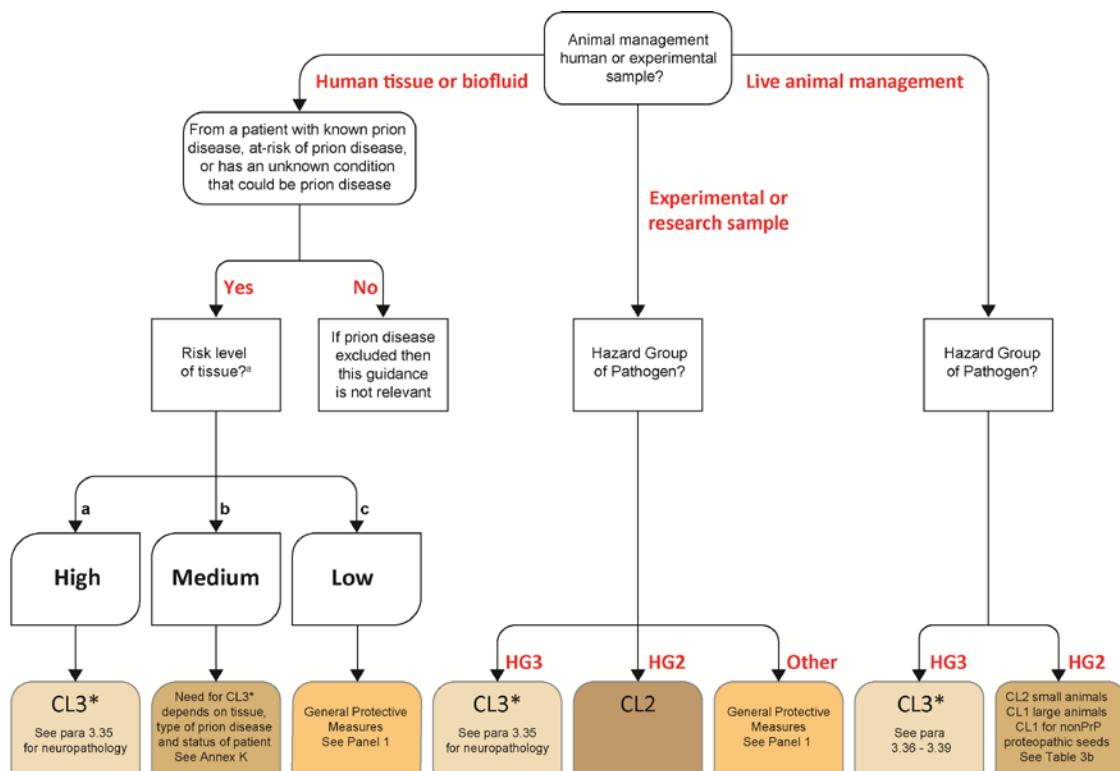


## PART 3

### Laboratory containment and control measures

Flowchart: How to use this document



CL\* = ACDP Containment Level (see [Table 3c](#)).

For General Protective Measures see [Panel 1](#).

HG= Hazard Group.

<sup>a</sup>High risk tissues include brain, spinal cord, dura mater, posterior eye, pituitary, cranial nerves and ganglia.

<sup>b</sup>Medium risk tissues include spinal ganglia and olfactory epithelium, and in the case of vCJD (but note vCJD is currently extremely rare), tonsil, spleen, appendix, thymus and gut associated lymphoid tissues.

<sup>c</sup>Low risk tissues include all others, most notably any patient-derived fluids like CSF, blood, urine, saliva.

General Protective measures only for asymptomatic “at-risk” patients.

See [Annex K](#) for further details.

