



METHODS TO EVALUATE NEW SURGICAL INSTRUMENT DECONTAMINATION TECHNOLOGIES

ISSUE

1. The Engineering and Science Advisory Committee into the decontamination of surgical instruments including prion removal (ESAC-Pr) is considering the evaluation and implementation of new decontamination technologies to reduce the risk of transmission of transmissible spongiform encephalopathies (TSEs) via surgical instruments. ESAC-Pr has asked SEAC to advise on the principles to consider when developing a scientifically robust and rigorous strategy to evaluate new prion decontamination methods.

BACKGROUND

2. Residual biological material can remain adherent to the surface of surgical instruments following standard cleaning and sterilisation¹. Therefore, potentially infectious material can be transferred from an infected patient to other patients via the instruments used. A small number of cases of iatrogenic transmission of CJD via contaminated surgical instruments have been reported². To date, no cases of iatrogenic transmission of vCJD via surgical instruments have been identified.
3. An initial risk assessment prepared by the Department of Health (DH) in 2000 and reviewed in 2005, both times accepted by SEAC, analysed the risk of transmission of prion diseases via surgical instruments³. It concluded, on the basis of the available data on tissue infectivity, the quantity of residual material on instruments after surgery and the effectiveness of current instrument decontamination practice, that the risks of vCJD transmission

¹ Murdoch *et al.* (2006) Surface decontamination of surgical instruments: an ongoing dilemma. *J. Hosp. Infect.* Epub.

² World Health Organisation (1997) Report of a WHO consultation on medicinal and other products in relation to human and animal transmissible spongiform encephalopathies. <http://www.who.int/biologicals/publications/en/BTSE97mar24.pdf>

³ Department of Health (2005) Assessing the risk of vCJD transmission via surgery: an interim review. <http://www.dh.gov.uk/assetRoot/04/11/35/42/04113542.pdf>

appear to be significant for certain types of surgery, in particular those involving the central nervous system and those encountering lymphoid tissue. Therefore, achieving high standards of instrument decontamination is of critical importance to reducing the risks. However, the limitations of current decontamination technology mean that instruments used on an infective patient could still be at significant risk of passing on vCJD.

4. Prions are capable of being adsorbed onto stainless steel surfaces⁴ and are unusually resistant to conventional chemical and physical sterilisation procedures⁵. Thus, they are relatively resistant to the normal cleaning and sterilisation practices used to decontaminate surgical instruments⁶. Many conventional decontamination procedures used on surgical instruments, although achieving very significant reductions in prion load, do not completely remove or inactivate prions⁷. Although effective chemical treatments to inactivate prions have been identified, they can irreversibly damage surgical instruments⁸.
5. A number of new alternative decontamination methods have been developed that are, or soon will become, commercially available. Many are reported to effectively remove, degrade and / or deactivate prions adsorbed onto stainless steel surfaces and to be compatible for use on surgical instruments. These reports are made on the basis of extrapolation of results from laboratory studies that have used experimental systems to model the clinical situation (i.e. decontamination of surgical instruments contaminated with CJD or vCJD prions).
6. ESAC-Pr is an advisory committee to DH. It is examining the growing body of research relating to prion decontamination of surgical instruments and is considering how this research could be transferred from the laboratory into practical surgical instrument reprocessing environments. It is considering guidelines for the independent evaluation of new commercialised decontamination methods.
7. ESAC-Pr is concerned about the validity of the model systems used to assess the effectiveness of decontamination methods and

⁴ Zobeley *et al.* (1999) Infectivity of scrapie prions bound to a stainless steel surface. *Mol. Med.* 5, 240-243.

⁵ Taylor *et al.* (1994) Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie. *Arch. Virol.* 139, 313-326.

⁶ McDonnell & Burke (2003) The challenge of prion decontamination. *CID.* 36, 1152-1154.

⁷ Smith *et al.* (2003) Prions and the oral cavity. *J. Dent. Res.* 82, 769-775.

