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COMMERCIAL-IN-CONFIDENCE

INACTIVATION OF SCRAPIE-LIKE AGENTS

Some implications for the use of bovine material in sterile medical devices in the UK

Produced, with reference to the Southwood Report, by STD PG1A

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NOTE: References are numbered in historical order of the year of publication.

Papers in which experimental work is published are distinguished from reviews, clinical reports and guidance documents by printing the reference number in bold-type.

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INACTIVATION OF SCRAPIE-LIKE ORGANISMS

1.0 Summary

Existing Department of Health guidance[5,6,15] recommends:

1) Autoclaving in a porous load cycle at
either 134°C +4 -0 for 6 cycles of 3 minutes, Hold Time at Temperature (HTAT)
or 134°C +4 -0 for 1 cycle of 18 minutes, HTAT.

2) sodium hypochlorite:

1% dilution of hypochlorite containing 10 000 ppm available chlorine
for 30 minutes for contaminated surfaces;
for 18 hours for laboratory equipment (excluding metals).

The Microbiology Advisory Committee has endorsed the recommendations of the Americal Neurological Association's Committee on Health Care Issues[23]. Those considered to be fully effective are:

1) Steam autoclaving for 1 hour at 132°C.
2) Immersion in 1N NaOH for 1 hour at room temperature.

This advice is to some extent at variance. In the American report hypochlorite is considered to be only partially effective. However, in a Department of Health sponsored research project in which disinfection studies were carried out with 2 strains of the scrapie agent[11], inactivation was effected on exposure to hypochlorite where 10,000 ppm available chlorine was present.

Many other inactivation procedures have been reported in experimental data and a number of review articles have been written but conclusions of some of them are conflicting. Some of these differences may be explained by the lack of standard methodology (eg differences in the concentration of brain tissue) and the difficulties of using the assay system required for the detection of these agents.

More research is needed to apply standardised methodology to the inactivation of different strains of the agents of scrapie and Creutzfeldt-Jakob Disease.

None of these results have been obtained with the agent of BSE. Recently a strain of the agent of BSE has been successfully transmitted to laboratory mice and inactivation studies are under consideration[38].

2.0 INTRODUCTION

The publication of the Southwood Report[33], February 1989, has drawn attention to some of the implications of the transmissible bovine spongiform encephalopathy (BSE) currently affecting a large number of cattle in the UK.

Inactivation studies have been carried out over the past few years in attempts to identify procedures which are effective in inactivating the agents of scrapie in sheep and Creutzfeldt-Jakob Disease in humans. Recently the agent of BSE has been successfully transmitted to laboratory stains of mice and inactivation studies with this agent are under consideration[38].

3.0 DEPARTMENT OF HEALTH GUIDANCE

3.1 Report of the Advisory Group on the Management of Patients with Spongiform Encephalopathy (Creutzfeldt-Jakob Disease (CJD))[5].

HMSO, November 1981.

Issued with DA (81) 22[6]. (See Appendix)

3.2 Management of Patients with Spongiform Encephalopathy (Creutzfeldt-Jakob Disease (CJD)). DA (84) 16[15]. (See Appendix)

The scope of this this document is only to update the previous advice on autoclaving.

As a result, Departmental advice became:

- a single cycle 134°C ($^{+4}$ -0) (30 lbs psi) for 18 minutes HTAT

- 6 separate cycles 134°C ($^{+4}$ -0) (30 lbs psi) for 3 minutes HTAT

and as an interim measure:

"The use of 1% dilution of hypochlorite containing 10,000 ppm available chlorine (freshly prepared dilute sodium hypochlorite BP) is recommended for use on contaimating surfaces leaving it for half an hour. For decontamination of laboratory equipment, other than metal (which is corroded by hypochlorites) soaking for 18 hours in a 1% dilution of hypochlorite containing 10,000 ppm available chlorine is advised."

This document[5] states that there is evidence that CJD agent will resist standard methods of disinfection and sterilization using

heat
formaldelyde
70% alcohol
ultraviolet radiation
ionising radiation

It also states that for the following methods their use is not recommended but that there is no evidence either way.

ether
chloroform
iodophors

Since this publication further work has been undertaken and is reviewed in the Appendix.

4.0 RESEARCH PUBLICATIONS

Research work has been carried out using the scrapie agent as a model for the Creutzfeldt-Jakob Disease (CJD) agent, and, later, with a mouse-adapted strain of the CJD agent. Infected brain is either macerated or homogenised in solution, exposed to the 'inactivating' procedure, and incoculated into mice. Methods which refer to the titre of infective agent rely on quantal titration assays in animals. As an example, incubation time prior to on-set of symptons is used as a measure of titre of infective agent in the inoculum. This type of assay inevitably lacks sensitivity. Little is known about the protective effect of the homogenate. One worker suggests that this could be considerable and demonstrates a similar finding using other viruses[13,17].