## Introduction

- 3 Prion diseases, or transmissible spongiform encephalopathies (TSEs), are fatal and transmissible neurodegenerative disorders of humans and animals. Prions, the infectious agent of prion diseases, are thought to comprise of assemblies of misfolded forms of prion protein (PrP). Prions propagate by binding and templating the misfolding of normal prion protein. They pose special issues for Health and Safety management in laboratory settings.
- 3.1 People have been infected with prions by medical or surgical procedures or percutaneous injury involving contaminated materials, such as contaminated surgical instruments, or contaminated treatments (such as hormone treatments). Strict control measures are therefore important to protect everyone who may be at risk (including lab workers, healthcare professionals and patients) from infection.
- 3.2 The tragic death of a French laboratory worker from variant Creutzfeldt-Jakob disease (vCJD) in 2019 highlights the importance of safe working with prions in laboratories (1). It is likely that this worker was infected when metal forceps contaminated with bovine spongiform encephalopathy (BSE)-derived prions penetrated the skin of her finger in a laboratory accident several years earlier.
- 3.3 Over recent years there has been increasing evidence of the ability of proteins (other than the prion protein) to adopt abnormal conformations, self-propagate and cause transmissible pathologies and diseases in humans and laboratory animals (2-5). Such proteins and peptides share a range of pathological properties with PrP prions and may be referred to as “prions,” “prion-like,” “prionoid,” or “proteopathic seeds” in the scientific literature but are also distinct from PrP prions in several ways, including importantly, that there are no known animal or human epidemics or established occupational risks. In this document we use the term “proteopathic seeds” and include only those proteins known to associate with human neurodegenerative diseases (amyloid-beta peptide, microtubule associated protein tau, alpha-synuclein, TDP-43 and FUS). Human-human transmission of proteopathic seeds have only been observed for amyloid beta in some specific circumstances that historically transmitted prion infection (e.g. use of cadaver derived human pituitary hormones or dura mater in neurosurgery), after long latencies (2).
- 3.4 This section (Part 3) gives advice on safe working practices to help prevent the unwanted transmission of prions and/or proteopathic seeds during laboratory work with such agents or material that contains or may contain them. It covers:
- all experimental work with preparations derived from tissues or body fluids from patients or animals who are diagnosed with, suspected to have, or are at-risk of any form of prion disease.
  - all diagnostic laboratory work with preparations derived from body fluids or tissues derived from patients or animals known or at-risk of a form of prion disease – this includes:

- handling, analysis and storage of cerebrospinal fluid (CSF) samples and other low risk tissues such as blood, urine and saliva from patients with suspected or at-risk of Creutzfeldt-Jakob disease (CJD) and other prion diseases
- work with animal tissues collected in the field for onward transfer to laboratories for investigation, for example, tissues collected for surveillance purposes
- experimental work with animals infected or suspected to be infected with prions.
- experimental work with synthetic, amplified, or concentrated (see glossary) laboratory preparations of prions or proteopathic seeds.
- this guidance does not cover tissues or body fluids from patients known to have neurodegenerative disorders other than prion disease (eg. Alzheimer's disease, Parkinson's disease, frontotemporal dementia, etc.) unless they are processed in a research or diagnostic laboratory to concentrate or amplify proteopathic seeds. Advice in [Panel 1](#) below may be considered in risk assessments of work with this material.

3.5 Safe laboratory management of prions and proteopathic seeds requires consideration of:

- whether the sample or setting is:
  - human tissues and biofluids for diagnosis or research,
  - experimental, including disease surveillance,
  - with live animals,
- the Hazard Group classification of the pathogen, and
- the type of tissue or biofluid (See [Flowchart p1](#)).

3.6 Guidance on work with any hosts or vectors in which genes encoding prion proteins have been cloned using genetic modification and where expression may be achieved is given in the Scientific Advisory Committee on Genetic Modification's Compendium of Guidance ([The SACGM Compendium of guidance - Part 2: Risk assessment of genetically modified microorganisms \(other than those associated with plants\) \(hse.gov.uk\)](#)).

3.7 Human tissues and biofluids are categorised as High, Medium and Low risk, according to the likely concentration of prions determined by bioassay or amplification assays (e.g. real time quaking induced conversion assay, RT-QuIC). High risk materials for human prion diseases include brain, cranial nerves/ganglia, spinal cord and posterior eye tissues (see Annexes A1 and A2 [Minimise transmission risk of CJD and vCJD in healthcare settings - GOV.UK \(www.gov.uk\)](#)). Medium risk materials include the spinal ganglia and olfactory epithelium; and in variant CJD, tonsil, thymus, appendix, spleen and gut associated lymphoid tissues. Note that vCJD is currently (2021) extremely rare, only one case has been diagnosed in the UK between 2016-2021 (6). The WHO has similarly classified animal tissues into risk categories <https://www.who.int/bloodproducts/tablestissueinfectivity.pdf>.