⁸ Brown *et al.* (2005) Effects on instruments of the World Health Organisation recommended protocols for decontamination after possible exposure to transmissible spongiform encephalopathy-contaminated tissue. *J. Biomed. Mater. Res. B. Appl. Biomater.* 72, 186-190.

the extrapolation of the results to the clinical situation. The committee has requested advice from SEAC on the relevance of such systems to the clinical situation and the scientific principles it should consider in developing strategies for independent evaluation of new methods to decontaminate surgical instruments of human prions.

8. This paper provides an overview of published experimental approaches and model systems that have been used to demonstrate the effectiveness of new decontamination methods⁹. At SEAC 93, the committee will be presented with an overview of DH research on novel methods to decontaminate surgical instruments.

PREVIOUS SEAC ADVICE

9. SEAC has considered secondary transmission of vCJD via instruments used in surgical and dental procedures on numerous occasions. The committee has concluded that, although the risk of transmission of vCJD via contaminated instruments is uncertain, it cannot be ruled out. The most important risk reduction measure is effective decontamination of instruments.
10. At SEAC 86 (March 2005), the committee was updated on DH funded research on novel technologies to reduce the risk of transmission of prion diseases via instruments used in surgery and dentistry. It was informed that new decontamination methods would need to be independently evaluated for efficacy and reliability prior to implementation within the NHS. ESAC-Pr would address the issue of independent validation for new technologies. SEAC strongly endorsed independent validation of new methods.

ASSESSMENT OF DECONTAMINATION

11. The effectiveness of a decontamination method can be demonstrated by comparison of the contamination before and after treatment. If the levels of contamination are measured, the reduction in contamination can be quantified.
12. A variety of methods have been used to demonstrate the effectiveness of new decontamination technologies. *In vitro* systems have been used to measure either biological material or

⁹ Literature searches were conducted using the PubMed search engine and the following search terms, some used in combination: transmissible spongiform encephalopathy, TSE, scrapie, bovine spongiform encephalopathy, BSE, Creutzfeldt-Jakob disease, CJD, prion, PrP^{Sc}, decontamination, sterilisation, surgery, dentistry, surgical instrument and dental instrument.

protein as a non-specific marker of contamination or abnormal prion protein (PrP^{Sc}) as a surrogate marker for the TSE agent. *In vivo* bioassays have been used to detect directly the contaminating TSE infectivity present. The contaminant is either in a liquid suspension or adsorbed onto a stainless steel surface to model contamination of a surgical instrument. A table that summarises published methods used to demonstrate the effectiveness of new decontamination methods is given at Annex 1.

13. *In vitro* systems have been used to measure the removal and / or degradation of contaminating material. These methods have the advantage that they can be conducted rapidly. However, they do not provide a measure of the level of infectivity present to allow direct assessment of the effectiveness of a decontamination method to remove, degrade and / or deactivate TSE agents.
14. A number of different *in vivo* systems have been developed to model transmission of infectivity via surgery and have been used to demonstrate the effectiveness of decontamination methods. In general, they involve implantation into rodents of TSE contaminated stainless steel wires, or in one case spheres, either treated using the decontamination method or untreated to compare the levels of infectivity adsorbed. A number of different TSE strains, animal models and sites of implantation have been used in these systems. Therefore, comparisons of the effectiveness of different decontamination methods are difficult. Furthermore, although many of the studies report a reduction in, or absence of, detectable contamination following treatment, few studies have performed titration experiments that allow reductions in contamination to be quantified.
15. Cell-based assays are under development that may, in the future, provide a rapid non-animal based system to quantify TSE infectivity on a steel surface¹⁰, however it is unclear when such systems might be fully developed.

Analytical considerations

16. Two important considerations in relation to an evaluation system for new decontamination methods would appear to be:
 - the ability of the system to measure sufficiently large reductions in contamination.

¹⁰ Lehmann (2006) Third IPFA International Scientific Workshop on TSEs and the Safety of Blood Components and Plasma Derivatives.

- the relevance of the system to decontamination of surgical instruments contaminated with human TSE agents.

