TECHNICAL REPORT

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Medical devices utilizing animal tissues and their derivatives —

Part 4: Principles for elimination and/or inactivation of transmissible spongiform encephalopathy (TSE) agents and validation assays for those processes

Dispositifs médicaux utilisant des tissus animaux et leurs dérivés —

Partie 4: Principes d'inactivation et/ou d'élimination des agents transmissibles de l'encéphalopathie spongiforme bovine (ESB) et essais de validation de ces procédés

Reference number
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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In exceptional circumstances, when a technical committee has collected data of a different kind from that which is normally published as an International Standard ("state of the art", for example), it may decide by a simple majority vote of its participating members to publish a Technical Report. A Technical Report is entirely informative in nature and does not have to be reviewed until the data it provides are considered to be no longer valid or useful.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TR 22442-4 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*, Subcommittee SC 1, *Tissue product safety*.

ISO 22442 consists of the following parts, under the general title *Medical devices utilizing animal tissues and their derivatives*:

— Part 1: *Application of risk management*

— Part 2: *Controls on sourcing, collection and handling*

— Part 3: *Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents*

— Part 4: *Principles for elimination and/or inactivation of transmissible spongiform encephalopathy (TSE) agents and validation assays for those processes* [Technical Report]

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Introduction

Certain medical devices utilize materials of animal origin.

Animal tissues and their derivatives are used in the design and manufacture of medical devices to provide performance characteristics that were chosen for advantages over non-animal based materials. The range and quantities of materials of animal origin in medical devices vary. These materials can comprise a major part of the device (e.g. bovine/porcine heart valves, bone substitutes for use in dental or orthopaedic applications, haemostatic devices), can be a product coating or impregnation (e.g. collagen, gelatine, heparin), or can be used in the device manufacturing process (e.g. tallow derivatives such as oleates and stearates, fetal calf serum, enzymes, culture media).

This document is a Technical Report (TR) to offer suggestions for designing and conducting validation assays to help determine if processes used in the manufacture of medical devices derived from non-viable animal tissues might serve to reduce the risk of iatrogenic transmission of transmissible spongiform encephalopathies (TSEs). This document will refer to the infective vector as “TSE agent” rather than prion to remain consistent with the other Parts of ISO 22442. Some current information on human tissues and TSEs is also presented which may be applied by analogy to other animal tissues.

Iatrogenic transmission of the human TSE Creutzfeldt-Jakob disease (CJD) has been convincingly attributed to exposure to the human dura mater allograft (Hannah, E. L., E. D. Belay, et al. 2001) used in surgery as a patching material and to hormones extracted from human pituitary glands (Mills, J. L., L. B. Schonberger, et al. 2004)—both non-viable tissues; recently, sub-clinical infection with the vCJD agent was detected at autopsy in a patient with hemophilia and plausibly attributed to his treatment with processed human plasma-derived coagulation factor (UK Health Protection Agency 2009 at:

http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1234859690542?p=1231252394302

and

http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_100357).

In addition, corneal grafts have transmitted CJD (Kennedy, Hogan et al, 2001) and several transfused red blood cell concentrates have transmitted variant CJD (vCJD) (Llewelyn, Hewitt et al 2004; Peden, Head et al 2004; Peden, Ritchie and Ironside 2005).

Exposure to the agent of bovine spongiform encephalopathy (BSE) has been responsible for more than 210 cases of vCJD worldwide, most of them thought to have resulted from dietary exposure to infected beef products. Although, except for the iatrogenic vCJD infections just described, no transmissions of a BSE-derived agent via medical or veterinary products have been recognized, there is no reason to doubt that a medical device contaminated with BSE agent of ruminant origin could transmit infection to a susceptible subject. Indeed, two veterinary vaccines derived from non-viable ovine tissues transmitted the ovine/caprine TSE scrapie to sheep (World Health Organization 2006). Humans are not known to have been infected with the scrapie agent.

This Technical Report generally uses terminology suggested by the World Health Organization (WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies, 2006 (World Health Organization 2006)), while recognizing that there is no international consensus regarding either preferred terminology, the probable molecular nature of the transmissible agents (the all-protein or “prion” theory (Prusiner 1982) currently most widely held) or the precise role of various forms of the host-coded prion protein in the replication of the infectious agents or pathogenesis of disease. The sole intent of the TR is to suggest strategies to validate the effectiveness of methods that might reduce the risk of accidentally transmitting TSEs by medical devices prepared using non-viable animal tissues.