Different 'inactivating' methods used and the conclusions drawn are summarised in the Appendix.

5.0 MICROBIOLOGY ADVISORY COMMITTE

The Microbiology Advisory Committee (MAC) consists of expert Medical Microbiologists from within the National Health Service Public Health Laboratory Services, and academia, and throughout the United Kingdom who meet to provide advice to the Department of Health on matters concerning sterilization and disinfection.

In 1984, the MAC commented on the Departmental Guidance DA (81) 22[6] and DA (84) 16[15], and requested clarification of the advice.

In 1985 the Departmental working group on CJD disbanded and the remit to update the information in the 2 departmental documents was passed to the MAC.

In 1987 Dr Fenton-Lewis (MED IMCD) attended an MAC meeting with an update on CJD. The committee felt that the advice given in DA (84) 16 was being followed and no problems had been reported. An on-going literature search of publications on CJD was initiated, which is continuing to date. Papers relating to inactivation data are considered by the Committee.

In 1988, the committee noted the publication of the document 'Precautions in handling tissues, fluids, and other contaminated materials from patients with documented or suspected Ceutzfeldt-Jakob Disease'[23]. This was produced by the committee on Health Care Issues of the American Neurological Association. The MAC did not feel that current advice needed to be altered in the light of this paper which is summarised below:

<u>Sterilization Procedures for Creutzfeldt-Jakob Disease Tissues and Contaminated Materials</u>

<u>Fully Effective (Recommended) Procedures</u>

Steam autoclaving for 1 hour at 132°C
Immersion in 1N sodium hydroxide for 1 hour at room temperature

<u>Partially Effective Procedures</u>

Steam autoclaving at either 121°C or 132° for 15 to 30 minutes
Immersion in 1N sodium hydroxide for 15 minutes, or lower concentrations (less than 0.5N) for 1 hour
Immersion in bleach (undiluted, or up to 1 in 10 dilution) for 1 hour

<u>Ineffective Procedures</u>

Boiling, ultraviolet irradiation, ethylene oxide sterilization, ethanol, formalin, beta-propiolactone, detergents, quarternary ammonium compounds, Lysol, alcoholic iodine, acetone, potassium permanganate.

6.0 BOVINE MATERIAL USED IN THE MANUFACTURE OF SURGICAL IMPLANTS AND BLOOD CONTACT MEDICAL DEVICES

The Southwood Report[33] recognises that the risk to human health from the agent of BSE is remote and theoretical. It is, however, a new risk.

Glutaraldehyde [4,10,12,19] formaldehyde[5,10,11,13,19] and ethylene oxide[19,23] are all reported to be ineffective methods for sterilization of material infected with the agents of CJD or scrapie.

Two companies within the United Kingdom are currently known to manufacture prostheses from bovine material.

manufacture heart/lung prosthesis from bovine material. manufacture bovine and porcine bioprostheses. The use of bovine material introduces implications for possible contamination by the agent of bovine spongiform encephalopathy.

The bovine material used by is exposed to 0.5% glutaraldehyde and 4% formaldehyde during manufacture of the prosthesis. The packaged device is exposed to ethylene oxide sterilization. Glutaraldelyde is also used in the treatment of bovine material by .

These procedures fall into the category of those "not recommended" in publications on the inactivation of scrapie-like agents and are not recommended in the Departmental Guidance[5,6,15].

Previous advice and research using the agents of CJD and scrapie, had concentrated on the decontamination of equipment; protection of health care workers from contaminated human material; human growth hormone; and dura mater. The methods developed may not be directly applicable or transferable to material of bovine origin for use in human implantation. A recent publication reports the effect of treating dura mater with salt solutions, hydrogen peroxide and acetone followed by irradiation with cobalt 60 (25KGy). In laboratory studies with scrapie-infested hamster dura mater, inclusion of NaOH into the protocol destroyed scrapie infectivity which was only reduced 90-99% with the standard procedure[34]. However, it should be noted that there is increasing doubt regarding the efficacy of treatment with NaOH solution [31,38]. In the use of human dura material it is recognized that an absolute guarantee of freedom from CJD cannot be given[36].

7.0 NON-STANDARD DECONTAMINATION PROCEDURES

Methods for validation of sterilization by "non-standard" procedures are currently under consideration by PG1A for eventual inclusion in the Guide to Good Manufacturing Practice for Sterile Medical Devices and Surgical Products[7]. This aims to provide guidelines for validation of methods of sterilization using liquid chemicals which are the only methods available for some devices.