Required reduction in contamination

17. The level of decontamination necessary to reduce transmission risks to acceptably low levels is uncertain and dependent on the tissue load on instruments and the infectivity of the contaminating tissues. Therefore, the level of reduction in infectivity that might be required from a decontamination method is unclear. However, inputs into the DH risk assessment on transmission of vCJD via surgery provide an indication that current cleaning practices reduce the level of contamination (i.e. clearance of material from the metal surface) by between 1 and 3 logs and autoclaving reduces infectivity (i.e. deactivation of TSE agents) by between 2 and 3 logs. This suggests that methods to quantify the effectiveness of new and improved decontamination methods should be capable of measuring more than a 5 log reduction in contamination. Methods capable of measuring reductions in this order may also allow the kinetics of decontamination to be assessed, affording some extrapolation to development of decontamination methods.

Relevance of the system to decontamination of surgical instruments

18. It is unclear whether the physico-chemical and infectious properties of TSE agents are modified by adsorption onto a metal surface. It is possible they may be more resistant towards decontamination and that desorption of prions from the surface into a patient is not necessary to transmit infection¹¹. Therefore, systems that assess the decontamination of prions bound to stainless steel surfaces may be of more relevance to the clinical situation compared with a similar assessment made with prions in a liquid suspension.
19. To model TSE transmission from contaminated surgical instruments, many of the studies summarised in Annex 1 have used contaminated stainless steel implanted into rodents. Most of the studies have used the intracranial route of implantation to model transmission via neurosurgical instruments and to provide an efficient route of transmission. One study used the intraperitoneal route of implantation to model general surgical procedures. It is assumed that the adsorption of TSE infectivity onto such stainless steel implants is reasonably representative of the potential binding of infectivity to surgical instruments. In addition, it is assumed that the decontamination method will

¹¹ Flechsig *et al.* (2001) Transmission of scrapie by steel-surface-bound prions. *Mol. Med.* 7, 679-684.

behave similarly when applied to surgical instruments as it does on these implants. In all the studies, exposure of the animal to the contaminated stainless steel is continuous to maximise the efficiency of transmission, rather than transient as would be expected during surgery.

20. In these experimental systems, preparations of brain homogenate have been used to represent the potential contaminant tissue, rather than homogenates of other tissues or whole tissues, to maximise the titre of infectivity of the test material and the level of contamination of the stainless steel implant.
21. A number of different TSE agents have been used in these rodent models. Most studies have used hamster adapted scrapie as a model TSE agent since this allows an inoculum of high infectivity titre to be prepared that enables a large reduction in infectivity to be measured. However, it is unclear whether the properties of this agent are representative of human TSE agents. Studies suggest that differences in the physico-chemical, biochemical and structural properties of TSE agents may exist^{12,13,14}. Thus, they may behave differently from one another when exposed to the decontamination conditions being tested. One study has shown that the sCJD agent is substantially more resistant than the hamster scrapie agent under certain decontamination conditions (acidified sodium dodecyl sulphate treatment of contaminated stainless steel wires)¹⁵. Therefore, in the absence of comparative data on the behaviour of different TSE agents under a range of conditions, it may be difficult to make reliable predictions about the behaviour of one TSE agent under decontamination conditions on the basis of the behaviour of another agent.
22. A few studies have used rodent adapted human TSE agent or transgenic mice that express the human prion protein gene to enable assessment of decontamination of human, or closely related, TSE strains. Generally these strains produce inocula of lower titres of infectivity compared with the hamster scrapie strain. Transgenic mice that over-express the prion protein gene and are relatively susceptible to TSE compared with wild-type mice have been used as bioassays to increase the sensitivity of the system.

¹² Legname *et al.* (2005) Strain-specific characteristics of mouse synthetic prions. *Proc. Natl. Acad. Sci.* 102, 2168-2173.

¹³ Somerville *et al.* (2002) Characterisation of thermodynamic diversity between transmissible spongiform encephalopathy agent strains and its theoretical implications. *J. Biol. Chem.* 277, 11084-11089.