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The following referenced documents are standards helpful for the proper interpretation of this document:

- ISO 22442-1, *Medical devices utilizing animal tissues and their derivatives — Part 1: Application of risk management*
- ISO 22442-2, *Medical devices utilizing animal tissues and their derivatives — Part 2: Controls on sourcing, collection and handling*
- ISO 22442-3, *Medical devices utilizing animal tissues and their derivatives — Part 3: Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents*
- ISO 14160, *Sterilization of health care products — Liquid chemical sterilizing agents for single-use medical devices utilizing animal tissues and their derivatives — Requirements for characterization, development, validation and routine control of a sterilization process for medical devices*

These documents include both normative and informative annexes also directly relevant to the topic of this ISO TR. All terms defined in those documents are used verbatim in this report.

Due to the lack of consistent process steps that can reliably eliminate TSEs, it is important that one must use low-risk source animals and tissues.

Although not directly applicable to validating methods purported to reduce the TSE risk from medical devices manufactured from non-viable animal tissues, UK and US competent authorities have solicited expert advice on desirable features of validation studies for devices intended to remove TSE infectivity from human blood potentially contaminated with TSE agents, and this advice may be helpful in evaluating methods for animal-derived tissues as well. These features included preliminary evaluation using TSE-spiked material with high titers of infectivity, selecting experimental agents relevant to the infection of concern, and accepting studies using assays for PrP^{TSE} as a preliminary screening strategy to dismiss unpromising methods. These methods were required to indicate significant reduction in infectivity demonstrated by bioassays in known susceptible experimental animals. To qualify a method as potentially useful, the assay needed to demonstrate similar results for the same candidate method with two TSE agent-bioassay combinations, whenever possible. These criteria should be met before concluding that the method offers sufficient promise to consider in practice. Demonstration that a method reduces TSE infectivity for tissues endogenously infected, and that the complete manufacturing process eliminates all detectable infectivity, while desirable, are not currently feasible. Very low titers of infectivity in most tissues outside the nervous system and limited animals known to be susceptible to naturally occurring TSE agents without adaptation to a new species are limiting factors. The lack of standard reference infected materials of known titer and biological properties from humans and animals with TSEs is thought to be an additional impediment to developing validation studies (World Health Organization (2006), Annex 2). Considering the extremely limited attempted validation efforts for methods for improving TSE safety of human blood-derived and other human tissue-derived medical products — products with demonstrated iatrogenic transmissions — care must be taken not to discourage new efforts to validate methods that might improve the TSE safety of medical devices derived from animal tissues.

It should be noted again that, as summarized above, animal tissues have not been directly implicated in causing any iatrogenic TSE infections of humans (Minor, Newham et al. 2004). However, experience with food-borne BSE and field transmissions of scrapie to sheep by ovine tissue-derived veterinary vaccines suggests that the risk of iatrogenic transmissions of TSEs (other than BSE) from animals to humans, while theoretical, remains worthy of continued attention.

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Medical devices utilizing animal tissues and their derivatives —

Part 4: Principles for elimination and/or inactivation of transmissible spongiform encephalopathy (TSE) agents and validation assays for those processes

1 Scope

This Technical Report offers suggestions for designing and conducting validation assays to help determine if processes used in the manufacture of medical devices derived from non-viable animal tissues might serve to reduce the risk of iatrogenic transmission of transmissible spongiform encephalopathies (TSEs).

The TSE-removal methods used to process animal tissues should also reduce the risk of transmitting TSE infections via non-viable tissues of human origin; this Technical Report does not address this issue. Some current information on human tissues and TSEs is presented which may be applied by analogy to other animal tissues.

This Technical Report does not intend to imply a need for validation of methods involving specific materials identified as having a "negligible risk" of contamination with TSE agents as listed in Annex C of ISO 22442-1:2007.

This Technical Report is intended to clarify final draft international standards included in the ISO 22442 series, as well as in ISO 14160.