- 3.8 Notable low risk materials include blood, urine, saliva, cerebrospinal fluid (CSF) and most peripheral (non-CNS) tissues. These need only general laboratory precautions (Panel 1) for managing prions because they are thought to contain only very low concentrations of the infectious agent.
- 3.9 Risk assessments should be done for all work with human samples, live animals, and research materials that might contain prions or proteopathic seeds. The risk assessment is informed by the containment level (CL) for the agent which is derived from the hazard grouping (HG). When working with an agent in a particular hazard group, the Control of Substances Hazardous to Health (COSHH) Regulations 2002 require that the CL selected must match the HG of the agent as a minimum.
- 3.10 Most prion agents are classified as HG3. However, they are in the Approved List of biological agents within HG3 (denoted 3\*) identified as presenting a more limited risk of infection for workers because they are not normally infectious by the airborne route. Those working with these agents may not necessarily need to use all the containment measures normally required at CL3, based on a risk assessment. Dispensing with some of the control measures from CL3 (termed CL3\*) does not imply that the work can be carried out at CL2, it simply allows certain physical containment requirements (particularly those aimed at controlling airborne infection, and the ability to fumigate an area, which are not relevant measures for prions) normally expected at CL3 to be dispensed with. All other aspects of the work, in particular, high levels of management and oversight, supervision and training, should reflect the high standards expected at CL3.

## Formal classification of prions (TSE agents)

Table 3a (4 tables below) shows the Hazard Group (HG) classification of the causative agents of transmissible spongiform encephalopathies as listed in the Health and Safety Commission's Approved List of Biological Agents (<http://www.hse.gov.uk/pubns/misc208.pdf>). 3\* see paragraph 3.10

**Table 3a Hazard groupings for high-risk tissues in prion diseases**

<b>Human prion diseases</b>	<b>HG</b>
Sporadic forms of human prion disease:	
Sporadic Creutzfeldt-Jakob disease agent	3*
Sporadic fatal insomnia agent	3*
Variably protease-resistant prionopathy agent	3*
Inherited forms of human prion disease:	
Familial Creutzfeldt-Jakob disease agent	3*
Fatal familial insomnia agent	3*
Gerstmann-Sträussler-Scheinker syndrome agent	3*
Acquired forms of human prion disease:	
Variant Creutzfeldt-Jakob disease agent	3*
Iatrogenic Creutzfeldt-Jakob disease agent	3*
Kuru agent	3*

<b>Animal prion diseases</b>	<b>HG</b>
Bovine spongiform encephalopathy (BSE) agent and other related animal prion diseases	3*
All strains related to or derived from BSE (including feline spongiform encephalopathy agent and spongiform encephalopathy agent in exotic ungulates)	3*
H-type BSE agent	3*
L-type BSE agent	3*
Uncharacterised animal strains (from point of view of zoonotic potential)	3*
Scrapie and scrapie-related agents	2
Atypical scrapie agent	2
Transmissible Mink Encephalopathy	2
Chronic Wasting Disease (CWD) agent, N American strains	2
European CWD (not yet fully characterised)	3*

<b>Laboratory strains of prion diseases</b>	<b>HG</b>
Any prion strain propagated in primates.	3*
Any prion strain propagated in animals engineered to express the human PrP gene (with or without inherited prion disease mutations) or other PrP sequences that might be predicted to adopt prion strains with transmissibility to humans.	3*
Human prion strains propagated in any species	3*

<b>Proteopathic Seeds</b>	<b>HG</b>
<p>Classification applies to concentrated, amplified or synthesized materials containing misfolded protein assemblies in forms known to share pathological properties with prions (including the ability to self-propagate) <i>and</i> are associated with human neurodegenerative diseases. Includes proteopathic seed forms of amyloid-beta peptide, tau protein, alpha-synuclein, TDP-43 and FUS. See Glossary for more detailed definitions.</p>	2

- 3.11 Risk assessments should be done for laboratory work with preparations of non-PrP proteopathic seeds. The hazards are greatest when handling *in vitro* materials containing concentrated, amplified, purified or synthesised material which has or could have seeding (self-propagating) functions in human. Risk assessment should also be done for the handling of materials derived from cells or animals that are inoculated with proteopathic seeds and engineered to express human forms of these proteins (or proteins sequences that might propagate the human protein). At least CL2 working with such materials are recommended.
- 3.12 **No additional measures are recommended for handling of any biofluid or tissue specimens from patients with non-prion neurodegenerative diseases (eg. Alzheimer's disease, Parkinson's disease, frontotemporal dementia, motor neuron disease / amyotrophic lateral sclerosis) who are not suspected to have CJD, beyond a consideration of general protective measures in risk assessment (see Panel 1 below).**
- 3.13 Based on the current Hazard Grouping of prion and proteopathic seed agents, the recommended overall Containment Levels are given in Table 3b below. Work is categorised according to the type of infectious agent and, for work with large and small animals. Work with human prion agents includes primary sources and any sub-passages of human derived agents in other species.

