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*Custodit vitam qui custodit sanitatem
Sed prior est sanitas quam sit curatio morbi
(Flos Medicinae Scholae Salerni)*

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Jung MJ., Pistolesi D. and Panà A.

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Prions, priondiseases and decontamination

Jung MJ.⁽¹⁾, Pistolesi D.⁽²⁾ and Panà A.⁽³⁾

⁽¹⁾ CH-8800 THALWIL/SVIZZERA SÄUMERSTRASSE 45 (FOR REPRINTS)

⁽²⁾ FEDEGARI SpA, ALBUZZANO PAVIA

⁽³⁾ CATTEDRA D'IGIENE, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"

Keywords *Prions decontamination in hospital units*

Summary Prions are extremely resistant to disinfection and sterilization methods used so far. The pathogenic prion protein core (called prion) consists of 142 aminoacids, is resistant to proteolytic enzymes, has a mass of 15 pikograms and is filtrabile. Fixed by dessication or chemicals may retain infectivity for years. It survives dry heat at 200 °C for 1-2 hours. Prions are fixed to stainless steel within minutes and remain infectious for long periods. Their pathogenetic properties depend on tertiary spatial structure (conformation) which is specific and transmissible in experiment.

The prion decontamination appears by far the most important area of the prion science because very little, or nothing, has been done in the majority of world hospitals to prevent iatrogenic transmission. The number of potentially infectious patients is not known. Therefore, patients undergoing neurosurgery, laryngeal or ophthalmic operations, orthodontal treatments and even anaesthetic or endoscopic applications should be classified into risk groups, even if clinically prion-disease inapparent. The use (or misuse) of disposable instruments is certainly not the final answer for all cases and classic decontamination procedures, if possible because of the character of medical devices, appear still of greatest importance.

We consider the high pathogen safety (HPS) autoclave from FEDEGARI as the best actual equipment for the effective decontamination of prions in the hospital practice. The investment costs are moderate and the handling is simple but must be careful. It appears practicable even in small specialized units.

Prioni, Malattie da Prioni e Decontaminazione

Parole chiave *Decontaminazione da prioni in ospedale*

Riassunto I prioni sono estremamente resistenti ai sistemi di disinfezione e sterilizzazione normalmente usati. La proteina prionica patogena consiste in 142 aminoacidi, è resistente agli enzimi proteolitici, ha una massa di 15 picogrammi ed è filtrabile. Se fissata mediante l'essiccamento o con sostanze chimiche essa può mantenere il suo grado di infettività per anni. Sopravvive al calore secco a 200°C per 1-2 ore. I prioni si fissano in pochi minuti sull'acciaio inossidabile e rimangono infettivi per lungo tempo. Le loro proprietà patologiche derivano dalla struttura spaziale terziaria che è specifica e sperimentalmente trasmissibile. La decontaminazione dei prioni costituisce il problema più rilevante poiché niente o molto poco è stato fatto nella maggior parte degli ospedali per prevenire la trasmissione iatrogena. Il numero dei pazienti potenzialmente infetti non è conosciuto; In ogni caso pazienti che hanno subito interventi di neurochirurgia o dell'apparato laringeo ed oftalmico, trattamenti ortodontali, applicazioni endoscopiche o che hanno subito anestesie, dovrebbero essere considerati gruppi a rischio anche se clinicamente privi di sintomi da prioni. L'uso di strumentario medico a perdere non può costituire la risposta decisiva e le classiche procedure di decontaminazione soprattutto per certi strumentari medici è ancora di estrema importanza. In questo lavoro viene descritta la notevole capacità di decontaminazione degli autoclavi Fedegari che costituiscono il migliore

sistema oggi per la decontaminazione dei prioni nella pratica ospedaliera. I costi di investimento sono moderati e l'uso è semplice e accurato; Il loro uso è particolarmente adatto in piccole unità mediche specializzate