This Technical Report builds upon the specific discussion in ISO 22442-3 relative to TSE agents and attempts to summarize the current state of the art in the arena of TSE agent elimination. As the understanding of inactivation and elimination of TSE agents evolves, this document will be revised when possible.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 22442-1, *Medical devices utilizing animal tissues and their derivatives — Part 1: Application of risk management*

ISO 22442-2, *Medical devices utilizing animal tissues and their derivatives — Part 2: Controls on sourcing, collection and handling*

ISO 22442-3, *Medical devices utilizing animal tissues and their derivatives — Part 3: Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents*

ISO 14160, *Sterilization of single-use medical devices incorporating materials of animal origin — Validation and routine control of sterilization by liquid chemical sterilants*

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3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 22442-1, ISO 22442-2, ISO 22442-3, and ISO 14160 apply.

4 Elimination of TSE agents: basic considerations

4.1 General

4.1.1 TSEs of concern

BSE of cattle is the only TSE of animal origin known so far to have transmitted disease to humans (i.e, a zoonosis). Scrapie, while of theoretical concern, has not been recognized as a zoonosis in spite of hundreds of years of experience. Nonetheless, some competent regulatory authorities have adopted precautionary policies that discourage the sourcing of ovine-derived and caprine-derived injectable materials from herds with a history of scrapie (e.g. transcripts of the FDA TSE Advisory Committee meeting 03 June 1999, accessed 16 July 2009 at http://www.fda.gov/ohrms/dockets/ac/cber99.htm#Transmissible%20Spongiform, European Union Directive from 2003 [OJ L 105, 26.4.2003 p. 18, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:105:0018:0023:EN:PDF], and Japanese notification 177 http://www.nihs.go.jp/cgtp/cgtp/guidline/03052001.pdf). The susceptibility of sheep and goats to infection with the BSE agent has posed another more recent concern (World Health Organization 2006). Although pigs have been experimentally infected with the BSE agent (Wells, Hawkins et al. 2003; Castilla, Gutierrez-Adan et al. 2004), they were not infected when exposed by the oral route and no naturally transmitted porcine TSE has been recognized, and most authorities remain generally satisfied that porcine tissues are an unlikely source of human exposure to any TSE agent. The same thing is true for tissues of other animals less commonly used in the manufacture of medical devices, such as horses. (Animals not susceptible to infection with TSE agents by the oral route have not been considered “TSE-relevant animal species” by EMEA; tissues of such species were not considered to be of concern regarding TSE risk in the manufacture of medicinal products for human or veterinary use in a 2004 note for guidance of the EMEA at http://www.emea.europa.eu/pdfs/human/bwp/TSE%20NFG%20410-rev2.pdf.)

4.1.2 Animal tissues of concern

Examples of animal tissues currently used in their non-viable form to manufacture medical devices include porcine heart valves, bovine pericardium, and bovine collagen. While none of these tissues has been demonstrated to contain a TSE agent (World Health Organization 2006), experiments with animal tissues have been very limited in number, and the assays used vary in sensitivity of detection. Furthermore, it is conceivable that almost any animal tissue collected as part of routine slaughter might be accidentally contaminated with higher-risk tissues. Regulations and procedures requiring removal, segregation and safe disposal of “specified-risk” materials from animal carcasses — especially from carcasses of older animals — should reduce but not completely eliminate this risk. The European Commission has recently proposed “risk-proportionate rules for animal by-products” to clarify and facilitate risk management in selection of source materials of animal origin (see EU regulation 1069/2009).

4.1.3 Tissues infected with TSE agents

Agents might contaminate tissues in two ways: (1) a tissue infected during the TSE disease process, or (2) infectivity introduced into the tissue from infected tissue (for example, due to contact with tissues of the nervous system, lymphoreticular cells or from blood in the tissues). This second or “exogenous” source of contamination with a TSE agent might occur during harvesting of the tissue from an infected source or from instruments or surfaces contaminated with TSE agent from a previously handled source. These distinctions are important for several reasons: Endogenously infected tissues (except for tissues of the CNS, which have been found to contain the great majority of total infectivity in the body of an infected animal) generally contain very small amounts of agent, so suitable models to validate methods for eliminating endogenous infectivity are logistically difficult to develop. Exogenous contamination is more easily modeled by intentionally spiking tissues with TSE agents of known provenance, biological characteristics and content of infectivity as defined by titrations in susceptible animals. Several strains of scrapie agent and one strain of BSE-derived agent

