td>Alzheimer’s disease, cerebral amyloid angiopathy</td>
</tr>
<tr>
<td></td>
<td>Amyloid British</td>
<td>Spongiform encephalopathies</td>
</tr>
<tr>
<td></td>
<td>Amyloid Danish</td>
<td>British familial dementia</td>
</tr>
<tr>
<td></td>
<td>α-Synuclein</td>
<td>Danish familial dementia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parkinson’s disease</td>
</tr>
</tbody>
</table>

- Incidence rates:
  - 1.5 in 100 Alzheimer (1 in 2 over 80 years of age)
Infectious Proteins

Misfolding Proteins are found in blood, lymphatic tissues and even breast milk and urine

Mathias Jucker, Tubingen University
We found that intraperitoneal inoculation with β-amyloid–rich extracts induced β-amyloidosis in the brains of β-amyloid precursor protein transgenic mice after [7-month] incubation time.

It has been confirmed beyond reasonable doubt that at least two amyloid diseases (CJD & Alzheimer) are infectious and can be transferred via both intracerebral and intraperitonital contact.

No firm evidence exists that the AD and other protein misfolding diseases are infectious outside the lab, regulators say. However few experts (including regulators) are willing to rule out the possibility that Alzheimer’s might be transmitted from person to person via blood transfusion or neurosurgery.
Protein Misfolding Diseases
Plaque Formation

Ahlzeimers plaque

CJD plaque
Protein Misfolding Diseases

Findings

Spontaneous generation of mammalian prions

Julie A. Edgeworth, Nathalie Gros, Jack Alden, Susan Joiner, Jonathan D. F. Wadsworth, Jackie Linehan, Sebastian Brandner, Graham S. Jackson, Charles Weissmann¹,², and John Collinge¹

The apparent “spontaneous generation” of infective prions from normal brain tissue could result if the metal surface, possibly with bound cofactors, catalyzed de novo formation of prions from normal cellular prion protein.
Preventative Measures

Pre-screening

- Use of questionnaires - there are no rapid screening tests available e.g. blood or saliva

- French regulatory authorities (ANSM) acknowledge inherently dubious value of such questionnaire and require compulsory prion decontamination of all instruments contacting brain, throat and eye (using a prion deactivating method/detergent)
Preventative Measures

WHO- Guidelines for Transmissible Spongiform Encephalopathies

Figure 1. Neurosurgical instruments following treatment by immersion in 1M NaOH and steam sterilization (gravity cycle) at 121°C for 30 minutes.
Infectious Proteins

Where to begin?

- The only logical place to introduce prion decontamination is before any thermal/chemical disinfection

- 1st by not allowing instruments to dry

- When it comes to cleaning, protein hydrolysis (cleaving) may be achieved with enzymatic and alkaline detergents, but which one to choose?
Infectious Proteins. When to decontaminate?


ANNEX C GENERAL PRINCIPLES OF DECONTAMINATION AND WASTE DISPOSAL

The Decontamination Cycle for reusable medical equipment
C2.

The reusable surgical instrument cycle

- ACQUISITION
  1. Purchase
  2. Loan

- CLEANING

- DISINFECTION
  (NEW PRION DEACTIVATION TECHNOLOGY)

- INSPECTION
  (& PROTEIN TESTING)

- PACKAGING
  1. Scrap
  2. Return to lender

- DISPOSAL

- STERILIZATION

- TRANSPORT

- STORAGE

- USE

- TRANSPORT

At all stages:
- Location
- Facilities
- Equipment
- Management
- Policies/Procedures

Diagram from Department of Health CFPP 01-01

NO!!! BEFORE disinfection (and fixing) proteins, not AFTER.
Detergent Choice

Enzymatic or Alkaline

- **Alkaline detergents** rely on a hydroxide ion concentration approximately 2-2.8 M/L for concentrates, typically at a pH >12
  - Source of electrochemical staining and corrosion
  - Additional need to neutralise

- **Enzymatic detergents** rely on proteolytic activity – at close to neutral pH < 8.5
  - Compatible with most instrument surfaces
  - No need to neutralise
## Stoichiometric Calculations

### Enzymatic or Alkaline

- In a standard CSSD wash (10 min @ 60°C, in 30 litres of water) the theoretical amount of protein digested*:

<table>
<thead>
<tr>
<th>Detergent Type</th>
<th>Water soluble protein</th>
<th>Denatured protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced Enzymatic Detergent with prion claim dosed at 3 ml/L</td>
<td>1,007,400 mg</td>
<td>105 mg</td>
</tr>
<tr>
<td>Alkaline detergent with prion claim at 10% w/w of NaOH dosed at 5ml /L</td>
<td>28,125 mg</td>
<td>0.3 mg</td>
</tr>
</tbody>
</table>

*Sava A Kritzler S Protein elimination from medical instruments Central service Jan 2013*
## Detergent Choice

Animal Prion vs. Human

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Estimated based on reduction from $10^4$ initial inoculation and positive control results (not shown)