Prions , Maladies à Prions et Décontamination

Mots-clé *Décontamination à prions dans les Hôpitaux.*

Résumé Les prions sont extrêmement résistants aux systèmes de désinfection et de stérilisation que l'on utilise couramment. La protéine pathogène du prion consiste à 142 acides aminés; elle est résistante aux enzymes protéolytiques, a une masse de 15 picogrammes et peut être filtrée. Lorsqu'elle est fixée par le séchage, ou bien par des substances chimiques, elle peut garder son degré d'infectiosité pendant des années. Elle survit à la chaleur sèche à 200°C pendant une ou deux heures. En quelques minutes seulement, les prions peuvent se fixer sur l'acier inoxydable et rester infectieux longtemps. Leur propriétés pathologiques tiennent à une structure spatiale tridimensionnelle et spécifique qui peut être transmise expérimentalement. La décontamination des prions reste un problème important puisque dans la plupart des hôpitaux il n'y a pas eu de mesures efficaces pour prévenir la transmission iatrogène. Le nombre des patients potentiellement infectés est encore inconnu. De toute manière, les patients qui ont été soumis à des soins neurochirurgicaux, à des interventions aux appareils laryngiens et ophtalmiques, à des traitements orthodontiques, à une chirurgie endoscopique ou à des anesthésies, doivent être considérés comme des groupes à risque, bien que cliniquement ils ne montrent aucun symptôme à prions. Le recours aux instruments chirurgicaux ne peut pas être considéré comme une solution définitive. Ainsi, les procédures traditionnelles de décontamination restent fondamentales, surtout en ce qui concerne certains instruments médicaux. Dans cette étude, les auteurs décrivent la capacité de décontamination des Autoclaves Fedegari, qui sont à présent le système le meilleur pour la décontamination des prions dans des milieux hospitaliers. Le coût d'investissement est assez modéré et leur emploi est facile et précis. En particulier, elles sont indiquées pour les petites unités médicales spécialisées.

Prionen, Prionenkrankheiten und dekontaminierungsmethoden

Schusselwörter *Prionen dekontaminierung in Sterilizationabteilungen*

Zusammenfassung Prionen sind äusserst resistent gegen bisher angewendete Desinfektions- und Sterilisationsmethoden. Das pathogene Prion Kern-Protein besteht aus 142 Aminosäuren und ist resistent gegen proteolytische Enzyme. Es wiegt 15 Pikogramm und ist filtrierbar. Durch Austrocknung oder chemisch fixierte Prionen behalten ihre Infektiosität über Jahre, und sie überstehen trockene Hitze (200°C) bis zwei Stunden. Prionen setzen sich minutenschnell fest an rostfreiem Stahl und bleiben längere Zeit infektiös. Ihre Pathogenität ist auf die räumliche Struktur (Konformation) zurückzuführen, die spezifisch ist und die im Experiment übertragbar ist.

Die Inaktivierung von Prionen scheint der wichtigste Bereich der Prionenforschung zu werden, weil in den meisten Spitälern der Welt wenig oder nichts unternommen wurde, um iatrogene Übertragungen zu verhindern. Die Zahl der potentiell infektiösen Patienten ist nicht bekannt. Daher sollten Patienten, die sich neurochirurgischen Eingriffen, oder Eingriffen am Kehlkopf oder am Auge, Kieferoperationen und Anästhesie oder Endoskopie-Behandlungen unterziehen, als Risiko-Gruppen eingestuft werden, selbst wenn sich eine Prion-Erkrankung klinisch nicht manifestiert. Der Gebrauch (oder Missbrauch) von Einweg-Instrumenten ist sicher nicht die endgültige Antwort für alle Anwendungen und klassische, den Eigenschaften von medizinischen Instrumenten angepasste Dekontaminierungs-Methoden sind immer noch von grösster Wichtigkeit.

Wir beurteilen den HPS (High Pathogen Safety) Autoklav der Firma FEDEGARI als die zur Zeit beste Ausrüstung zur wirksamen Inaktivierung von Prionen für Spitäler. Die Anschaffungskosten sind zumutbar und die Bedienung ist einfach, muss aber zuverlässig erfolgen. Der HPS Autoklav eignet sich auch für kleine spezialisierte Abteilungen.

Introduction

Prions are unprecedented infectious pathogens causing invariably fatal neurodegenerative diseases by an entirely novel mechanism. They are devoid of nucleic acids (!) because they are resistant to methods which inactivate this genetic material (Nobel prize 1), ^(1,2). They are formed post translation (synthesis of proteins) through conversion of normal cellular prion protein, PrP^C, encoded by Exon 2 of the prion protein gene, PRNP ⁽³⁾ into pathological prion protein isoform PrP^{Sc} (Sc from scrapie). This conversion does not involve a chemical process and the aminoacid sequence (primary structure) is identical in both isoforms,

but the secondary structure differs in Beta-sheet ⁽⁴⁾ contents (Figure 1) - PrP^{Sc} is also partially resistant to proteolysis and insoluble in non-denaturing detergents. The tertiary, three-dimensional structure (conformation) is responsible for strain characteristics of prions responsible for various diseases. Studies on transgenic animals argue that PrP^{Sc} acts as a template upon which PrP^C is refolded into a nascent PrP^{Sc} molecule through a process facilitated by another, protein X ⁽⁵⁾ (Figure 2). This, self-perpetuating process results in geometrically increasing PrP^{Sc} concentration, which is deposited mainly in brain but on other places as well.