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(301V) adapted to propagate to high titers in rodents have been especially useful to model exogenously infected materials (see C.1 of ISO 22442-3:2007 and Annex D of ISO 22442-03:2007. The selection of model agents for validation studies is constrained by several considerations:

a) although the actual unpassaged TSE agent likely to be present in the tissue of concern might be considered most “relevant” for purposes of validating inactivation/removal studies, the infectivity titers in such agents are usually both unknown and lower than those of rodent-adapted agents (Wells 2007); and

b) or handling of the BSE agent and rodent-adapted strains derived from BSE agent, regulatory authorities may require high-containment research facilities not widely available. In general, studies with rodent-adapted scrapie agent have been considered acceptable, keeping in mind that reported resistance of various strains of TSE to inactivation procedures has not been uniform (Peretz, Supattapone et al. 2006)

5 Potential methods to eliminate TSE agents

5.1 Methods for inactivating infectivity

5.1.1 General

Most authorities recommend that, whenever possible, TSE agents potentially contaminating source materials be inactivated rather than simply separated from the raw materials. The presence of residual active agent might pose a continuing danger of later accidental contamination of a tissue-derived product as well as contaminating manufacturing facilities and non-disposable equipment. However, unfortunately, most of the physical and chemical methods known to be effective for inactivating TSE agents are relatively harsh and often impair the functional properties of tissues. For sanitizing the facilities and processing equipment that cannot be destroyed after single use or protected from direct contact with potentially contaminated materials, decontamination methods that reliably inactivate TSE agents are preferred to those that simply remove the agents.

5.1.2 Physical methods for inactivating TSE infectivity

5.1.2.1 Heat

When suspensions of tissue from scrapie-infected rodents were rapidly heated with continuous stirring, relatively rapid loss of infectivity occurred within a few minutes (Rohwer 1984; Rohwer 1984), reaching the assay limit of detection. However, macerates and dried preparations of TSE agents retained some infectivity even after long exposures to heat (Asher, Pomeroy et al. 1986; Asher, Pomeroy et al. 1987; Taylor, Fraser et al. 1994). Dry heat appears to be less effective than moist heat for inactivating TSE agents (Taylor 2001). (Indeed, two reports suggesting surgical transmissions of iatrogenic CJD involved instruments that had been sterilized by exposure to dry heat (Nevin, McMenemy et al. 1960; Foncin, Gaches et al. 1980).) One series of studies even found small amounts of infectivity in ash from scrapie agent incinerated in an oven at temperatures up to 600 °C (Brown, Liberski, et al. 1990), though not at a higher temperature (Brown, Rau et al. 2004). Tissue devices are unlikely to tolerate heat treatments adequate to inactivate TSE agents without loss of function.

There have been suggestions that for adequate decontamination of TSE agents one should specify exposure to steam in a porous-load autoclave for at least 18 minutes at 20-bar pressure. This has been shown to be ineffective (Taylor and McBride 1987). A WHO Consultation (World Health Organization 1999) warned that such treatment was unlikely to decontaminate a surface soiled with dried-on tissue from a TSE-contaminated source. A consultant to the US CDC cautioned that many pathogens survive heat and chemical treatments when contaminated surfaces have not been thoroughly cleaned and that thorough cleaning of critical surfaces must be employed for safe reuse of all medical devices (Rutala W, FDA TSE Advisory Committee, 17 July 2003 accessed 16 July 2009 at http://www.fda.gov/ohrms/dockets/ac/03/slides/3969s1.htm); the same consultant (Rutala 2010) noted, however, that the extreme rarity of iatrogenic transmissions of CJD attributed to surgical instruments (none documented in medical literature since 1980) suggests that current standard methods for sanitization and terminal sterilization of surgical instruments are probably effective in removing sufficient CJD agent to reduce the risk to undetectable levels.

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This observed efficacy is likely a combined effect of cleaning and moist heat and the potential efficacy of either method individually has been shown in the laboratory to be insufficient. (Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 2001, UK Department of Health 2009)

5.1.2.2 Radiation

The TSE agents have resisted inactivation by both ultraviolet and ionizing radiations (Alper, Haig et al. 1966; Latarjet, Muel et al. 1970; Latarjet 1979).

