

Prions Fact Sheet

What is a Prion?

A Prion is an extremely small piece of protein that can infect healthy humans. They are regarded as sub-viral particles, i.e. Prions are smaller than the smallest viruses. The common names given to Prions and their diseases are Spongiform Encephalopathy, Bovine Spongiform Encephalopathy (BSE), Transmissible Spongiform Encephalopathy (TSE), Creutzfeldt Jacob Disease (CJD), Variant Creutzfeldt Jacob Disease (vCJD), Mad Cow Disease, Gerstmann-Straussler-Scheinker disease (GSS), Fatal Familial Insomnia (FFI) or Kuru. And in animals Scrapie (sheep), Chronic Wasting Disease (CWD)(Deer), Mad Cow Disease, and Transmissible Mink Encephalopathy (TME)(Mink). There are also reports of Spongiform Encephalopathies effecting large macropods and other mammals elsewhere including the Continental Americas.

What diseases are caused by Prions?

Prions can cause a specific type of progressive disease of the central nervous system leading to accelerating memory loss, dementia, progressive paralysis and eventually death. These groups of illnesses are usually referred to as Neurodegenerative Illnesses and Disorders. But there is generally a long latency or incubating period between the time of infection and the development of the disease (range is >2 years to <40 years but generally around >10 to <20 years).

How can you be infected?

There is a small number of people who seem to be susceptible to random development, and this latent potential for spongiform encephalopathy caused by Prions is genetically transmitted. The **Known** routes of becoming infected include eating infected body parts, particularly from the Central Nervous System or brains of those infected (Kuru), eating infected animals, receiving transplanted cadaver-explanted cornea, cadaver-explanted pituitary hormones, cadaver-explanted Dura mater grafts, surgical wound during handling of infected body parts, neurosurgical instruments contaminated with CNS material following surgical interventions with infected individuals, and blood donations from an infected donor. Apart from illness occurring sporadically in the general community amongst genetically susceptible population groups, only a small number of the population seem to be susceptible to extraneous infection when exposed to causative agent/protein.

Other instruments or objects that could potentially harbour and transmit the infectious agent/Prion are all neurosurgical implements and instruments, and cadaver explanted neural or dental body parts or devices. It should be noted that expression of the protein associated with infection, in individuals who are infected, is significantly present in neurological tissues and even the human tonsils. Retrospective studies have been conducted on tonsil tissue archives in the United Kingdom.

Who is susceptible to infection?

The most significant risk factor following exposure for symptomatic infection is genetic, i.e. inside the material of your DNA. But other non-susceptible people may be affected and infected, albeit with longer incubation or latency. Some studies on the background level of infection have yielded non-uniform results. CJD like disease's amongst nonsusceptible genetic populations has not been reported.

What makes a Prion different from other Infectious Human Pathogens?

A Prion is very difficult to kill because its infectivity is not subject to the normal rules of replication. It appears to be a wrongly folded protein that simply hooks onto a

commonly found host protein intracellular production organelle. Once genetically susceptible individuals are infected, Prions then cause the subsequent development of the host protein organelle to replicate the form of the infectious protein. As the host protein is commonly produced in certain types of cells, eventually all of the locations where the host protein would otherwise normally develop in healthy individuals, those cells (post infection) will produce this new and distorted infectious version of the original protein.

The infectious protein then causes a progressively degenerative illness as the infectious protein accumulates throughout the cells in the body. Where this protein accumulates (particularly in nervous tissues), the host cells affected develop large protein based fibrous looking clumps (called Fibrils), which are clustered in brain, nervous system and lymphoid tissues. These clumps are rich in Copper ions (Cu^{2+}) which is possibly artifact due to the normal role of the protein in regulation of intracellular copper. An excess of manganese at the time of cellular infection may also play some role in infection but this may also be an artifact due to the intended normal function of these proteins units within healthy cells.

Simply put, a Prion is a bit of rogue protein that when it gets into a susceptible host, causes the cells in that person to produce more and more of the protein which interferes with central nervous system functions until the susceptible infected person dies. These pieces of rogue protein can be singular or clumped together, but when in 'clumps' can act as insulators for the innermost intact Prion proteins.