Prion protein polypeptide consists of 254 aminoacids (AA) with (Figure 3) 22 AA signal sequences at N-terminal and 23

Figure 1
Secondary structure of PrP

PERCENT	PrP ^C	PrP ^{Sc}	PrP27-30
ALFA HELIX	42	30	21
BETA SHEET	3	43	54

PNAS 90, 10962, 1993

Figure 2
Conversion PrP^C→PrP^{Sc}

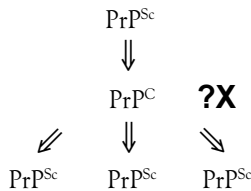


Figure 3
Prion Protein (PrP) Isoform

Codon	1	23	51	91	102	231	254
PrP ^C	209	AMINOACIDS (AA)					
PrP ^{Sc}	209	AMINOACIDS (AA)					
	LIMITED PROTEOLYSIS (K)						
PRION PrP 27-30	142 AMINOCID (AA)						

From Prusiner S,B, Nobel Lecture, 1998. Univ. California
Ball H.L. Italfarmaco Research Centre, Milano, 1996
Ball H.L. Univ. California, 2001

AA signal sequences at C-terminal; mature prion protein (both PrP^C and PrP^{Sc}) have 209 AA. The major conformational change occurring during PrP^C→PrP^{Sc} conversion has been largely localized to a region bounded by residues AA 90 and 112. Treatment with a proteolytic enzyme (Proteinase K) completely digests PrP^C, but only circa 67 AA are digested from the N-terminus of PrP^{Sc} thus generating the prion with 142 AA; it was detected more than 20 years ago in pathological materials of deceased patients at autopsy. It was declared as PrP27-30 according to its molecular weight and mass of circa 15 pikograms (10^{-9.0/mg}). Prions are filtrabile (contrary to some publications) and thus resemble viruses ^(6,7,8).

Prion diseases are (A) Genetic by mutations on PRNP including (a) point mutation (*), (b) deletions and (c) insertions, (B) Infectious (but not contagious) by Iatrogenic transmission and new variant CJD and (C) Sporadic as sporadic CJD (sCJD) of unknown aetiology with infection suspected but not proved ^(9,10,11). This form is the most frequent. It is of greatest importance that they are all experimentally and iatrogenic transmissible (*Table 1*).

Table 1
HUMAN TRANSMISSIBLES SPONGIFORM ENCEPHALOPATHIES

DISEASE	GENETIC ABNORMALITIES
CJ D/FAMILIAL GSS FFI	POINT MUTATIONS AT CODON PrP 102, 105, 117, 145, 160, 171, 178, 180, 183, 187, 188, 198, 200, 202, 208, 210, 212, 217, 232
CJD/SPORADIC /IATROGEN /VARIANT FI /SPORADIC	NONE NONE NONE NONE
OTHER	DELETIONS BETWEEN PrP 51-91 ONE OCTAPEPTIDE (24bp) TWO OCTAPEPTIDES (48 bp) INSERTIONS BETWEEN PrP 51-91 ONE, TWO, FOUR, FIVE, SIX, SEVEN, EIGHT, NINE, OCTAPEPTIDES
CJD	CREUTZFELDT-JAKOB DISEASE
GSS	GERSTMANN-STRAEUSSLER-SCHEINKER SYNDROME
FFI	FATAL FAMILIAL INSOMNIA
FI	FATAL SPORADIC INSOMNIA

Prion diseases are histopathologically characterized by spongiform vacuolization affecting any part of the cellular gray matter, astrocytic proliferation and neuronal loss, because of degeneration and, sometimes, deposition of amyloid plaques. There is no inflammation and no detectable antibodies thus differing from typical viral encephalitis. Progressive dementia is the hallmark of CJD and psychiatric symptoms in the early stage of vCJD; ataxia may also be dominant in some prion diseases. The diagnosis is confirmative at autopsy. It is not possible to test the infectivity in the patient during the preclinical phase of the disease. Similarly, this is also true for subclinically BSE infected cattle.