5.1.3 Chemical methods for inactivating TSE infectivity

5.1.3.1 Alkaline hydrolysis treatments

Exposure to sodium hydroxide (≥ 1N, especially at elevated temperatures) has been found effective in removing infectivity from both aqueous suspensions and tissues dried onto surfaces (Taylor 2000) and is widely used in laboratories dealing with TSE agents (Brown, Rohwer et al. 1984; Baron, Safar et al. 2001). The hazards posed by NaOH (which is caustic, especially when hot, and potentially explosive in contact with aluminum (http://www.certified-lye.com/safety.html) are well known. The corrosive properties of NaOH for autoclaves seem to have been exaggerated, so long as solutions are carefully contained to prevent direct contact with critical surfaces of the chamber (Brown and Merritt 2003; Brown, Merritt et al. 2005).

Calcium hydroxide treatments, widely used in the manufacture of gelatins, appeared to be much less effective than NaOH in removing scrapie and BSE infectivity from spiked preparations of animal bones, although the addition of heat was highly effective (Grobben, Steele et al. 2005; Grobben, Steele et al. 2006a; Grobben, Steele et al. 2006b).

A number of alkaline-based formulations (mixtures of chemicals with alkaline sources) are claimed to be as effective as low concentrations of alkalinity. These effects appear to be formulation-specific and will depend on the formulation, temperature and concentration of the specific product. These treatments are less destructive to tissues, but the studies to date have not compared their efficacy to higher concentrations of alkali treatments.

5.1.3.2 Acid treatments

TSE agents have been substantially if not completely inactivated by exposures to concentrated formic acid (Brown, Liberski et al. 1990) and, more recently, to acetic acid in a solution of sodium dodecyl sulfate (Peretz, Supattapone et al. 2006).

5.1.3.3 Halide and other oxidizing agent treatments

Sodium hypochlorite (household chlorine bleach at concentrations ≥ 5%) has been found effective in removing TSE infectivity from scrapie-contaminated suspensions and surfaces (Taylor 2000) and is widely used in situations where the corrosive effects on metals are not a problem (Brown and Merritt 2003; Taylor 2004). Chloramine and other halides have been found less effective (Asher 1986). Liquid hydrogen peroxide has also been found to lack utility for decontaminating TSE agents (Taylor 2004), although in low-temperature gaseous form it might be more effective (Langeveld, Wang et al. 2003; Fichet, Comoy et al. 2004; Yakovleva, Janiak et al. 2004; Yan, Stitz et al. 2004; Fichet, Antloga et al. 2007).

5.1.3.4 Treatments with phenolic disinfectants

A proprietary phenolic disinfectant has been reported to eliminate scrapie infectivity from contaminated suspension to the limit of detection (Ernst and Race 1993; Race and Raymond 2004). Treatment with phenol itself failed to eliminate infectivity (Asher, Gibbs et al. 1986).

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5.1.3.5 Protease treatments

Stimulated by the widely-accepted prion theory, studies investigating effects on infectivity of TSE agents from several protease treatments have been investigated, yielding variable results (Langeveld, Wang et al. 2003; Fichet, Comoy et al. 2004; Yakovleva, Janiak et al. 2004; Yan, Stitz et al. 2004; Jackson, McKintosh et al. 2005). As noted above, thorough cleaning is undoubtedly important in freeing surfaces of infectivity (Rutala and Weber 2004;), and protease treatments probably facilitate that process. Whether proteases have an additional specific effect of inactivating TSE agents by cleaving the prion protein is less clear.

5.1.3.6 Guanidine and other chaotropic chemical treatments

Treatments with guanidine (Manuelidis 1997) and other chaotropic chemicals (Prusiner, Groth et al. 1993) have been found to reduce infectivity. Some reports suggested that the apparent inactivation was reversible when the chaotropic chemical was removed (Prusiner, Groth et al. 1993; McKenzie, Bartz et al. 1998).

5.1.3.7 Combined treatments

Limited experience suggests that combinations of TSE-inactivating treatments having different chemical and physical actions might be more effective than either treatment used separately (Fichet, Comoy et al. 2004). Examples of potentially effective combined treatments have included NaOH with heat (Taguchi, Tamai et.al. 1991), including boiling in neutral detergent (Taylor 2004), and acetic acid in detergent solution (Peretz, Supattapone et al. 2006). Potential damage to autoclaves may be mitigated by the use of covered containers to isolate the NaOH from the autoclave interior (Brown and Merritt 2003; Brown, Merritt et al. 2005).

