Veterinary Laboratories Agency

BSE and scrapie: Guidelines on safe working procedures in histopathology laboratories and post-mortem rooms

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1. BSE and scrapie agents

These guidelines are based on the following assumptions:

1.1 There is compelling scientific evidence that the BSE agent may be transmitted to humans through the consumption of infected bovine tissue. It is therefore considered prudent to adopt higher levels of safety and disease security for the BSE agent compared with those which are in existence for working with the scrapie agent.

1.2 Non-pregnant live cattle or sheep do not present a microbiological hazard with respect to unconventional agent diseases.

1.3 Pregnant cattle or sheep present a potential hazard at parturition and abortion when the placenta, associated fluids and skin of the foetus may be contaminated with the agent. There is evidence for this in natural scrapie but not so far in natural BSE. The situation in regard to experimental BSE is unknown and therefore the hazard should be respected.

1.4 Brain, spinal cord, their protective fluids and membranes, eye (including retina), pituitary glands, peripheral nerves and ganglia, spleen, thymus, tonsil, lymph nodes, other lymphoreticular tissues (including Peyer's patches in the intestine) and placentae of clinically affected animals are most likely to have significant or detectable titres of agent. In addition, infectivity can be found in some species occasionally in adrenal gland, pancreas, lung and liver but usually at a low titre where this has been measured.

1.5 Formalin and glutaraldehyde fixation does not inactivate scrapie-like agents. Formalin and presumably glutaraldehyde confer an extraordinary resistance to subsequent porous load autoclaving at 134^{o}C - 138^{o}C for 18 minutes.

1.6 In discussing safety one must distinguish between safety for man and animals, and disease security in the context of animal experimentation though these are clearly linked.

1.7 The Advisory Committee on Dangerous Pathogens (ACDP) has categorised pathogens according to hazard and categories of containment, (ACDP 1995). The human TSE agents: CJD, GSS and kuru are ascribed to Hazard Group 3. The transmissible BSE agent is now known to be the same as that which causes nvCJD and therefore it too is now categorised as a Hazard Group 3 pathogen. However, as there is no evidence that any of the TSE agents are transmissible by the airborne route and therefore derogation from full Containment Level 3 is normally permitted.

1.8 These guidelines do not over-ride any safety control measures demanded or recommended by the Health and Safety Executive (HSE) or the VLA Safety Officer.

2. Accident procedures

2.1 Personal injuries should be treated immediately under the supervision of a qualified First Aider, further advice should be sought if necessary and the accident reported in accordance with local and laboratory guidelines.

3. *Post mortem* room procedures

3.1 In the context of these guidelines, and in relation to necropsies on adult cattle clinically suspected to have BSE or experimentally infected with the BSE/scrapie agent (including the conceptus of any such animal), protective clothing shall be worn as follows:

3.1.1 All persons within the PM room who are not directly handling the carcase:

- Wellington boots
- Long-sleeved, back-fastening water-proof gown
- Water-proof leggings
- Disposable particulate respirators (dust masks).

3.1.2 All persons directly handling the carcase, tissues from it or instruments and other materials that have been contaminated by the carcase or its tissues:

- Wellington boots
- Long-sleeved, back-fastening water-proof gown
- Water-proof leggings
- Cut-proof gloves worn under latex or rubber gloves
- Disposable particulate respirators (dust masks).

(In certain circumstances *e.g.* where there is a need for delicacy of touch, it is permissible to wear a cut-proof glove on the at-risk hand only; *i.e.* the left hand for right handed people).

3.1.3 All persons directly and closely associated with all sawing operations and actions likely to create aerosols or when brain, spinal cord, eye, spleen, thymus, intestine or other lymphatic tissues are handled in an unfixed state:

- Wellington boots
- Long-sleeved, back-fastening water-proof gown
- Water-proof leggings
- Cut-proof gloves worn under latex or rubber gloves

Positive pressure powered respirator which gives full eye, face and respiratory protection for the operator.