¹⁴ Peretz *et al.* (2002) A change in the conformation of prions accompanies the emergence of a new prion strain. *Neuron.* 34, 854-856.

¹⁵ Peretz *et al.* (2006) Inactivation of prions by acidic sodium dodecyl sulphate. *J. Virol.* 80, 322-331.

Summary

23. A number of different experimental systems have been used to demonstrate the effectiveness of new decontamination methods. Few studies have quantified the effectiveness of methods to reduce contamination. Thus, comparisons of the effectiveness of methods to reduce contamination are difficult.
24. The utility of evaluation systems to make reliable predictions about the effectiveness of new decontamination methods in the clinical setting is unclear. Ideally, an evaluation system would model the properties of human TSE agents bound to surgical instruments as closely as possible, would allow large reductions in infectivity to be quantified and would be applicable to a wide variety of decontamination methods.

ADVICE SOUGHT FROM THE COMMITTEE

25. ESAC-Pr is concerned about the validity of extrapolating results from experimental systems used to assess decontamination methods to the clinical situation. SEAC is asked to provide advice about the reliability of such extrapolations and for advice that can be used to develop a scientifically robust strategy for the evaluation and comparison of the effectiveness of new commercial decontamination products for human TSE agents.
26. The committee is asked to:
 - affirm its endorsement of independent evaluation of the efficiency and reliability of new decontamination methods prior to implementation.
 - provide scientific advice on the most appropriate experimental system or systems (if use of more than one system is appropriate) to evaluate and compare the effectiveness of new decontamination methods. In particular, advice is sought on the most appropriate form of contaminant(s), strain of TSE agent(s) and experimental system(s) (i.e. *in vitro* and/or *in vivo*) to use that will allow the most reliable evaluation possible of the effectiveness of new decontamination methods (see paragraphs 11 to 24 above).
 - produce a statement of its consideration for ESAC-Pr.

Summary table of published methods to demonstrate the effectiveness of decontamination methods (members of the committee have been provided with copies of the original research papers referenced in this table with this paper)

Analytical method	Decontamination treatment	Method of assessment of decontamination and effectiveness of decontamination treatment	Reference
<u>Physico-chemical analysis</u>			
Electron microscopy of: (i) Stainless steel spheres contaminated with hamster scrapie brain homogenate. (ii) Surgical instruments from sterile service departments.	Soaked in water then gas-plasma treated at 25°C for one hour.	Presence / absence of detectable residual material (resolution of > 5 nm) before and after decontamination. Absence of detectable residues after decontamination treatment.	Baxter <i>et al.</i> (2005) ¹⁶
Light microscopy of surgical instruments contaminated with mouse brain protein then stained with fluorescent dye (SPYRO Ruby).	None tested.	Level of detection of 85 pg/mm ² .	Lipscomb <i>et al.</i> (2006) ¹⁷
<u>Biochemical analysis</u>			
Immunoassay with chemiluminescence detection of PrP ^{Sc} on mouse scrapie brain homogenate contaminated stainless steel wires.	2M NaOH, 1 hour	Presence/absence of detectable prion protein after decontamination (level of detection of 0.3 ng of prion protein). Absence of detectable prion protein after decontamination treatment.	Flechsigg <i>et al.</i> (2001) ¹⁸
Western blot of PrP ^{Sc} in suspension of vCJD brain homogenate.	(i) Single and pairs of proteolytic enzymes over temperature range. (ii) SDS at 100°C,	Quantitation of the intensity of immunoreactive PrP ^{Sc} bands (limit of detection equivalent to 5nL of 10% (w/v) vCJD brain homogenate). Approx. 5 log reduction after decontamination treatment	Jackson <i>et al.</i> (2005) ¹⁹

¹⁶ Baxter *et al.* (2005) Elimination of transmissible spongiform encephalopathy infectivity and decontamination of surgical instruments by using radio-frequency gas-plasma treatment. *J. Gen. Virol.* 86, 2393-2399.