**Table 3b Containment Levels recommended for work with prion and proteopathic seed agents**

Laboratory work with:	Overall Laboratory Containment Level	Animal Containment Level
<i>Human prion diseases (except low risk tissues)</i> <i>Material from any prion disease showing zoonotic characteristics, such as BSE and the animal diseases epidemiologically or biochemically linked to BSE.</i> <i>Infectious studies involving uncharacterised prion strains e.g. prion disease from reindeer and moose in Europe or where effect of the prion strain host is not known</i>	3* (*with risk assessed derogations)	3 <sup>a</sup> (*with risk assessed derogations)
<i>Scrapie agents (classical and atypical) in their natural host (sheep or goats); wild type rodents or transgenic mice expressing mammalian PrP other than bovine or human PrP.</i> <i>Characterised (North American) CWD in natural host.</i>	2	2 - small animals 1 <sup>a</sup> - large animals
<i>Concentrated, purified, amplified, or synthesized forms of proteopathic seeds (see Table 3a)</i>	2	1

<sup>a</sup>Applies to the risk of human infection, BSE, classical scrapie and CWD are notifiable diseases ([Notifiable diseases in animals - GOV.UK \(www.gov.uk\)](http://www.gov.uk/government/collections/notifiable-diseases-in-animals)) under the Animal Health Act 1981 (as amended) in the UK so for classical scrapie and CWD in large animals at CL1, additional controls have to be in place to contain it and prevent spread outside the experiment. For 3\* see paragraph 3.10.

3.14 There may be circumstances in which it is known that the frequency of infection in a population of samples is very low, for example, a prevalence study in a large deer population that is expected to have zero or close to zero CWD infections. In this circumstance it may be reasonable for a risk

assessment to consider initial laboratory handling of samples at a lower level of containment than is usually recommended for the pathogen.

3.15 Before any decision to change the containment conditions is considered, a local risk assessment must be completed and the Health and Safety Executive (HSE) must give their consent (see COSHH Schedule 3, Part II that details the containment measures for health and veterinary care facilities, laboratories and animal room). The risk assessment should consider (see 3.36-3.39 for live animal work):

- the nature of the work;
- the quantity and type of material being handled; and
- the procedures and equipment that will be used – consider the potential for:
  - dispersal of the agent,
  - contamination of workers,
  - contamination of equipment or surfaces at all stages of the activity including handling processing and disposal (noting that fumigation is not effective)
  - contamination of equipment or surfaces during the setting up, servicing and maintenance of the equipment,
  - the potential danger from penetrative wounds,
  - the adherence of prion to metals such as stainless steel (noting that autoclaving will not completely remove prions, although it will reduce the level of infectivity, so consider single use disposable equipment)

3.16 Having completed the risk assessment, local rules/standard operating procedures should then be prepared detailing safe working practices.

#### **General approach to safe working practices applicable to all laboratory work with prions and proteopathic seeds**

3.17 This general approach applies to all laboratory work whether human or animal diagnostics or experimental (research) work.

3.18 '*The management, design and operation of microbiological containment laboratories*' [HSE: Management and Operation of microbiological containment laboratories](#) provides guidance on the management of biological agents including prions, in the laboratory environment. The guidance sets out the standards for CL2 and CL3 microbiological laboratories, and it should be read in conjunction with this guidance which sets out the specific and/or additional requirements for work with prions. The ACDP guidance '*Working safely with research animals: Management of infection risks*' <https://www.hse.gov.uk/pubns/priced/animal-research.pdf> should also be read in conjunction with this guidance. The essential features of CL2 and CL3, as required by COSHH, are shown in Table 3c below.

**Table 3c Containment measures for CL2 and CL3 laboratories**

<b>Containment measure: Air handling</b>	<b>Containment level 2</b>	<b>Containment level 3 (Note 3.10 re CL3*)</b>
The workplace is to be maintained at an air pressure negative to atmosphere	No	Yes
Input and extract air to the workplace are to be filtered using high efficiency particulate absorption (HEPA) or equivalent	No	Yes, on extract air

<b>Containment measure: Security and access</b>	<b>Containment level 2</b>	<b>Containment level 3 (Note 3.10 re CL3*)</b>
Access is to be restricted to authorised people only	Yes	Yes
The workplace is to be separated from any other activities in the same building	No	Yes
Efficient vector control, e.g. rodents and insects	Yes, for animal containment	Yes, for animal containment
An observation window, or alternative, is to be present so that occupants can be seen	No	Yes
Safe storage of biological agents	Yes	Yes
A laboratory is to contain its own equipment	No	Yes, as far as is reasonably practicable
Disinfection and disposal procedures		
The workplace is to be sealable to permit disinfection	No	Yes
Specified disinfection procedures	Yes	Yes
Surfaces impervious to water and easy to clean	Yes, for bench	Yes, for bench and floor (and for walls for animal containment)
Surfaces resistant to acids, alkalis, solvents and disinfectants	Yes, for bench	Yes, for bench and floor (and for walls for animal containment)
Incinerator for disposal of animal carcasses	Accessible	Accessible