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Concentration</th>
<th>Temperature (°C)</th>
<th>Time (mins)</th>
<th>% Deaths Observed</th>
<th>Mortality* Average ± (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic Cleaner</td>
<td>0.8%</td>
<td>43</td>
<td>5</td>
<td>100</td>
<td>149±15</td>
</tr>
<tr>
<td>(Klenzyme®)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline Cleaner (Hamo 100)</td>
<td>1.6%</td>
<td>43</td>
<td>15</td>
<td>0</td>
<td>&gt;365</td>
</tr>
<tr>
<td>NaOH</td>
<td>1N</td>
<td>20</td>
<td>60</td>
<td>0</td>
<td>&gt;365</td>
</tr>
<tr>
<td>NaOCl</td>
<td>2.5%</td>
<td>20</td>
<td>60</td>
<td>0</td>
<td>&gt;365</td>
</tr>
<tr>
<td>Environ® LpK®</td>
<td>5%</td>
<td>20</td>
<td>30</td>
<td>0</td>
<td>&gt;365</td>
</tr>
</tbody>
</table>

*(Fichet et al Lancet)*

263K scrapie animal prion strain testing
Animal Prion vs. Human

Fig 4. Evaluation of "standard" and commercially available decontamination procedures on human vCJD prions. Steel wires contaminated with 10% human vCJD brain homogenate were treated with...
Unexpectedly, among these commercial formulations, **only one product was found to be completely efficient** in removing vCJD-associated seeding activity. **More disturbingly**… **one treatment could be considered as inefficient** on vCJD as 100% of wires tested were detected as positive after the first Surf-PMCA round, similar to control-wires treated with water only…
### Detergent Choice

#### M1000- Human Prion Strain Testing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation ± SEM (days)</th>
<th>Approximate log dose (LD$_{50}$ units)</th>
<th>Number affected/total</th>
<th>Transmission rate</th>
<th>Approximate log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treat</td>
<td>71 ± 2</td>
<td>5.0</td>
<td>4/4</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>1N NaOH 1 hr</td>
<td>130 ± 19</td>
<td>2.4-3.0</td>
<td>9/10</td>
<td>90</td>
<td>2-2.6</td>
</tr>
<tr>
<td>134°C 18 min</td>
<td>120 ± 5</td>
<td>2.9</td>
<td>10/10</td>
<td>100</td>
<td>2.1</td>
</tr>
<tr>
<td>134°C 3 min</td>
<td>104 ± 3</td>
<td>3.6</td>
<td>9/9</td>
<td>100</td>
<td>1.4</td>
</tr>
<tr>
<td>121°C 20 min</td>
<td>106 ± 2</td>
<td>3.5</td>
<td>10/10</td>
<td>100</td>
<td>1.5</td>
</tr>
<tr>
<td>1% RMEC A 50°C 30 min</td>
<td>189 ± 11</td>
<td>&lt;1</td>
<td>7/10</td>
<td>70</td>
<td>4-5</td>
</tr>
<tr>
<td>0.3% RMEC B 60°C 30 min</td>
<td>147 ± 13</td>
<td>1.7</td>
<td>6/10</td>
<td>60</td>
<td>3.3</td>
</tr>
<tr>
<td>RMEC B + 134°C 3 min</td>
<td>166</td>
<td>0.9</td>
<td>1/10</td>
<td>10</td>
<td>4.1*</td>
</tr>
<tr>
<td>RMEC B + 121°C 20 min</td>
<td>-</td>
<td>-</td>
<td>0/10</td>
<td>0</td>
<td>&gt;5*</td>
</tr>
</tbody>
</table>

**Summary of bioassay data clinical mice 280 days post implantation.**

*Infection was confirmed in all clinical mice by western blot detection of PrP$^{Sc}$.  
*RMEC B treatment protocols + autoclaving indicate that prion deactivation of the M1000 prion strain by chemical (RMEC) and steam sterilization (autoclave) protocols are additive.*
Detergent Choice
Protein Digestion

Enzymatic Detergent with prion claim
**Full Digestion**

Alkaline Detergent with prion claim
**Negligible Digestion**

Enzymatic Detergent with no prion claim
**Partial Digestion**

Digestion of common SOLUBLE blood proteins fibrin, albumin, haemoglobin
From: N. Cheetham, Comparative efficacy of medical instrument cleaning products in digesting some blood proteins. Aust Infection Control, September 2005
1) Using a complex combination of surfactants, the hydrophobic core of the proteins is unfolded.

- The whole protein molecule becomes accessible and protease enzymes cleave the peptide bonds, breaking the structure into small innocuous fragments. These fragments are easily water soluble.
**Conclusion**

- Creutzfeldt-Jakob Disease is transmissible.

- Alzheimer’s in lab studies has been proven to be transmissible, suggesting the potential danger with human transmissibility.

- Stainless steel is one of Even a native healthy human protein could be converted into infectious prion when attached to stainless steel.

- Current decontamination methods do not address these potential threats.

- Protocol should include pre-screening, procedure specific instrument segregation, not allowing instruments to dry and lastly a validated prion inactivating detergent.

- Advanced enzymatic formulations with validated prion deactivation claims on true human prion offer 300-1200 greater protein cleaving power than alkaline detergents.

- With the current uncertainty over what protein (or part of protein) causes the prion-type abnormalities, the only assured way to prevent prion transmission via reusable medical instruments is to completely **REMOVE and DESTROY ALL Proteins** using known validated biochemical protocols.
Thank You
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