Adsorption of proteins to surgical stainless steel surfaces and assessment of SSD decontamination procedures

14th World Sterilization Congress
8th National Sterilization Disinfection Congress of Turkey

6th-9th November 2013

Bill Keevil PhD
Oldest trepanned skull, found at a neolithic burial site of Ensisheim in France, is more than 7,000 years old.

Trepanation was practised by the Ancient Egyptians, Chinese, Indians, Romans, Greeks and the early Mesoamerican civilizations.
Over 5000 years of potential iatrogenic infections...

**Bronze Age Brain Surgeons - Turkey**

Archaeologist Önder Bilgi uncovered 14 skulls with rectangular cut marks - trepanation.

Believes the Ikiztepe people used shards of volcanic glass - obsidian “scalpels,” found elsewhere on the site, to treat brain tumors and fight-related head injuries, and to relieve pressure from hemorrhaging.

The 4,400-year-old skull of an early neurosurgery patient. Ikiztepe archive
Over 2000 years of potential iatrogenic infections...

Roman era scalpel/dissector found at Silchester (Hampshire)

Surgical instrument set, Persian, 18th century (the Science Museum, London)
Over 2000 years of potential iatrogenic infections...

Eye surgery (1195)

The Extraction of the Stone of Madness
(Hieronymus Bosch; circa 1500)
Over 200 years of reducing the risks of iatrogenic infection

Ignaz Philipp Semmelweis (1818–1865)
Louis Pasteur (1822-1895)
Ferdinand Cohn (1828-1898)
Robert Koch (1843-1910)
Over 20 years of having to deal with a new problem

Stanley Prusiner (born 1942)

proteinaceous+infectious= “Prion” (1982)

Nobel Prize 1997

vCJD crisis (first?) peak in UK in 2000

Current estimate of 1:2000 of UK population

† Prompted new requirements (and a new market) in the field of decontamination to target PrPSc
Today

Modern Sterile Services Department Staff, UK


The Health and Social Care Act 2008

Choice Framework for local Policy and Procedures (May 2012…)

Increasing choice of new equipment and cleaning products

Practical and financial pressure
Today

The five main functions performed in a hospital sterile service department

and the practical requirements

RAPID
SAFE
EFFICIENT
SIMPLE
RELIABLE
COST-EFFECTIVE
Decontamination!
Decontamination!
<table>
<thead>
<tr>
<th>Steps in infection control</th>
<th>Tools available</th>
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<tr>
<td>Locate (and identify) the infectious agent(s)</td>
<td>Eyes, Detection kits, bioassays</td>
</tr>
<tr>
<td>Eliminate (or neutralize?) the infectious agent(s)</td>
<td>Equipment (AWDs, AERs) and chemicals</td>
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<tr>
<td>Prevent introducing new infectious agent(s)</td>
<td>SOPs (based on RA)</td>
</tr>
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</table>
The (expected) action of AWDs
Tools currently in place for infection control, with a consideration for “prions”

Detection kits
How clean is clean?

Sensitivity of QC assay

Ideal scenario
Tools currently in place for infection control

Detection kits: Ninhydrin

Figure 2 Ninhydrin test sensitivity results giving a minimum level of detection observed by 75% of volunteers (MLD75) sensitivity level of 9.25 µg (95% confidence interval 8.6—10.0 µg).

Lipscomb et al., JHI 2006
Episcopic Differential Interference Contrast with Epifluorescence (EDIC/EF) microscopy.

Keevil et al., Water Sci Technol 2003
Lipscomb et al., JHI 2006
EDIC/ EF

Limit of detection well beyond necessary and practical values (over 2-log more sensitive than WB).

Total proteins (SYPRO Ruby)
MLD$_{75}$ = 175 pg/mm$^2$ (95% CI 104 – 286 pg/mm$^2$)
~ 5 femtomoles

Amyloid proteins (Thioflavin T)
Prp$^{Sc}$ – 1 μm / 1pg aggregates
~ 30 attomoles

ThT and SR non toxic at concentrations bound to contaminated surfaces.
Deemed “clean” surgical instruments observed under EDIC/EF microscopy
Deemed “clean” surgical instruments observed under EDIC/EF microscopy.

Residual crystalline and proteinaceous deposits on forceps.

Lipscomb et al., JCM 2006
How clean is clean?

Sensitivity of QC assay

Residual contamination

Ninhydrin standard LOD
How clean is clean? What is acceptable?

Sensitivity of QC assay

Residual contamination

Expanded scale

EDIC/EF LOD
Tools currently in place for infection control

Detection kits

Equipment and chemicals
So what does this mean?

The five main functions performed in a hospital sterile service department

...and where it can partly fail.

WHAT’S REMAINING: BIOFILM/ LIVE BACTERIA? ENDOTOXINS? PRIONS? CHEMICAL RESIDUES?
The measured action of AWDs:

Total protein load
Deemed “clean” surgical instruments observed under EDIC/EF: dual stain looking for amyloid

Thioflavin T (bright blue) and SYPRO Ruby (amber) dual staining observed on (a-e) a suction cannulae and (f) diathermy forceps from a neurosurgery set that were fully processed through a sterile service department. White bars are 100 μm, red bar is 10 μm.

Hervé et al., JMM 2009
Cleaning limitations

Protein removal action of various enzymatic cleaners

Hervé et al., JHI 2010
Cleaning limitations

Remaining contamination on surfaces
(proportion of hydrophobic amyloid-rich proteins)

Hervé et al., JHI 2010
The measured action of AWDs

Total protein and amyloid load
The action of cleaning chemistries
1: “good” enzymatic cleaner

Prion aggregate
The action of cleaning chemistries
2: alkaline cleaner (pH>12)

Prion aggregate
The problems with incomplete decontamination
The problems with incomplete decontamination

A. baumannii
The problems with incomplete decontamination
The problems with incomplete decontamination
Aggregate breakup = infectivity increase?
Incomplete cleaning leaves residual infectivity and increases bioavailability.

The evidence from animal infectivity studies

The detectable residual protein level is only an indicator!

Hervé et al., in preparation
Tools currently in place for infection control

Detection kits

Equipment and chemicals

Procedures
Wet or dry?

Secker et al., JHI 2011
Conclusions

PrP$^{Sc}$ and other hydrophobic proteins are the most challenging potential contaminants identified so far. There is a need to improve/confirm the efficacy of decontamination methods, particularly against prions. Current chemistries apparently reduce the risk of infectivity based on animal assays, but there is no validated assay to confirm action on human strains. Most enzymatic cleaners appear better than alkaline at total protein removal. **Only strong alkali treatment has been proven to reduce significantly potential residual prion infectivity.** Future protocols may rely on combinations of both or radically new approaches.
Discussion

This study shows that currently marketed cleaning chemistries and recent decontamination protocols do not completely suppress the threat from iatrogenic CJD.

These findings should be taken into account for risk assessment purposes and re-evaluating instrument handling and decontamination practices.
Acknowledgements

SSDs (England)
CEA (France)
The Pirbright Institute
NCJDSU
Public Health England
English Department of Health
Fondation Alliance BioSecure
Teşekkürler!

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