However, consideration has currently been given to the decontamination of bacteria and fungi and not to assessment of the activity of a process against scrapie-like organisms.

8.0 CONCLUSIONS

1. The use of autoclaving at 134°C for 18 mins (or 6 cycles of 3 mins), sodium hypochlorite (10,000 ppm available chlorine) in the UK; and steam autoclaving at 132°C for 60 min, and 1N NaOH in the US, are methods which are recommended for inactivating material contaminated with scrapie-like agents.

2. Numerous compounds have been tested for their ability to inactivate these agents but for many of them the findings or intepretations either are conflicting or indicate that the process is unsuitable.

3. Bovine material is used in the manufacture of bioprostheses sold in the United Kingdom. The material will not withstand the processes outlined in 1. above. Consequently the sterilization processes used are not those recommended in Departmental guidance and are considered not to be effective in inactivating scrapie-like organisms.

4. There is no evidence to suggest that the sterilization methods used by manufacturers are effective against these agents, and some evidence which suggests that these methods are ineffective[4,5,10,11,12,13,19]. In particular there is evidence which indicates that formaldehyde is ineffective[5,10,11,13,19].

5. More research is required, using standardised methodology, to provide reliable data on the inactivation kinetics obtained with numerous compounds, before more recommendations can be made.

APPENDIX

Publications which have been cited in this appendix include reports of experimentation, reviews and guidance.

The data are separated into those "claimed to be effective" or "recommended", and those not. They demonstrate that advice has been given in many publications but contradictions are found. These may be explained by the lack of standardisation of methodology; differences in the agent or strain of agent used; difficulties in working with the agent due to the requirements for animal assay, the long incubation period, the tendency to aggregate, differences in titre of the agent in different assay systems, and difficulties in assessing the titre of the agent.

Quantitation of scrapie titres relies ultimately upon quantal titration assays in animals. This lacks sensitivity. The thermal and chemical resistance which is detected may be explained as due to a resistant subpopulation, to increased resistence due to aggregation of the agent, or due to interaction between the brain homogenate and the scrapie or CJD agent[13].

There is disagreement between workers in the field as to whether the agent of CJD should be used in activation studies to determine the requirements for CJD or whether the 22A strain of scrapie should be used. Those arguing for the latter claim that it is more suitable because different strains of scrapie have been identified and cloned in the laboratory and 22A strain is one known to possess high thermal resistance[24,31]. Such data have not been acquired for the agent of CJD. The KFu strain of CJD is one which has been cloned into laboratory animals.

Differences in conclusions may also be attributable to changes in methodology over the years. Methods that were initially considered effective and even recommended may later have been shown to be less trustworthy. This has been demonstrated with sodium hypochlorite, 1N sodium hydroxide and autoclaving of formal-fixed tissues. In these 3 examples, recent data contradict guidance which has been given.

	METHODS "CLAIMED TO BE EFFECTIVE" OR "RECOMMEDED"	METHODS "CLAIMED TO BE INEFFECTIVE" OR "NOT RECOMMENDED"
1. Autoclaving using steam	121-124°C for 90 min (5,28) 121°C for 15 min for CJD (Kfu strain) (12) 121°C for 15-30min for CJD (23) 121°C for at least 50 min for CJD (10,12) 121°C for 60 min (19) 126°C for 60-120min for scrapie using brain homogenates (11) 132°C ofr 15-30 min for CJD (18) 132°C for 60 min (22,23,27) 134°C for 18 min or 6 cycles of 3 min (5,27,33,35) 136°C for 4-32 min for scrapie (11) 126-129°C for 60 min (5,28) 136 - 138°C for 18 min or for 6 x 3 min (5,28,33)	121°C for 60 min (3,8,31,33) 121°C for 60 min for scrapie using brain homogenates (22) 121°C for 60 min for scrapie using brain macerates (26) 126°C for 30 min for scrapie (11,25) 126°C for 120 min for 1 strain of scrapio (11,25,26) 240°C for 1 minute for scrapie and CJD (33) 126°C for 120 to 240 min for scrapie (33)
2. Processing using Ethylene Oxide		Ineffective (19,23,28)
3. Heat Inactivation	45°C for 360 min achieved 2 log reduction CJD Kfu 80°C for 30 min " " " "	80°C] ineffective for scrapie (23,28) 100°C] heat (5,33)
4. Boiling in water		Ineffective (11,19,23,33)
5. Sodium Hypochlorite	undiluted or up to 1 in 10 dilution for 60 min for CJD (23) 0.5% for 60 min for CJD agent (10) 2.0% available chlorine (33) 2.5% for scrapie or CJD (22,28) 5.0% for scrapie (19) 1000 ppm available chlorine for 4-16 hrs] for 4 log 10000ppm available chlorine for 30 mins] reduction of scrapie(11) 10000ppm available chlorine for 30 min for CJD (5) 10000ppm available chlorine for 18 hrs for CJD (5) 1.0N sodium hyppchlorite (37)	5% for CJD (31) 2.5% for scrapie or CJD (35)