3.2 Where a carcase is hung from a hoist and, for example, the worker is to use long sweeping strokes of the knife, then the wearing of a chain mail apron and chain mail gloves is strongly recommended.

3.3 The officer in charge of the necropsy is responsible for determining what other level of protection may be necessary for other procedures in relation to the risks from the BSE agent. All staff must be made aware of the need for further protection from chemicals and conventional disease agents that may need special precautions for their safe use and handling. Where novel situations arise, advise should be sought from the Safety Officer.

3.4 After use, and within the confines of the PM room, re-useable clothing should be doused with hypochlorite containing 20,000 ppm available chlorine and thoroughly cleansed of organic matter, followed by rinsing in clean water prior to final cleaning and disinfection by the laboratory attendant wearing appropriate protective clothing.

4. Decontamination procedures

4.1 Acceptable decontamination procedures are:

4.2 Physical inactivation

4.2.1 For equipment and waste but excluding fluids and surfaces:

Porous load autoclaving at a hold temperature of 134^{o}C - 138^{o}C for 18 minutes at 30 lb/in^{2} or six separate cycles of 3 minutes each (DHSS, 1984). It should be noted that at the lower end of this temperature range, inactivation of the agent may not be completely effective (Taylor *et al*, 1994).

4.2.2 Downward or upward displacement autoclaves **must not** be used.

4.3 Chemical inactivation

4.3.1 For equipment, spillages and surfaces:

a) Disinfect with sodium hypochlorite containing 20,000 ppm available chlorine in the final product applied for a minimum of one hour at 20^{o}C for surfaces, or overnight for equipment. Alternatively, if this is contra-indicated for example due to corrosive effects, then:

b) 2N sodium hydroxide for a minimum of one hour at 20^{o}C for surfaces or overnight for equipment. Inactivation is not completely effective against high titres of the agent or where material is protected by dried organic material (Taylor *et al*, 1994). Constant re-wetting of surfaces may be necessary.

5. Live animals

5.1 Non-pregnant

5.1.1 Protective clothing must be worn followed by disinfection and cleaning procedures as specified in the code of practice.

5.2 Pregnant

5.2.1 As 5.1 but during the period of parturition, and for three days following complete placental separation, the animal shall be isolated. Any tissues or uterine discharges not required for further examination must be incinerated. Three days after placental separation the environment of the cow or sheep must be disinfected, cleaned and the material disposed of by incineration and the accommodation disinfected again. The hindquarters and contaminated parts must be cleaned before these procedures. Disinfection must be repeated after the animal finally leaves the isolation facility if this is later.

5.3 Transgenic animals

5.3.1 Any work with transgenic animals carrying single or multicopies of the *PrP* gene or PrP derived from them (particularly if this is primate, including human, PrP) or any work with tissues derived from such animals may present increased risks. Specialist advice should be sought before work is commenced. However, if work planned with animals or tissues from transgenic animals carrying the human *PrP* gene is authorised by the Chief Executive of the VLA, and has been assessed for risks by the VLA Genetic Manipulation Safety Committee, then such work must be conducted at the level appropriate for human TSE - normally Containment Level 3.

6. Dead animals and tissues

6.1 Appropriate protective clothing must be worn during necropsy and at all times when fresh, unfixed, partially fixed or frozen higher risk tissues are handled.

6.2 Fixed or partially fixed tissues in sealed containers may be removed to the Pathology Section. Fresh tissue in sealed leak-proof unbreakable containers may be removed to the Virology Department.

6.3 All unwanted tissue and disposable items that have been directly or indirectly in contact with potentially affected tissues or animals must be incinerated.

6.4 Instruments and other reusable items must be autoclaved or disinfected and cleaned according to Para 4 above. Staff who have direct contact with higher risk tissues must be kept to the minimum necessary.

6.5 There are two types of incinerator, one designed to safely burn biological material and one to deal with all other waste including clinical waste. Therefore carcasses and waste from them and nothing else must be placed in trolleys dedicated for the carcase incinerator. All other contaminated waste (syringes, gloves, paper and plastic etc.) must be placed in bags and then into containers designated for clinical waste.