<b>Containment measure: Protective equipment and procedures</b>	<b>Containment level 2</b>	<b>Containment level 3 (Note 3.10 re CL3*)</b>
Infected material, including any animal, is to be handled in a safety cabinet, isolator or other suitable containment	Yes	Yes

- 3.19 General, basic protective measures should be used wherever there is a risk of exposure to potentially infectious material, including prions and/or proteopathic seed preparations as defined in Table 3a. These measures are summarised in Panel 1 below.

### Panel 1: General Protective Measures

- Laboratory staff must read and understand the Codes of Practice that are specific for their laboratory. These MUST be followed at all times
- Never work alone in a laboratory without informing others of your presence there
- Apply general good hygiene measures, never eat, drink, smoke, apply make-up in a laboratory or pipette by mouth.
- Disposable gloves should be worn at all times when handling prions and/or proteopathic seeds. Consider the use of suitable special hand protection such as armoured glove(s) (<https://www.hse.gov.uk/biosafety/blood-borne-viruses/use-of-gloves.htm#what-gloves-should-be-worn>) where the use of sharp instruments is essential e.g. in post-mortem examinations or the collection of human or animal brain/spinal cord
- All workers in the laboratory must cover cuts and abrasions with a waterproof dressing before entering the laboratory
- Wear the appropriate protective clothing routinely for all work with prions and/or proteopathic seeds. This includes wearing eye protection or full-face visor to protect eyes and mucous membranes from splashes with potentially infectious material. If a liquid-repellent laboratory coat is not available a disposable plastic apron should be worn over existing laboratory coat, or disposable gown.
- Manual handling of CSF, blood, urine or other biological material should preferably be undertaken on non-absorbent surfaces in a Microbiological Safety Cabinet (MSC) if one is available.
- Use closed systems such as sealed centrifuge buckets and fully enclosed auto-analysers, or when manually handling samples, a Microbiological Safety Cabinet (MSC) to protect against splashing of material when mixing, centrifuging or homogenising samples (see next bullet for what to do if an MSC is not available).
- If an MSC is not available, risk assessments should consider the potential for dispersal of the agent, for contamination of workers, equipment or surfaces at all stages of the activity, for example using disposable plastic trays when aliquoting CSF on an open bench, and have protocols in place for dealing with spillage (see [INFOBOX1](#)).
- Keep your working area clean and free from obstructions. Do not leave coats, bags or unnecessary books on the benches or on floors to trip over.
- Remove waste material from your area as soon as possible and dispose of it in the appropriate waste container.
- When you have finished, leave your area tidy and follow any instructions for clearing and cleaning equipment.

- Minimise the use of sharps (needles, knives, scissors and laboratory glassware) wherever possible. Any use of sharps must be risk assessed.
- Remove protective equipment (e.g. your laboratory coat) and wash your hands thoroughly before leaving the laboratory
- Use plastic single-use disposable items (containers, pipettes, inoculating loops and other such instruments); in the case of large items this could be interpreted as specified parts of the item e.g. dedicated ultracentrifuge rotors or electron microscope grids
- Use recommended decontamination procedures – see [INFOBOX1](#)

3.20 **Cleaning and decontamination.** As many of the standard methods of decontamination cannot ensure complete inactivation of prions and/or proteopathic seeds, the emphasis must be on prevention of spillages, single use equipment and the removal of the agent by specific cleaning protocols (See [INFOBOX1](#) for decontamination methods, and [Annex C](#)) (7). An annual check of competency at handling spillages (“spill drill”) is recommended.

**INFOBOX1**

**Examples of procedures for decontamination of prions**

These procedures may be relevant to routine work or after a spillage.

Spillages or contamination with low risk human biofluids can follow standard local procedures.

Any work involving unsealed biological material should be performed in a defined area such as over a plastic spill tray. In the event of a spillage outside of a spill tray, the area should be decontaminated before cleaning.

Laboratory equipment that has been exposed to prion infection and is not in a dedicated location within a CL3 or 3\* (see paragraph 3.10) laboratory should be decontaminated after the exposure.