Variant Creutzfeldt-Jakob Disease (vCJD)

The interest in prion diseases increased in the past decade since the description of the vCJD in Great Britain ⁽¹³⁾. The link between Bovine spongiform encephalopathy (BSE) and vCJD was first noted by a young physician in 1988 ⁽¹⁴⁾ and declared, by the British government, as possible and probably, in 1996. The disease was named “variant” CJD because of clinical differences (psychiatric symptoms predominant) and young age (median 28 years; the youngest 12 years). Reasons for that may be (a) the age-dependent susceptibility to infection, (b) age-dependent incubation period because of earlier clinical onset and (c) age-dependent exposure because younger eat riskier food. By 6th October 2003 ⁽¹⁵⁾ 143 cases were observed in Great Britain (Table 2), six in France ⁽¹⁶⁾, two in Italy ^(17,18) and one in Republic Ireland, Hong Kong ⁽¹⁹⁾, Miami ⁽²⁰⁾ and Canada ⁽²¹⁾. The future of vCJD is not well understood because of the extremely long incubation period (decades) which sometimes may

Table 2
Data from CJD Surveillance Unit

<i>Deaths</i>	
Deaths from definite vCJD (confirmed):	101
Deaths from probable vCJD (without neuropathological confirmation):	33
Deaths from probable vCJD (neuropathological confirmation pending):	3
Number of deaths from definite or probable vCJD:	137
<i>Alive</i>	
Number of definite/probable vCJD cases still alive:	6
Total number of definite or probable vCJD cases (dead and alive):	143

(Table updated 6 October 2003)

be longer than the life expectancy. A world-known italo-swiss scientist (22) means that hundred millions of European people are BSE/vCJD contacted. The link BSE→vCJD is confirmed by (1) biochemical analysis of prion strains (Western blot)⁽²³⁾, (2) comparison of neuropathology and incubation times on serial passages in laboratory animals⁽²⁴⁾ (3) experiments on transgenic animals carrying human or bovine PrP (or both) which were cross infected⁽²⁵⁾, (4) typical similarity of vCJD/BSE clinical pictures⁽²⁶⁾ and (5) similarities by nuclear magnetic resonance (NMR), Nobel prize 2⁽²⁷⁾ of vCJD and BSE prions. Enormous work has been invested in such works and one strain typization requested up to two million British pounds.

The interest in prion diseases has significantly increased as evident from scientific publications. One medical article of general interest is usually followed by ten others, supposed to be more important, but by hundred articles on prions.

Bovine Spongiform Encephalopathy (BSE)

BSE has been, besides scrapie, the best known animal prion disease identified in Great Britain in November 1986. BSE epidemiologically exploded and by 2001 over 177.000 cases of BSE had been confirmed only in GB⁽²⁸⁾. Over 2,500.000 cattle were slaughtered to stop the epidemic, but 750.000 infected cattle entered human food chain. The disease in cattle originated from supplementary cattle feed containing meat and bone meal (MBM) contaminated by a prion agent from cattle or sheep; the serious rendering procedures were drastically reduced this time (decreasing the temperature during processing and stopping the solvent extraction). This was quickly realized and use of MBM stopped (feed ban) in 1988. However, the MBM export was continued for some time and many cases of BSE have slowly developed in West - but not East - European region (probably because shortage on money to pay for British MBM). Cases of BSE were also found in Arab Emirates, Falkland Islands, Canada and recently in Japan, where the use of MBM was allowed until recently (Japan scandal)⁽²⁹⁾. Italy has also exported MBM to Slovenia. BSE free countries are USA and the Middle-and South-America and some Asian countries. The control of cattle for BSE during slaughtering for consumer protection is not very effective because the test we use give false negative⁽³⁰⁾ results in the preclinical stage of the disease and the same is true for human infections. Decontamination of prions appears therefore of elementary importance.

Decontamination of Prions

It has been long known, that prions are unusually resistant to disinfection and sterilization by physical and chemical methods in common use for decontamination of infectious pathogens⁽³¹⁻³⁵⁾. It is a difficult task to gain a consensus opinion on what constitutes optimal and practical conditions for decontamination of prions. Numerous studies have been conducted, but they do not reflect the reprocessing procedures for instruments in a clinical setting which are critical for iatrogenic transmission. A contact time of 5 minutes with prion infected tissue (brain in experiments suffices to render steel highly infectious⁽³⁶⁻³⁷⁾. Infectivity bound to steel-wires persists for long particularly if dried or treated with disinfectants fixing proteins as i.e. formaldehyde⁽³⁸⁾; it may fix prions for years. As what we know now non-disposable instruments used in neurosurgery, ophthalmology, otorhinolaryngology (dangerous lymphatic - and paralympathic tissue in the throat and tongue), instruments used in anaesthesia, flexible gastroscopes (prions were found in intestine) and orthodontal surgery should be regarded as critical. In its risk assessment the Dept. of Health (Great Britain) estimated that transmission on instruments could increase the size of any given epidemic by 10 percent. Seven hundred variant CJD cases are expected (!) unless steps were taken to eradicate this route of transmission⁽³⁹⁾.