7. Fixed tissue

7.1 Tissue blocking and cutting

- Anti-cut gloves should be worn under latex or rubber (Marigold or similar) gloves

- A visor or face mask and safety spectacles should be worn during blocking. All blocking should be performed under suitable local exhaust extraction *e.g.* Grosslab blocking station or other suitable control system.

- When work is carried out over down-drafted benching *e.g.* Afos benching, care must be taken not to use oversize cutting boards as this will interfere with the airflow across the benching

- Latex gloves should be worn when handling tissue pots

- Latex or rubber gloves should be worn when handling fixatives or decalcification or tissue treatment fluids. These should be used in a suitably ventilated or local exhaust extraction area.

7.2 Cassetting, processing and embedding

- Latex gloves should be worn for all cassetting, tissue processing and embedding procedures.

- Gloves and eye protection should be worn when changing tissue processor reagents.

- The embedding room floor must be kept free of wax debris. Splashing of hot wax must be avoided.

Tissues should be carefully transferred from processors to embedding centres to avoid the spillage of molten wax.

7.3 Sectioning

- Anti-cut gloves should be worn under powder-free latex disposable gloves. They must be worn for all sectioning procedures.

- Dedicated back fastening laboratory gowns should be worn during sectioning. These should remain in the Sectioning Room and autoclaved prior to despatch to the laundry. Disposable paper boiler suits may be used.

- Face masks and safety spectacles should be worn for block trimming and during cleaning of the work area. Masks and safety spectacles should be worn and the production of aerosols and dust should be minimised by careful attention to working procedures. Where local exhaust ventilation (*e.g.* Nederman extraction) is available, it must be used at all times with extraction hoods positioned directly above the work-station. All debris should be carefully cleaned away from the work area using suction nozzles.

- Disposable paper caps and disposable shoe protection is recommended for all operations to protect the person and to prevent spread of wax debris to other laboratories and offices. Shoe protection must be removed before leaving the room and placed in a covered receptacle.

- Latex gloves must be worn for mounting wax blocks onto wooden/plastic blocks.

- Special attention must be paid to personal habits - touching the face and particularly the eyes must be avoided and pencils, pens and diamond engravers etc. should not be placed in the mouth.

- All work stations must be kept clear of wax either by vacuum suction or by carefully brushing debris into plastic bags positioned near to the bench. All collected debris must be sealed in a bag and incinerated.

- Work benches must be disinfected by swabbing with 20% hypochlorite or with proprietary hypochlorite wipes when sectioning is completed.

- Microtomes should be cleaned using Histoclear and then swabbed with 20% hypochlorite and finally rinsed with water prior to servicing.

- Engineers should wear gloves and disposable over-shoes when servicing equipment in the laboratory.

7.4 Routine, special and histochemical staining

- Powder free latex gloves should be worn for all section staining and mounting procedures.

- Gloves should be worn during the preparation of all solutions and reagents.

- Nitrile gloves must be worn if contact with xylene is anticipated.

- Eye protection should be worn for all preparative procedures and when there is a risk of splashing. Dust masks should be worn when weighing powdered dyes and chromogen powders.

- Visors should be worn when working with acids or alkalis.

- All procedures employing or generating pungent or noxious fumes should be carried out under exhausted ventilation.

7.5 Block filing and retrieval

- Latex gloves should be worn when handling paraffin wax blocks.

- To minimise handling filed blocks consideration should be given to storing the blocks in snap-fastening plastic bags.

- Wherever possible separate storage areas should be used to segregate routine diagnostic material from BSE wax blocks. It should be noted that all adult bovine CNS could theoretically be infected with the BSE agent.

7.6 Handling glassware

- Latex gloves should be worn for collecting and washing used glassware and instruments

- All sample pots should be rinsed with water to remove fixative, decontaminated in 20% hypochlorite overnight and then rinsed again in water before loading into a dishwasher or for manual cleaning procedures.

8. Frozen tissues

8.1 Frozen tissues from animals infected or affected by TSE agents should be labelled appropriately and stored in dedicated containers, freezers or identified compartments of larger freezers.