¹⁷ Lipscomb *et al.* (2006) Rapid detection for the sensitive detection of protein contamination on surgical instruments. *J.* ¹⁸ Flechsigg *et al.* (2001) Transmission of scrapie by steel-surface-bound prions. *Mol. Med.* 7, 679-684.

¹⁹ Jackson *et al.* (2005) An enzyme-detergent method for effective prion decontamination of surgical steel. *J. Gen. Virol.* 86, 869-878.

Western blot of PrP ^{Sc} in hamster scrapie brain homogenate or eluate from hamster scrapie brain homogenate contaminated iron powder.	proteinase K then pronase. 10-100 mM NaOH, 15 mins.	(ii). Quantitation of the intensity of immunoreactive PrP ^{Sc} bands. Absence of detectable PrP ^{Sc} in eluate from iron powder after treatment with 100mM NaOH.	Kasermann & Kempf (2003) ²⁰
Western blot of PrP ^{Sc} in eluate from stainless steel wires contaminated with hamster scrapie brain homogenate.	(i) 0.1-1.0 M NaOH (ii) 1.0-2.5% Na hypochlorite (iii) Peracetic acid (iv) Urea (v) Commercial cleaners	Semi-quantitation of intensity of immunoreactive PrP ^{Sc} bands (i.e. classified on range of very strong to faint signals).	Lemmer <i>et al.</i> (2004) ²¹
Bioassays			
Stainless steel spheres contaminated with hamster scrapie brain homogenate implanted intraperitoneally into hamsters.	Soaked in water then gas-plasma treated at 25°C for one hour.	Attack rate (clinical signs) and incubation period. No decontamination - 5/5 transmissions. Using decontamination treatment - 0/5 transmissions.	Baxter <i>et al.</i> (2005)
Stainless steel wires contaminated with hamster scrapie brain homogenate implanted intracranially into hamsters.	(i) 1M NaOH, 20°C, one hour. (ii) Autoclave dry 134°C, 18 min. (iii) Autoclave in water 134°C, 18 min. (iv) Enzymatic cleaner 43°C, 5min. (v) Enzymatic cleaner 43°C, 5min plus (ii). (vi) Alkaline cleaner (vii) Peracetic acid, 55°C, 12 min.	Estimated log reduction in contamination made on basis of comparisons of attack rate (clinical signs) and incubation time after decontamination treatment with titration curve of infectivity on wires. Using decontamination treatment (i) >5.6, (ii) 4-4.5, (iii) >5.6, (iv) 3.5, (v) 5 (vi) >5.6 (vii) 3.5 (viii) >5.6	Fichet <i>et al.</i> (2004) ²²

²⁰ Kasermann & Kempf (2003) Sodium hydroxide renders the prion protein PrP^{Sc} sensitive to proteinase K. *J. Gen. Virol.* 84, 3173-3176.

²¹ Lemmer *et al.* (2004) Decontamination of surgical instruments from prion proteins: *in vitro* studies on the detachment, destabilization and degradation of PrP^{Sc} bound to steel surfaces. *J Gen. Virol.* 85, 3805-3816.

²² Fichet *et al.* (2004) Novel methods for the disinfection of prion-contaminated medical devices. *Lancet.* 364, 521-526.