What denatures a Prion?

Prions are not alive and are never alive even in an infected person. Because the Prion is no more than a piece of rogue protein, and it does not contain any genetic material such as DNA or RNA, it is not dependant on gene sequence for its replication. The folded shape of the protein also enables it to avoid degradation by the normal intracellular enzymes that usually control protein molecules and sub-units within healthy cells. Once outside the body Prions are resistant to most of the normal Sterilising processes. To denature or disintegrate this protein a more aggressive response is required. Normal Sterilizing approaches have been shown to be ineffective against Prions - even when present at low levels.

The following materials have been shown in the literature to be effective in destroying Prions:

1. 1 Normal Sodium Hydroxide (4% solution) or Potassium Hydroxide (6% solution);
2. 20,000 ppm (mg/L) of Sodium Hypochlorite;
3. Autoclaving at higher pressure for longer periods than normally indicated. This is to ensure that where the Prion Proteins are clumped together, that the heat and pressure is able to fully penetrate to the center of the clumps where intact proteins may be harboured;

Other chemical means of achieving at least sufficient denaturation or clump separation have been investigated and published, but none can guarantee to totally denature all Prion proteins, nor can they guarantee complete separation of clumps. Data claims in this area are still at the peer review stage and some articles are already contradicting others. None of the methods published thus far follow a path that represents a clear route for total Prion destruction except where the instruments surfaces are degraded or compromised, or the above methods are also included.

The following materials and methods have some data suggesting efficacy against Prions:

4. Multi-step processes where protein digestion is achieved through multiple

means including alkaline detergents using Sodium Hydroxide or Potassium Hydroxide and detergents at elevated temperatures (pH must be pH >11 at >90°C),

5. Multi-step processes where protein digestion is achieved through multiple means including alkaline detergents and/or Proteinaceous Enzyme Detergent - in some cases injected simultaneously (pH must be pH >11 at >55°C), and then subject to normal sterilising processes;

6. Multi-step process where protein digestion is done using an alkaline detergent system with >500ppm effective alkali at >50°C and then uses Peracetic Acid >2,000 ppm at >53°C

What destroys a Prion has been shown to be aggressive chemicals or physical processes that interrupt and denature all proteins. The protein must be fully denatured to stop infection.

It should be noted that one of the problems in testing has been until recently access to samples with cost effective analytical systems. The principles of deactivation are principally known methods of protein digestion with added washing machine applications adjusted to reach the necessary performance criteria. Peracetic acid alone is no more effective than Glutaraldehyde or other Sterilant materials including ETO. Likewise, alkaline systems alone require extreme concentrations and long exposure times at room temperature (> 60 minutes) to be effective.

Earlier studies did show that shorter exposure times to alkaline materials could be achieved using heated solutions but in reality how would healthcare facilities handle 4% (concentration \geq 40g/L) caustic heated to 100°C for 10 minutes?

What are the risks of Prion contamination?

Most instruments are not at risk for Prions. The incidence of Prion Infection in the wider Australian population is very rare. The 'bubble' of infections due to Mad Cow Disease in the UK has now receded. After the initial fear that up to 30,000 people in the UK and elsewhere could be affected by the UK "outbreak", the numbers of actual infections appears to have resulted in around 2,000 fatalities. No accurate or clinically defensible method of assessing the asymptomatic carriers is available at this time.

It has been suggested that up to 37% of UK residents had the most susceptible genetic profile for the risk of being infected and affected by the Prion Protein (see Murdoch et al.).

The risk of Mad Cow Disease in Australia is much lower. There have been no reported cases of vCJD in Australia. Rare and sporadic cases of CJD have been reported over a long period. Vigilance over the various decontamination processes in high risk cases and for "at risk", "high risk" or known CJD patients must be maintained. The Australian Infection Control Guidelines set out the best and most accurate indications for risk and risk management for the Australian Health Care System.

Latest Evidence on Protein Contamination of Instruments

Several studies have now demonstrated that instrument cleaning measures can be improved. In the case of flexible endoscopes, the risk of biofilm contamination is high. Studies have shown that enzymatic detergents are inferior to formulated detergent and cleaning products designed for Biofilm removal. The presence of biofilm contamination in one study showed 100% of discarded internal working channels from endoscopes had evidence of biofilm contamination. Pertinently enzymatic cleaning products had been the normally indicated product for cleaning of reusable endoscopes for all of the 1990's. Only more recently has AS/NZS 4187:2003 recognised the importance of detergent alternatives.