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	METHODS "CLAIMED TO BE EFFECTIVE" OR "RECOMMEDED"	METHODS "CLAIMED TO BE INEFFECTIVE" OR "NOT RECOMMENDED"
6. Sodium Hyrdoxide	0.1N to 1.0N for scrapie (4,5,19) 1.0N for 60 min for CJD (16,23,33) 1.0N for 60 min for scrapie (33) 1.0N for 5-10 min for skin decontamination (16,18,27) 1.0N for scrapie and CJD (28)	1.0N for 60 min (31, 38) 1.0N for 24 hrs (14)
7. Other Reagents	0.002M potassium permanganate (19) Potassium permangate for ophthalmologists instruments (18) phenol extraction (19) strong detergents (19)	formol fixation (31) formalin (1,19,23) formalin-fixation of tissue for 1 year (21) formaldehyde (5,10,11,13,19,28,37) glutaraldehyde (4,10,12,19,28,37) phenol (9,19) 4% hycolin (11) lysol (23,37) QAC's (19,23) 70% alcohol (5,11,19) ethanol (4,12,20,23,28) acetone (19,23,28) chloroform (5,19,28) Iodine (8,19) alcoholic iodine (23) 0.2% 0.4% and 0.8% potassium permanganate (10,11,22,23) 5% sodium dodecyl sulphate (SDS) (10,11) low concentrations SDS (9) 4M and 8M urea (16,22) urea (9) 1.0M HN_3 (22) 1.0N HCl (22) alkylating agents (33) organic solvents (33) concentrated salt solutions (33) many detergents (19,23,33) 100u M cis-dichlorodiammine (22) 500u M Platinum II (22) hydroxylamine (29) sodium deoxycholate (10) Triton X-100 (10) sodium metaperiodate (8) chlorine dioxide (8) 2mM Zn^{2+} hydrolysis (9) Bpropiolactone (19,23,28) hydrogen peroxide (8,19) ether (5,19) EDTA (19,28) nucleases (19,28) iodophores (5) KSCN (9)
8. Irradiation		Ineffective (23) cobalt 60 irradiation (2.5 Mrad) ineffective for suture material (18) UV irradiation (5,23) (2540A°) (19,28,33) ionising radiation (gamma rays) 5,19,33)

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	METHODS "CLAIMED TO BE EFFECTIVE" OR "RECOMMEDED"	METHODS "CLAIMED TO BE INEFFECTIVE" OR "NOT RECOMMENDED"
9. Ultrasonic energy		Ineffective (19)
10. Combinations of Methods	i. Fixation in formol + autoclaving at 134°C - 138°C for 18 min (for histopathological examination of tissue). (27) ii. Salt solutions, H_2O_2, and acetone + 0.1mol/l or 1.0mol/l NaOH for 60 min, then irradiation (for dura mater)(34) iii. 15% phenolized formalin for several weeks (30) (for autopsy material)	i. Fixation in formol + autoclaving at 126°C for 30 min (27). ii. Salt solutions, H_2O_2, and acetone for several weeks; then Co60 irradiation (25K Gy) (34) iii. Glass bead sterilizer (21) (for CJD) for scrapie - contaminated needles exposed to dry heat at 240°C iv. formal fixation + autoclaving at 134°C for 18 min (for scrapie-infected brain tissue) (27,32) v. 70% alcohol + formaldehyde vapour (for electrodes used on patients with CJD)(2,29) vi. formalin fixation, dehydration in methanol, clearing in cholorform, embedding in paraffin, (for brain tissue) (8)
11. Methods used on scrapie agent partially purified from hamster brain	Proteinase K and trypsin digestion chemical modification with diethyl pyrocarbonate phenol. Sodium dodcyl sulphate potassium cynate urea diethyl pyrocarbonate (9)	RN ase degradation DN ase proteases Zn^{2+} catalysed hydrolysis psoralen photoadduct formation NH_2OH 4 'aminomethyl 1-4, 5' 8-trimethyl - pyrocarbonate sodium dodecyl sulphate. (9)
Filtration	Untreated 25nm filter for CJD from human growth homone (21)	100nm filter for CJD from human growth homone (21)

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