WHO recommended methods for prion destruction are exposure to a hypochlorite solution containing a final concentration of >20,000 ppm free chlorine or 1M NaOH for 1 hour at room temperature.

Where such treatments are not possible (for example because of reactions with chemicals or surfaces) consideration should be given to alternatives that, although not formally validated, may have inactivating action against prions and/or proteopathic seeds.

These could include: Autoclave at  $\geq 134^{\circ}\text{C}$  for  $\geq 20$  minutes (7).

Exposure to high concentrations of ionic detergents in aqueous solution at elevated temperature, eg >2% w/v SDS at  $>45^{\circ}\text{C}$ .

Exposure to strong chaotropes such as guanidine hydrochloride (>5M) or guanidine isothiocyanate (>3M).

Treatment with high concentrations of broad specificity alkaline proteases, eg proteinase K at >1mg/ml.

[Sequential treatments are likely to increase the probability of inactivation]

## Handling Accidents

- 3.21 Accident and emergency procedures should follow the procedures given in the COSSH guidance. Specific guidance in relation to prions is given below.
- 3.22 There should be plans in place (including competency testing) in the laboratory to deal with accidents involving prions or proteopathic seeds, for example dealing with spillages or first aid arrangements for inoculation injuries. Employees must report immediately to their employer, or the appropriate contact responsible for health and safety, any accident or incident that results in the release of prions.
- 3.23 The training of employees working with prions or proteopathic seeds should include highlighting the readily foreseeable incidents that could occur and the procedures for dealing with accidents, incidents and emergencies, and the name of the person or people to whom accidents should be reported. Competency should be tested at intervals.
- 3.24 Spillages should be handled according to local procedures (see [INFOBOX1](#) and [Annexes C](#) and [K](#) and [Part 4](#) for more guidance).
- 3.25 Any inoculation injury with prions or proteopathic seeds (or material that contains these agents) if not bleeding should be gently encouraged to bleed, washed (not scrubbed) with warm soapy water and covered with a waterproof dressing. Similarly, any contamination of skin should also be removed with soapy water. Disinfectants should not be put onto cuts or broken skin, as this could worsen the injury and impair the body's localised defence reaction.
- 3.26 Employees may be distressed about a potential exposure and should be offered local psychological support if needed. National services (eg. National Prion Clinic, [www.nationalprionclinic.org](http://www.nationalprionclinic.org), and National CJD Research and Surveillance Unit, <https://www.cjd.ed.ac.uk/>, can provide additional advice if needed).
- 3.27 An official local record should be kept of any incident that involves the potential escape of prion agents into the environment. An official local record should be kept of any inoculation or mucosal membrane (eg. face splash) incident involving prion or proteopathic seeds. Except in rare circumstances, incidents will need to be reported to HSE under RIDDOR (see Part 2, paragraph 2.25 [Part2\\_rev\\_May03.doc \(publishing.service.gov.uk\)](#)). Due to the length of incubation period of human TSEs these records should be retained for 40 years.

## Transport of specimens

- 3.28 Specimens from human and animal origin, known or suspected to contain prions are classed as Category B infectious substances for the purposes of transport. Packaging needs to be UN3373 compliant. Guidance on the

transport of this material is given in Annex D and [Transportation of infectious substances - Blood borne viruses \(BBV\) \(hse.gov.uk\)](https://www.hse.gov.uk).

## Diagnostic laboratories

- 3.29 A range of laboratory tests may be required for the investigation of patients with a clinical diagnosis of, or suspected CJD. Similarly, veterinary diagnostic tests will be needed in herds where animal prion diseases may be suspected. Diagnostic-type tests may also be carried out on human or animal tissues for surveillance purposes.
- 3.30 Brain and spinal cord samples (and related tissues like posterior eye, pituitary and cranial nerves/ganglia) from suspected prion disease patients/ patients being investigated for prion disease present the greatest risk of exposure to prions as compared to biofluid and peripheral tissue specimens because of the much higher titres of infectivity. These and medium risk tissues should be managed at CL3 or 3\* (until formalin-fixed and formic acid treated) and preferably, at a specialist neuropathology laboratory familiar with this work. Note the following recommendations for peripheral tissues:
- Samples containing lymphoreticular tissue (eg. tonsil, appendix, spleen, gut associated tissues) contain medium risk infectivity for patients with or suspected to have variant CJD (but note para 3.7 variant CJD (2021) is currently very rare).
  - Olfactory mucosa and spinal ganglia are medium risk for patients with or suspected to have any form of prion disease.
  - **No specialist precautions above general protective measures are necessary for medium risk tissues from asymptomatic “at-risk” individuals.**
  - see Annexes A1, A2 and K for detailed description of tissues and patient status.
- 3.31 Diagnostic biofluid specimens such as blood, urine, CSF and saliva from suspected prion disease patients can be handled and stored using general laboratory precautions for handling prion disease specimens ([General Protective Measures](#)). Measures based on [General Protective Measures](#) should be included in local Standard Operating Procedures including for instrument decontamination, disposal of samples and single use equipment. Examples of the factors that might be considered by a CSF laboratory in local risk assessments are suggested in the [INFOBOX2](#). **Importantly, all CSF specimen processing, where these include samples taken from patients with neurodegenerative dementias, should be handled in a way that considers the possibility that the sample came from a patient with prion disease ([General Protective Measuresable 3d](#)).** Processing of samples from patients with suspected CJD should not be delayed until confirmatory tests are available.