Recommendations to prevent cross-transmission of infections from medical device have been based on prion infectivity studies by inoculating animals (mostly mice) intra-cerebrally prior and after the decontamination experiment. The infectivity titer of inoculated materials ranged usually between $10^{8.0}$ and $10^{10.0}$. The titer decrease after decontamination was accepted as degree of effectiveness⁽⁴⁰⁾. When the first decontamination result is a 7-log reduction of infectivity and 1-log in following decontamination cycles this would in total contribute to surgical transmission of 1.5 - 4 extra cases per 1000 primary infections. In the case of 5-log and 1-log reduction about 3.5 - 11 additional cases per 100 primary operations might be expected⁽⁴¹⁾. All these tests are very labour intensive and very few laboratories are able to do this.

A wide variety of practices and procedures for disinfection and sterilization or reprocessing medical instruments potentially contaminated with prions were published (World Health Organization⁽⁴²⁾ Steering Scientific committee of European Commission⁽⁴³⁾ U.S. Food and Drug Administration, Center for

Disease Control/National Center for Infectious Diseases USA ⁽⁴⁴⁾ American Association of Neurology ⁽⁴⁵⁾ American Society of Microbiology ⁽⁴⁶⁾ Pharmaceutical Regulation Affairs ⁽⁴⁷⁾ Association for Professionals in Infection Control, APIC ⁽⁴⁸⁾ Health Departments of several European countries etc). It is of interest that Swith Health Department ⁽⁴⁹⁾ proposal for prion decontamination corresponds to that of the British published 1984 (!). There is a long list of chemicals tested for their decontaminating activity; the lists include practically all disinfectants used so far and will not be repeated here. Many of these chemicals decrease the infectivity titer of prions (regularly between $10^{8.0}$ and $10^{10.0}$) for one to five logarithms but NONE WAS REALLY EFFECTIVE. The only two, believed to be affective, were NaOCl and NaOH, but recent studies gave variable results as to decontaminating effect. They are effective in combination with autoclave.

NaOCl (42) solution (usually 20.000/per mil) continuously evolve chlorine and must be kept tightly sealed and away from light. The released chlorine may create a potential respiratory hazard and should be used in well-ventilated or closed rooms. It does not corrode glass or aluminium but is corrosive to both stainless steel and autoclaves. It cannot be used as an instrument bath in the autoclave.

NaOH is easier to handle. It should be prepared fresh before use as 1N solution (40 g NaOH/liter water) or less ⁽⁵⁰⁻⁵¹⁾ NaOH does not corrode stainless steel with some exceptions, but is corrosive to glass and aluminium. NaOH may be used as an instrument bath in the gravity load autoclave. 30 minutes autoclaving is more than sufficient and the temperature is not important (usually 121°C).

The combination autoclave + NaOH has been the most effective prion decontamination process ⁽⁵²⁻⁶⁰⁾. It is unimportant whether instruments are treated with NaOH prior to autoclaving. The results autoclave + NaOH since 1988 (!) were always satisfactory even the NaOH concentration was decreased to 0.1N. Most of experiments with prion decontamination, particularly autoclaving, were done by David N. Taylor from Edinburgh EH13 9DX (scrapie, BSE and CJD strains). He also showed that the porous load autoclave temperatures of 132 - 136°C recommended in many countries are, under circumstances not safe ⁽⁶¹⁾ depending on several factors (intact tissue, tissue macerates or suspensions, drying of proteins by temperature fixation, vacuum or chemicals). This results in a resistant sub-population ⁽⁶²⁻⁶³⁾ which cannot be decontaminated thereafter even with higher autoclave temperatures (138°C).