Recent studies with branded enzyme cleaners have shown some promise, but as unregulated materials issues on ageing are yet to be addressed with respect to efficacy claims and no clear evidence on consistent performance has become available through the published literature and peer review is continuing even on the published references as further studies are continuing to be published. It seems likely that enzyme cleaners may assist in “un-clumping” of protein units but this mechanism requires further work and studies that link solution life and Prion sub-types to consistent outcomes.

It is important to note that because the protein unit ‘Prions’ are not “alive”, doing infective enumeration is also difficult, thus the infective dose is not quantified in any way that is linked quantitatively to infection. Thus, claims as to “kill” are not being made, because the evidence is not strong and is limited to “decontamination”. Reliable denaturation studies that are validated are not yet commercially available.

On rigid instruments of all sorts, more recent studies have repeatedly demonstrated the presence of recoverable volumes/masses of Proteinaceous materials from instruments post processing and immediately prior to re-use. The mass of the soil recovered has varied from pico-grams to milligrams. The upper end of the published range demonstrated 45mg of a particular instrument. Latest studies suggest a failure rate for true cleanliness between 38% and 100% of fully washed and “sterilized”, ‘procedure ready’ instruments.

Prions are strands of protein of around 30,000 Dalton units. Many proteins have around 600,000+ Dalton units of mass. It has been estimated that 1 micro gram of protein residue would contain 10^{14} individual protein molecules (Baxter et al.). So 45mg of Proteinaceous mass represents a major risk level (possibly up to 45 times 10^{17} potentially infective protein units). The risk of residual protein remaining on instruments and medical devices used on “at risk”, “high risk” or known CJD patient risk groups should not be understated, albeit the frequency of those risk groups is rare.

One important study (Lipscomb et al.) showed that despite presence of protein the validation systems used to ensure viable microbes were destroyed was effective. In that study, neither the evidence of colonization nor the presence of bacterial Endotoxin was found as evidence in the “ready for reuse” instruments surveyed. The risk of protein residues remains the focus of further study.

What Works?

Interestingly information on cleaning systems used in the dairy industry or under modern HACCP systems is instructive. Alkaline cleaning systems that are sufficiently strong, with sufficient exposure time, and with sufficient heat have been demonstrated under recent reports to be highly effective in removing and disintegrating protein remnants. Some enzyme systems can assist, but these have only been shown to work in combination with sufficient alkali (greater than 500mg/L). All studies have shown that pH, time and temperature are the key variables.

What is most concerning is that the trade-off cost benefits are not fully appreciated.

There is not a single study that links any gain to the following four factors being

- i) the risk of infection occurring in the Australian context;
- ii) the Risk of reaching an infective dose through normal processing systems (i.e. the current methods of instrument processing under AS/NZS 4187:2003);
- iii) the potential for damage to otherwise well maintained surgical devices and with
- iv) the overall cost of the reprocessing system.

What about current Visual Indicator Systems?

These have been shown to be almost irrelevant, except as pacifiers (Stewart 2007). Obviously if the contamination risks are only detectable after considerable chemical digestion and / or scanning electron microscopy (SEM), then a system that relies on the naked eye is somewhat limited. Most test strips and cleaning verification tools also rely on proper placement within the automated cleaning machine. Wrong placement of the test strips inside a Washer Disinfector will yield false positive outcomes.

What this does say about the broader effectiveness of test strips used within the automated washers is that the efficiency and efficacy of some instrument washing machines is less than desirable.

Testing of the available verification systems also indicates that at this time no reliable field based tool exists to indicate the efficacy of the cleaning systems at the microscopic level. To detect residual proteins some investigators have used fairly extreme chemistry methods and chemicals that sacrifice the instruments to detect what are very small quantities of residual protein. Clearly this cannot be a normal model for the chemistry and science used in instrument reprocessing.

How Should I clean my instruments?