## INFOBOX2

**Examples of factors that should be taken into account by a CSF laboratory that processes samples from patients with neurodegenerative diseases (also see [Panel 1](#))**

Personal Protective Equipment

- a) Eye protection to avoid potential splashes eg safety glasses, visor
- b) Spill-resistant gown or disposable plastic apron
- c) Gloves and protective covering of all cuts and abrasions

Manual procedures undertaken in a quiet area of the laboratory

- a) Separate room, consider using CL2/3 lab if one is available, or MSC
- b) Area of laboratory with limited passing footfall
- c) Undertaking work at quieter times, eg lunch-time, end or beginning of day

Working environment

- a) Bench covered in spill resistant covering which is easy to decontaminate such as Benchkote or on plastic trays that can be disposed of in case of spillages
- b) Disposable equipment used wherever possible and consumables to be disposed of in small sharps bins that can be removed from laboratory immediately after use, according to local laboratory guidelines.
- c) Avoid using sharps such as scalpels, glass Pasteur pipettes
- d) Non-disposable items such as glass counting chambers to be decontaminated according to local laboratory guidelines

Storage and disposal of CSF samples

- a) CSF samples from patients with suspected prion disease can be stored alongside other CSF samples, according to local laboratory guidelines. There is no need to store separately
- b) CSF samples from patients with suspected prion disease can be disposed of alongside other CSF samples, according to local laboratory guidelines. No additional precautions are required.

- 3.32 Disposal of used laboratory consumables. General laboratory consumables such as pipettes, pipette tips, paper towels, sample tubes etc must be disposed of according to the local laboratory Standard Operating Procedures. No additional precautions need to be undertaken.
- 3.33 CSF blood, urine, and saliva samples should be stored and disposed of according to local laboratory SOPs. No additional precautions need to be taken.
- 3.34 Disposal of CSF, blood, urine and saliva. Biological samples from patients should be disposed according to the local laboratory Standard Operating Procedures. No additional precautions need to be undertaken.

## Neuropathology

- 3.35 Standard formalin is used for optimal fixation of whole brain for general histopathology purposes. Formalin-fixed prion infected tissues have reduced infectivity, but are not decontaminated, and local risk assessments should take this into account. Similarly, tissue for electron microscopy fixed in glutaraldehyde retains its infectivity, although this risk is considered to be negligible and no special precautions are required beyond disposal of grids as infectious waste. Formalin-fixed prion infected tissue can be decontaminated with formic acid treatment – see Annex C for details (Note: formic acid treatment has not been shown to be effective for non-formalin-fixed material). Once tissue blocks are fixed and formic acid-treated, they no longer need to be managed like frozen material, sections can be cut on a standard microtome (preferably using a disposable knife) and processed as usual. We recommend that debris (wax shavings) from section cutting should be contained and disposed of by incineration, but again the risks are considered to be very small from these materials. Archive material stored in paraffin blocks or as mounted slides (unless known to be formic acid treated) may still have some residual prion infectivity which should be considered in risk assessments when filing these materials.

### **INFOBOX3**

#### **General protective measures when undertaking an autopsy where prion disease is possible (any neurodegenerative disease)**

- Laboratory staff must read and understand the Codes of Practice that are specific for their mortuary. These MUST be followed at all times
- The number of staff in the mortuary should be kept to a minimum and all staff should wear the required PPE.
- A full autopsy examination can be undertaken safely following guidelines and use of appropriate PPE.
- The minimum PPE recommended is: a face mask with attached visor; surgical scrubs; boots with steel toecaps; water resistant gown; waterproof apron that extends to boots; surgical gloves, cut-resistant gloves, further surgical gloves.
- Scissors and forceps should have blunt ends to avoid skin puncture. Equipment should be disposable or decontaminated as outlined in section 3.20
- Brain cuts should use disposable knives, and each knife should be disposed of at the end of the examination.
- Gown, apron, and surgical gloves should all be disposed of at the end of the autopsy, using a designated and appropriate clinical waste bag for incineration.
- When the autopsy has been completed, the area should be appropriately decontaminated (see [INFOBOX1](#)).