8.2 Higher risk tissues (Para 1.4) must be dealt with according to specific codes of practice laid down after consultation with the Safety Officer and after conducting a full risk assessment

8.3 For lower risk tissues being prepared for frozen sectioning, laboratory coats and latex gloves should be worn. Once prepared the material is to be treated as for other unfixed tissue (Para 6).

9. Disease security

9.1 No fresh, unfixed or frozen tissue from BSE animals shall be released from VLA laboratories without the knowledge and agreement of the Chief Executive of the VLA.

10. Risk assessments and codes of practice

10.1 Local management must conduct a full risk assessment as required under the COSHH regulations and they must ensure that staff who are at risk from being exposed to TSE agents are not exposed to those agents. All staff who are at risk from possible exposure must follow all guidelines and adhere to any control measures which are recommended as a consequence of any risk assessment.

10.2 Each Department or Centre shall have a code of practice for handling BSE, scrapie or other unconventional transmissible agent-infected tissues based upon advice in these guidelines Additional scientific advice may be obtained from the Virology Department SROII (Mr M Dawson), the Safety Officer, the Health and Safety Executive or other reputable source.

10.3 All staff who work with infected tissues or animals must be acquainted with the code. Copies should be made available to staff in sections dealing with BSE or scrapie. Copies should also be lodged with the Virology Department SROII and the Safety Officer.

10.4 Codes of practice should be reviewed at least once annually or at other times if necessary and be amended if appropriate. Such changes must be brought to the attention of workers. Any member of staff is encouraged to offer advice or criticism on these guidelines by writing to the Safety Officer. All communications will be considered for inclusion at the next review date, or before if they are judged to significantly improve health and safety.

D W Redwood
Safety Manager

References and Bibliography

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R Bradley. *BSE and scrapie - Guidelines on some safety aspects - CVL*. Central Veterinary Laboratory, unpublished guidance document 1995.

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PERSONAL PROTECTION

The Advisory Committee on Dangerous Pathogens consider that if BSE is transmissible to humans in the occupational setting, the most likely routes are through specified bovine materials contaminating open wounds and open lesions on the skin, splashing mucous membranes (eyes and mouth) or exceptionally by ingestion. Transmission by the airborne route, *i.e.* by the inhalation of infectious airborne particles, is considered to be the least likely route of exposure.

The COSHH regulations require a hierarchy of control measures to protect those at occupational risk of exposure from biological (and other) agents. Thus it important to make every effort to separate the hazard from the worker by engineering controls *i.e.* a microbiological safety cabinet or other form of exhaust ventilation. Where this is impractical *e.g.* for *post mortem* examination of carcases, the staff at risk have to be adequately protected from exposure by personal protective equipment and by the observance of high standards of personal hygiene.

Protection of skin

Scratches, blisters, grazes and other breaks in the skin must be covered by a water-proof first aid dressing. Staff who suffer from medical conditions which result in skin lesions should be discouraged from working in *post mortem* rooms.

The use of sharp instruments should be kept to a minimum. Special care must be taken to ensure that surgical instruments are not lost under viscera or in the body cavity. A shallow enamel or plastic tray placed at one corner of the autopsy table in which all instruments are laid is a simple and inexpensive method of reducing the risk of cut and stab injuries.

Always discard used hypodermic needles directly into a sharps container. Never attempt to resheath needles.

Always use disposable plastic scalpel blade removers.

Cut-resistant gloves are available for varying levels of protection. None of them protect against stab injuries. Chain mail gloves offer complete protection but do not offer the delicacy of touch which is often required by the pathologist. These gloves will generally be worn by the slaughterman or the pathologist who is making long sweeping strokes to open the body cavity and for personnel who are at risk from band saw operations. The gloves are difficult to clean, therefore rubber gloves should be worn on top of the chain mail gloves.