For most instruments your existing and standard approach as per AS/NZS 4187:2003 is sufficient. Where specific carrier risks are identified, additional precautions in cleaning may be required. A full informational guideline is available in the Australian Infection Control Guidelines 2004.

Strongly alkaline cleaners, when used above pH 12 and above 80°C with a 10 minutes cleaning cycle have been shown to be effective at decontamination of Prions. However, some of the studies do not indicate protein removal as much as denaturation of the protein thus decontaminating the instrument.

End users should be aware that any implied claims for PRION effectiveness must have full TGA approval pre-market and so inquirers should contact the TGA to verify any claims. The standard of proof required for a “kill-claim” in this area does not appear to have been reached at this time thus some marketing is being constructed around product performance to avoid the regulators specific review.

Note: elevated pH levels (in excess of pH 9 - 10) will cause corrosion and physical damage to many instruments. Use of elevated alkaline cleaning solutions should be avoided in most Medical Devices where more than one metal or alloy is present or where any metal other than high quality Stainless Steel is used in the construction of the Medical Device.

Instruments or Medical Devices containing Aluminium are particularly vulnerable to alkali corrosion and care should be taken to check on device manufacturer recommendations prior to alkaline decontamination procedures of any instrument, particularly where a more complex device is being used with more than one type of metal present. Customers should very carefully consider the alkali levels recommended through false negative cleaning indications through use of certain “cleaning validation tools”.

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References

“The Australian Infection Control Guidelines for the prevention of transmission of infectious diseases in the healthcare setting”, Australian Government, Department of Health and Ageing, 2004

"Halting the Spread of Human Prion Disease – exceptional measures for an exceptional problem", Stephenson J; J Hosp Inf, 65(S2), pp 14 -18, 2007

"Decontamination of surfaces contaminated with Prion-Infected material with oxidizing agent based formulations", McDonnell GE, Kaiser HJ, Antioga KM, & Scocos JA; United States Patent and Trademarks Office (USPTO), USP # 7001873, 2006 (application priority date 2002)

"Novel methods for disinfection of prion-contaminated medical devices", Fichet G, Comoy E, Duval C, and others, The Lancet, vol 364, pp 521-526, 2004

"Efficacy Against Infectious Prions in Instrument Reprocessing", Rudin D; Inf Control Today, vol 10 (6), March 2006

"A study in Washing and Sterilisation", Rosenberg U, Materials Management Magazine, February 2006
"Comparative Study of Surgical Instruments from Sterile Service Departments for the presence of Residual Gram-negative Endotoxin and Proteinaceous Deposits", Lipscomb IP, Sihota AK & Keevil CW; J Clin Microbiol., ASM, August 2006

"Enzymatic detergent treatment protocol that reduces protease-resistant prion protein load and infectivity from surgical-steel monofilaments contaminated with a human derived prion strain", Lawson VA, Stewart JD & Masters CL, J General Virology, vol. 88, pp 2905-2914, 2007

"Surface decontamination of surgical instruments: an ongoing dilemma", Murdoch H, Taylor D, Dickinson J, Walker JT, Perrett D, Raven NDH, Sutton JM, J Hosp Inf, vol 63, pp432-438, 2006

"Quantitative analysis of residual protein contamination on reprocessed surgical instruments", Baxter RL, Baxter HC, Campbell GA, Grant K, Jones A, Richardson P, Whittaker G, J Hosp Inf, vol 63, pp 439-444, 2006

"Comparative study of surgical instruments from sterile service departments for the presence of residual Gram-negative endotoxin and Proteinaceous deposits", Lipscomb IP, Sihota AK, Keevil CW, J Clinical Microbiol, August 2006

"Investigating Current Methods of Soil Testing and Cleaning Validation" Alison Stewart, New Zealand Sterilising Journal, 2007

"A physiological role for healthy proteins" Treiber C, et al, Biochemistry J (German: translation summary in Royal Society of Chemistry, under "World News", by Micheal Gross, May 2006)

ISO 17664:2004 "Sterilisation of Medical Devices – information to be provided by the Manufacturer for the processing of resterilisable medical devices"; International Standards Organisation
Health Technical Memorandum (HTM) 2030, British Government: National Health Service, 1997