	(viii) Phenolic cleaner 20°C, 30min. (ix) Enzymatic cleaner 43°C, 5min plus vapourised hydrogen peroxide.	(ix) >5.6 log reduction in contamination.	
Stainless steel wires contaminated with mouse scrapie brain homogenate implanted intracranially into wild-type mice.	(i) Autoclave 134°C, 20 min. (ii) SDS, proteinase K then pronase at 40°C, one hour. (iii) 2M NaOH & autoclave.	Attack rate and incubation period. No decontamination - 5/7 transmissions. Using decontamination treatment (i) 0/9, (ii) 0/10, (iii) 0/10 transmissions.	Jackson <i>et al.</i> (2005)
Stainless steel wires contaminated with mouse scrapie brain homogenate implanted intracranially into reporter mice that over express prion protein gene.	(i) Autoclave 134°C, 20 min. (ii) SDS, proteinase K then pronase at 40°C, one hour.	Attack rate (clinical and neuropathological signs) and incubation time. No decontamination – 17/17 transmissions. Using decontamination treatment (i) 13/13, (ii) 1/18 transmissions.	Jackson <i>et al.</i> (2005)
Stainless steel wires contaminated with hamster scrapie brain homogenate implanted intracranially into hamsters or transgenic mice over expressing hamster prion protein gene.	(i) 4% SDS, 1% acetic acid, 65°C, 2 hours. (ii) (i) plus autoclave.	Attack rate and incubation time. Estimated log reduction in contamination made on basis of comparisons of incubation time with titration curve of infectivity on wires (limit of detection of approx. 9 log reduction in infectivity). No treatment 100% transmissions, 55 day incubation time. Using decontamination treatment (i) 14% transmissions, >400 day incubation time. (ii) No transmissions (> 8 log reduction).	Peretz <i>et al.</i> (2006) ²³
Stainless steel wires contaminated with sCJD brain homogenate implanted intracranially into transgenic mice expressing mouse-human	(i) 4% SDS, 1% acetic acid, 65°C, 2 hours. (ii) (i) plus autoclave.	Estimated log reduction in contamination made on basis of comparisons of incubation time with titration curve of infectivity on wires (limit of detection of approx. 6 log	Peretz <i>et al.</i> (2006)

²³ Peretz *et al.* (2006) Inactivation of prions by acidic sodium dodecyl sulphate. *J. Virol.* 80, 322-331.

chimeria prion protein transgene.		reduction in infectivity). No treatment 100% transmissions, 181 day incubation time. Using decontamination treatment (i) 64% transmission, 259 day incubation time. (ii) No transmissions (> 5 log reduction).	
Intracranial inoculation of suspension of mouse BSE brain homogenate into mice.	Range of thermostable proteases at 50-90°C and pH 7-12.	Estimated log reduction in contamination made on basis of comparison of incubation time with titration curve of infectivity (limit of detection of approx. 7 log reduction in infectivity). No treatment – 136 days incubation time. Using properase, 60°C, pH 12, 30min – 148 days incubation time (approx. 3 log reduction).	McLeod <i>et al.</i> (2004) ²⁴
Intracranial inoculation of suspension of hamster scrapie brain homogenate into hamsters.	Steris Corp. disinfectants: (i) Environ LpH (phenolic solvents) (ii) LpH-SE (phenolic solvents and acids).	Attack rate (clinical signs) and incubation time. No decontamination – 4/4 transmissions. Using decontamination treatment (i) 0/3, (ii) 4/4 transmissions.	Race & Raymond (2004) ²⁵
Stainless steel wires contaminated with mouse scrapie brain homogenate implanted intracranially into hamsters.	(i) Autoclave 134°C for 18min. (ii) Hydrogen peroxide, 10 min. (iii) Enzymatic detergent, 30 min. (iv) Peracetic acid, 5 min. (v) Alkaline detergent with aldehyde at 70°C.	Attack rate (clinical signs) and incubation period. No decontamination – 5/5 transmissions. Using decontamination treatment (i) 1/10, (ii) 3/10, (iii) 10/10 (iv) 10/10, (v) 2/10 transmissions.	Yan <i>et al.</i> (2004) ²⁶

²⁴ MacLeod *et al.* (2004) Proteolytic inactivation of the bovine spongiform encephalopathy agent. *Biochem. Biophys. Res. Commun.* 317, 1165-1170.

²⁵ Race & Raymond (2004) Inactivation of transmissible spongiform encephalopathy (prion) agents by Environ LpH. *J. Virol.* 78, 2164-2165.

²⁶ Yan *et al.* (2004) Infectivity of prion protein bound to stainless steel wires: a model for testing decontamination procedures for transmissible spongiform encephalopathies. *Infect. Control. Hosp. Epidemiol.* 25, 280-283.