The method of reprocessing prion-contaminated instruments as proposed by the WHO ⁽⁴²⁾ includes (1) decontamination by NaOH or NaOCl for 30 or 60 minutes followed by GL-autoclaving at 121°C for 30 minutes, (2) cleaning and (3) routine PL-autoclaving at 134°C. This is absolutely correct, but most modern hospitals do not have GL-autoclaves because they were replaced by PL-types. Modern high-vacuum PL-autoclaves are not suited for prion decontamination because the vacuum-evacuated chamber is rapidly filled with steam thus suddenly increasing the temperature on the surfaces. This tends to fix the dried tissue film on the instruments thus considerably reducing the decontamination. In GL-autoclaves the temperature increase is less progressive and the risk of protein fixation is reduced. On the other hand, the majority of people prefer to perform cleaning before decontamination, but if so, the cleaning material must be treated as infectious waste and even the cleaning station (!) must be decontaminated ⁽⁴²⁾.

The autoclave enabling the prion decontamination performances are ⁽⁶⁴⁾ available from FEDEGARI. The vertical autoclave chamber has a diameter of 40 cm and a depth of 60 cm. It was constructed for decontamination purposes and is completely electronic regulated. There is, intentionally, no vacuum pump and the air inside the autoclave chamber is not evacuated. The mixture steam/air formed consequently is maintained homogenous by means of a ventilator, with a magnetic connection, installed under the autoclave cover to exclude the forcing out of the mixture formed at the beginning of the cycle. There is no contamination danger for the personnel. The autoclave construction is particularly robust because the autoclave temperature may arise to 141°C and the steam pressure to 5,7 bar during the decontamination cycle (*The steam pressure at 141°C is 3.70 bar and the partial pressure of the air, heated at 141°C is 1.50 bar, the total pressure is 5.20 bar. The remaining 0.50 bar appears as the margin of security*). The condensate formed during the cycle as well as the one formed at the beginning of the process are maintained in the chamber until the end of the cycle close to ambient temperature.

The autoclave described here may be used for two types of decontamination (A) Steam/heat at 134°C for 18 minutes (Europe) or for 60-90 minutes (USA) or with any temperature up to 141°C or (B) Steam/Heat at 121°C with 1N NaOH 30 minutes or with lower temperatures because even boiling for 10 minutes was effective ⁽⁶⁵⁾. The effect with method (B) is absolute if performed simultaneously. This is with this autoclave possible using special containment pans from

polypropylene ⁽⁶⁶⁾ or from stainless steel; there is no corrosion damage for the autoclave. Medical instruments should be immersed into NaOH solution or, if not possible because of instruments, into water with some additive.

It is known that some medical devices cannot be decontaminated by heat and moisture. Disinfectants have been widely used for this purpose although practically ineffective. The only completely safe way to prevent transmission by this route is to use single-use instruments. Disposable tonsillectomy equipment along with disposable anaesthetic equipment was introduced in Summer 2001⁽⁶⁷⁾. Disposable tracheal tubes have been widely used for at least 15 years, but laryngoscopes are reused between patients, however. It must be remembered, that the lymphoreticular tissue of the oropharynx appears highly infectious $\geq (10^6 \text{ID}_{50} \text{g}^{-1})$ thus justifying such decision. However, soon after the introduction of disposable equipments the Medical Device Agency began to receive an increased number of reports of adverse incidence (also severe) following tonsil and adenoid surgery. The use of disposable laryngoscopes and disposable sheets led to significant increase of complaints. It seemed necessary to review the Dept. Of Health proposal to use only disposable anaesthetic equipment for tonsillectomy. On the other hand British senior operating practitioners are not prepared to put a laryngoscope cleaned in their units into their own mouth ⁽⁶⁸⁾. The authors also do not believe ⁽³⁹⁾ that cleaning and sterilization have reduced the risk of iatrogenic transmission in the same way as using disposable instruments. The risk of disposable instruments are probably no greater than of re-usable with adequate quality control. Disposable instruments are a small price to prevent such a risk.

The problem of prion decontamination will develop more important with time because of (1) up to 100 million of Europeans have probably contacted with BSE prions, variant CJD (22) and they will remain as risks persons for the next 40-50 years because of long incubation time, (2) it is possible that up to 35 percent of continuously increasing sporadic CJD (swiss increase 200 percent) resulted from medical interventions ⁽⁶⁹⁻⁷⁰⁾ and (3) CJD is not one disease but a syndrom of many different forms of the disease (see Table 1). Some of genetic mutations, although clinically inaparent, were found in about 2 percent of healthy population. Having this in mind we certainly do not enough to stop artificial transmission by medical interventions which are avoidable.

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(*) *Point mutations are the change in ONE nucleotide base pair (bp) in the complete human genome off 7x10⁹ bp in each diploid cell (12).*

M.J Jung

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