5.1.3.8 Potential processes to remove TSE agents from non-viable tissue-derived medical devices that have been investigated

We are not aware of experimental validation with TSE agents with of any methods similar to those used with tissue-derived devices to reduce spiked infectivity, except for one study using NaOH to remove scrapie agent from hamster dura mater (Diringer and Braig 1989) and another of a meat product experimentally spiked with scrapie agent that apparently remained palatable after exposure to high temperature and pressure (Brown, Meyer et al. 2003); neither treatment — while resulting in a substantial loss of infectivity — reduced spiked scrapie infectivity below the level of detection.

NOTE A variety of methods currently used to sterilize non-viable human tissue-derived devices have been reported in the medical literature (including gamma irradiation with or without radioprotectants, and various chemical cleansers and sterilants, among them detergents, hydrogen peroxide, alcohols, acidification (McAllister, Joyce et al. 2007) and sodium hydroxide treatment (Hannah, Belay et al. 2001)); at least some of those procedures seemed to impair the functional properties of the devices (McAllister, Joyce et al. 2007). Some methods have been claimed to reduce spiked bacteria substantially (sterility assurance level [SAL] 10-3, a level recommended by FDA for surgically implanted human cells, tissues and tissue-derived products, SAL 10-6 for implanted medical devices that can withstand sterilization).

5.1.3.9 Ineffective treatments

Ethylene oxide gas, alcohols, mercurial disinfectants and a number of other treatments commonly used to sanitize or sterilize surfaces have not been found useful for decontamination of TSE agents; research with such treatments has been limited (Asher 1986). Aldehydes not only failed to inactivate TSE agents, they may have stabilized the infectivity (Taylor and McBride 1987; Brown, Liberski et al. 1990). More ineffective treatments may be found in the document from the UK government (DoH ACDP TSE WG – annex C), which lists ineffective treatments.

5.2 Methods for removing TSE infectivity without inactivating infectivity

Although physical methods such as chromatography, sedimentation, filtration and partition of fractions offer some promise for reducing amounts of TSE infectivity in complex biological mixtures (e.g., blood and its components and plasma and its fractions (Foster 2004)), it is unlikely that selective removal of infectivity from intact non-viable tissues would be feasible.

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6 Experimental validation of methods for eliminating TSE agents from medical devices utilizing non-viable animal tissues

6.1 General

Although limited study has been attempted using methods expected to reduce if not eliminate TSE agents exogenously contaminating human non-viable tissues, few if any studies have directly attempted to decontaminate animal-derived tissues. As outlined in ISO 22442, all parts, the more appropriate method for reducing risk is careful selection of low-risk source animals and tissues and good manufacturing processes (GMPs) to reduce opportunities for exogenous contamination with neural tissues and, to a lesser extent, lymphoreticular and intestinal tissues (McAllister and Joyce 2007).

6.2 Defining of product families for purposes of designing TSE process validation studies

For purposes of this document, the definitions of product families described in AAMI TIR37:2007 Section 4 that are appropriate to evaluation of validation assays for elimination/removal of TSE agents from medical devices utilizing non-viable animal tissues should apply here as well. The products selected for experimental validation should, as closely as feasible, resemble the manufactured product of concern and be considered by one or more expert consultants to constitute an acceptable realistic representative of a product family (master product, equivalent product or simulated product as defined in 4.3.2 – 4.3.4 of AAMI TIR37:2007.

6.3 Selection and testing of product for establishing and verifying the infecting dose of TSE agent

For purposes of this document, the considerations for nature of the test product sampling conditions, sample item portions, considerations regarding use of multiple batches, assays of fluids extracted from test products (direct assays of whole articles in animals generally being unfeasible) should be similar to those described in Clause 5 of AAMI TIR37:2007 that are appropriate to evaluation of validation assays for elimination/removal of TSE agents from medical devices utilizing non-viable animal tissues should apply here as well. Note that assays of TSE agents, whether by preliminary immunoassays of protease-resistant PrP^{TSE} or — especially — by subsequent bioassay in animals are considerably more technically difficult, time-consuming (months or years for bioassays) and expensive than are cultures of bacteria and fungi, and that must be taken into account when selecting numbers of replicates for testing.