Gloves incorporating woven cut resistant filaments such as Dyneema™, Kevlar™ or Silicon/Spectra™ fibre are not totally cut-proof and will offer varying degrees of protection according to their thickness.
Recommended gloves (in order of increased protection) are:

- Spectra™ Stretch,
- Spectra™ Heavyweight,
- Neptune Kevlar™,
- Polar Bear™ Supreme
- Euroflex™ Chainmail.

There are many more similar gloves by other manufacturers which will offer similar protection.

Forearm protectors are also available in the form of Kevlar or chainmail sleeves.

Protection of eyes

In the PM room there is a risk from fragments of bone, shards of broken glass, splashes of corrosive or infections material and irritation from dusts and vapours. Safety spectacles and visors are lightweight, comfortable and inexpensive. They do however only offer limited protection - mainly from impact and splash injuries. For better protection antimist goggles are more appropriate. Where protection is needed from dust, vapours and aerosols, glasses and goggles offer only very limited protection. Both goggles and spectacles are available in a wide range of styles and there is little to choose between them. They will all conform to the relevant British or European Standard.

Full protection of the eyes against all risks will be provided by a full face respirator or a positive pressure helmet (see below).

Respiratory protection.

High levels of protection to the eyes for respiration can be achieved by a full face respirator fitted with a P3 particle filter. They are however tiring to use, limit the field of vision and have to be properly fitted to ensure an efficient face seal. Normally they should not be shared for reasons of hygiene and proper fit.

Positive pressure helmets provide a stream of filtered air down over the face and offer good protection combined with a wide field of vision. As they are maintained at positive pressure, the problems associated with an efficient face seal do not arise.

As mentioned above, the ACDP consider that the risk from aerosol contamination from TSEs is very low. Naked prion protein is very unlikely to be generated in *post mortem* procedures; it is probable that the infectious agent will be bound up in relatively large aggregates of tissue or body fluid droplets.

HEPA filtration is therefore not entirely necessary and a filter matrix with a P3 rating will provide adequate protection.

Positive pressure helmets are more costly than full face respirators (£200+ compared with £80+) but the advantages will outweigh the disadvantages. It is important to note that particle filters (*i.e.* HEPA, P2 &P3) will not filter out gases such as formalin vapour. Where, for example, brain perfusions are carried out using glutaraldehyde and/or formalin then a full face respirator with an acid gas filter canister must be used.

Recommended helmets are:

- Pulsafe Clearflow Turbovisor
- Helmet Integrated Systems PurePlus PF23
- MSA Cobra Powered Helmet Respirator
- Racal Breath Easy BE1 helmet system
- Racal Airlite

Both Racal systems use Belt packs to hold the rechargeable batteries and filter canisters. The other systems incorporate lightweight rechargeable batteries and filter pads in the helmet.

Recommended disposable “dust masks” are:

3M 8822
3M 8835
3M 6000 series (reuseable half mask)

PERSONAL PROTECTIVE EQUIPMENT SUPPLIERS

Agents and general suppliers

Arco Ltd (Head Office)
PO Box 21
Waverley Street
Hull HU1 2SJ
Tel: 01482 222522
(Ring for the address of your nearest ARCO branch)

Brindus
Unit 67
Butterfly Avenue
Dartford Trade Park
Dartford
Kent DA1 1JG
Tel: 01322 274141

RS Components UK
PO Box 99
Corby
Northants NN17 9RS
Tel: 01536 444141

Greenham Trading Ltd
Greenham House
671 London Road
Isleworth
Middlesex TW7 4EX
Tel: 0181 5601244
(Ring for the address of your nearest Greenham branch)

Manufacturers

Helmet Integrated Systems Ltd
Moat factory
Wheathampstead
St Albans
Herts AL4 8QT
Tel: 01582 834211

Pulsafe Safety Products Ltd
Respiratory Protection Division
Landsberg
Lichfield Road Industrial Estate
Tamworth
Staffs B79 7XB
Tel: 01827 55564

MSA (Britain) Ltd
East Shawhead
Coatbridge
Scotland ML5 4TD
Tel: 01236 424966

Racal Health and Safety Ltd
Folly Lane
Warrington
Cheshire WA5 5NY
Tel: 01925 234656

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