6.4 TSE agent spiking materials

Limited but accumulating evidence suggests that, while all TSE agents share most biological and physical properties, including unusual resistance to inactivation by a variety of treatments, strains have differed substantially in that resistance (Taylor 2000) (Peretz, Supattapone et al. 2006). It seems prudent to select for spiking a tissue infected with a strain of TSE agent more rather than less resistant to the inactivation procedure under study so long as the spike material is relevant to the manufacturing process. While it might be argued that worst-case scenarios are unrealistic representations of manufacturing situations, the logistics of validation models have usually required that tissues containing very high concentrations of TSE agent (usually meaning infected brain tissue suspensions, fragments or macerates) be employed as spiking materials. It must be acknowledged that such materials, most often prepared from brains of rodents infected with rodent-adapted strains of TSE agent (especially scrapie agent) might not closely resemble naturally infected tissues either in amounts or biological and physical properties of the agent or in some properties of the matrix (tissue of origin). However, in general, it has not been feasible to validate processes for eliminating TSE agents from raw materials except by spiking with rodent-adapted TSE agent strains in rodent brain tissues for several reasons.

a) Titers of TSE agent in naturally infected tissues are generally not known, nor is the susceptibility of available rodents to infection with field isolates (often poor). Tissues other than brain have much lower titers of both PrP^{TSE} (often not detectable) and infectivity than brain.

b) Well characterized standard reference materials prepared from tissues of naturally infected animals are not yet widely available.

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c) Transgenic mice (or other rodents — e.g., European bank voles (Nonno, Di Bari et al. 2006) consistently susceptible to infection with field isolates of TSE agents are not generally available except in a few research laboratories.

6.5 Availability of bioassay animals (conventional and transgenic mice, other rodents, farm animals)

Rodent-adapted TSE agents, commonly derived from strains of sheep scrapie but recently from BSE as well, have been used in pilot studies to investigate methods for eliminating infectivity experimentally spiked into various raw materials or intermediates. The 263K hamster-adapted strain of scrapie agent (Nazor 2005) and similar strains (Peretz, Scott et al. 2001) assayed in golden Syrian hamsters have been especially useful. The 301V mouse-adapted strain of BSE agent assayed in conventional mice might be more predictive of the probable behavior of BSE agent in bovine tissues (Grobben, Steele et al. 2005).

Transgenic mice engineered to express amino acid sequences of other species in the *PRNP* gene may be useful in certain situations but pose several problems.

a) Mice overexpressing prion proteins have developed non-transmissible neurological diseases histopathologically resembling TSEs later in life, which sometimes confused the interpretation of infectivity assays (Nazor, Seward, et al. 2007).

b) Most lines of PRNP–transgenic mice can currently be obtained only as gifts from the developers.

Compared with some strains of conventional mice, the superiority of mice expressing bovine *PRNP* amino acid sequences has not been rigorously demonstrated to date, although some lines may prove to be more sensitive. (Human-derived TSE agents infrequently transmit disease to conventional mice, and — since assays using nonhuman primates have become increasingly unfeasible — transgenic mice expressing human *PRNP* amino acid sequences appear to offer the most practical assays of infectivity.) As noted above, European bank voles have been described as highly susceptible to a number of TSE agents (Cartoni, Schinina et al. 2005; Nonno, Di Bari et al. 2006; World Health Organization 2006; Cartoni, Schinina et al. 2007), however they appeared to be relatively less sensitive than mice to the BSE agent and to the emerging Nor98 strain of scrapie agent, and, in any case, colony-raised voles are not widely available.

It might be argued that methods for eliminating the infectivity of animal-derived TSE agents should most appropriately be studied using materials from naturally infected animals assayed in susceptible animals of the same species; although that has been done to a limited extent to investigate pathogenesis of BSE by assays in calves (World Health Organization 2006; Masujin, Matthews et al. 2007) and scrapie in sheep [(Hadlow, Race et al. 1979; Hunter, Foster et al. 2002; Hunter and Houston 2002; Hunter 2003), it is generally not practical to study elimination of TSE infectivity in large animals.

6.6 Potential development of cell culture assays for infectivity

Several cell lines have been described that support the propagation of selected TSE agents [(Klohn, Stoltze et al. 2003; Kocisko, Morrey et al. 2004; Kocisko, Engel et al. 2005; World Health Organization 2006; Liu, Sun et al. 2008). Although the cells showed no recognizable cytopathic effect, PrP^{TSE} could be detected. Such a cell culture assay has been reported to be much more sensitive than direct detection of PrP^{TSE} in the original infected tissue. Unfortunately, cell culture assays have not been developed for detection of BSE agents, field isolates of scrapie agents from sheep, or human-derived TSE agents. In addition, false-positive PrP^{TSE} results have posed a problem (Vorberg, Raines et al. 2004). The development of reliable and sensitive cell-culture-based assays for animal TSE agents would greatly facilitate the validation of methods to eliminate the agents from animal-derived materials.

6.7 Correlations between PrP^{TSE} and infectivity assays

Although PrP^{TSE} has usually been detected in tissues and tissue extracts containing TSE infectivity, a number of reports failed to demonstrate PrP^{TSE} in materials containing considerable amounts of infectivity (Lasmezas, Deslys et al. 1997; Manuelidis, Fritch et al. 1997; Manson, Jamieson et al. 1999; Race, Meade-White et al. 2002; Barron, Campbell et al. 2007; Piccardo, Manson et al. 2007). Furthermore, immunoreactive proteins

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with properties of PrP^{TSE} have been found in tissues that did not contain detectable agent transmitting spongiform encephalopathy. Several investigators have asserted that it is possible to generate TSE infectivity from tissues of animals that were never exposed to the agents (Yuan, Xiao et al. 2006; Deleault, Harris et al. 2007; Castilla 2008; Jackson et al. 2009). This confusing situation urgently requires clarification (Aguzzi 2007) but serves as a caution when interpreting results of assays for PrP^{TSE}.

Although several reports claimed that innovative assays for PrP^{TSE} were more sensitive than rodent bioassays of infectivity (Safar, Cohen et al. 2000; Safar, Scott et al. 2002; Lee, Long et al. 2005; Safar, Geschwind et al. 2005; Castilla, Saa et al. 2006; Soto, Estrada et al. 2006), those claims remain to be independently verified in comparative blinded and randomized studies.

6.8 Reductions in infectivity compared with failure to detect at limits of detection

Confirmed reductions in amounts of infectivity (or, as a preliminary step, PrP^{TSE}) estimated by assays can be considered informative so long as the tests have used robust methods with a sufficient number of replicate experiments and assays within each experiment to assure that results are repeatable. Just as for estimates of reductions in infectivity for other infectious agents, considerable caution must be exercised when relying on results of variable assays, and especially when reductions are less than 1000-fold. For that reason, some authorities have recommended that two validated infectivity-reducing processes, each based on a different chemical-physical principle (“orthogonality” (Trejo, Hotta et al. 2003)) be employed in any manufacturing scheme claiming to reduce the risk of contamination. For biological products, FDA policies have recommended that each of the two processes reduce spiked infectivity by at least ten-thousand fold before asserting a claiming that a manufacturing method is likely to reduce the risk of contamination with a defined pathogen to an acceptable level (Farshid, Taffs et al. 2005).

6.9 Determining numbers of replicate validations needed to support inferences of reduction in infectivity rather than variations in assay performance

The number of repeated tests and replicate assays in a test depend in large measure on the variability of the assay and the process under study. The proposed method should be robust, meaning that validation tests should be conducted under all conditions likely to be encountered in the process at manufacturing scale — pH, ionic strength, temperature and organic load.

6.10 Requirements for step-wise reductions in PrP^{TSE} and infectivity verses whole-process validation

Although it would be especially reassuring to validate the effectiveness of an entire manufacturing process by eliminating substantial amounts of relevant TSE infectivity spiked into real starting material in a realistic pilot model, yielding a functional end product completely free of detectable infectivity, such validation may not be achievable for several reasons. The input infectivity in starting material may be modest; various steps may greatly dilute the amount of original materials reaching the final product. This dilution introduces a sampling problem in the design of the experiment. If there is no infectious dose in a sample due to dilution, one will see a false-negative in the assay used. The solution to this problem is to test more samples, which is often not feasible. Surface-volume relationships are not maintained in scaled-down pilot models. Therefore, as for viral validations of biologic products (QA5 Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, International Conference on Harmonisation, 1998, accessed 16 July 2009 at http://www.emea.europa.eu/pdfs/human/ich/029595en.pdf) it is more often necessary to spike individual intermediate steps with model TSE agents, attempting to simulate realistic process conditions and conditioning of the intermediates, and then to infer probable overall effectiveness of a whole production scheme by adding orthogonal log reduction factors to yield a probable total log reduction value, keeping in mind attendant uncertainties.

It is understood that reduction factors from steps with similar inactivation or elimination mechanisms should not be added into a cumulative reduction factor. Further guidance on validation studies may be found in ISO 22442-3.

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