## Research work with live infected animals

- 3.36 In general, live animals infected with prions do not pose a significant risk of exposure to prions. However, the nature of experimental work with such animals means that there will be procedures/tasks that increase the risk of exposure, that need to be managed by risk assessment (see 3.14-16).
- 3.37 Before any decision to change the containment conditions is considered, a local risk assessment must be completed and the HSE must give their consent (see COSHH Schedule 3, Part II that details the containment measures for health and veterinary care facilities, laboratories and animal room). The risk assessment should consider the general points made in para 3.15.

For live animal work this should additionally consider:

- the type and strain of prion, particularly if it has been used or derived from previous animal experiments
- what is known about how the host species handles prions e.g. peripheral lympho-reticular involvement, excretion in body fluids or at parturition in diseases such as scrapie in sheep
- the field epidemiology evidence available e.g with BSE the lack of infection spread between animals or those involved with their husbandry and slaughter
- method of dosing e.g. oral dosing has the potential for prion to be excreted in the faeces for a period after dosing, inoculation by injection has the potential risk of leakage for contaminated material from entry wound. The risk of leakage should be minimised with techniques such as the use of sealants and dependent on what is injected this should be considered high hazard with associated control measures e.g. disposable mouse boxes
- the lack of airborne spread from live animals incubating the infection
- carcasses and other associated material, e.g. tissue samples, from all animals experimentally infected with prions should be disposed of by incineration

## Collection of specimens

- 3.38 The risk assessment of the type of sample required will affect the control measures required. The WHO has classified animal tissues into high and low risk categories <https://www.who.int/bloodproducts/tablestissueinfectivity.pdf> in a similar way to the human. As well as restraint and sedation, the taking of body fluids should avoid exposure to mucous membranes of the eyes and mouth, with the use of face protection. Procedures should also be in place to avoid accidental puncture wounds (See [Panel 1](#) for prompts of measures to be taken).

## Disposal of waste

- 3.39 All waste streams from animals infected with prions should be risk assessed. High risk material that is known to contain HG3\* prions (Table 3b) or any used sharps should either be autoclaved then incinerated or go direct to incineration on site dependent on what is risk assessed as safer. Bedding and faecal waste during any initial shedding phase from dosing must also be treated in a similar manner.

Where there is epidemiological evidence that the faeces is not infectious to humans e.g. BSE in cattle, this waste stream can be disposed of in the normal way (e.g. by landfill burial or spreading on land with waste water being discharged to the sewer system). In the absence of satisfactory evidence, a precautionary approach should be taken, meaning that waste should be incinerated and in the case of the liquid effluent, chemically treated by a recognised method such as sodium hydroxide.

Whilst some of the waste managed at CL1 or CL2 presents a very low risk to humans, infection in waste and body excretions can be infectious to other animals, such as waste from sheep scrapie and CWD, and infectivity is known to persist in the environment. Classical scrapie and CWD are also [notifiable diseases](#) ([Notifiable diseases in animals - GOV.UK \(www.gov.uk\)](#)), therefore unless the waste is going for incineration, measures should be agreed with DEFRA to protect the environment and transmission to other animals.

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## Glossary

<b>Proteopathic seeds, prionoids, prion-like proteins</b>	Misfolded protein assemblies usually found in aggregated states in neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, frontotemporal dementia, motor neuron disease, and that share some pathological properties with prions. Excludes monomeric proteins in their native state.
<b>Prion</b>	The infectious agent of human and animal prion diseases, composed solely or predominantly of misfolded forms of cellular prion protein.
<b>Transmissible Spongiform Encephalopathies</b>	Prion diseases.
<b>Synthetic proteopathic seeds</b>	Proteopathic seeds made from purely recombinant material
<b>Amplified proteopathic seeds</b>	Proteopathic seeds made using methodologies like protein misfolding cyclic amplification (PMCA) or real-time quaking induced conversion (RT-QuIC) that may involve use of substrates that are either synthetic or derived from mammalian tissues
<b>Concentrated proteopathic seeds</b>	Proteopathic seed preparations concentrated from mammalian tissues or biofluid, eg. brain tissue from patients with Alzheimer's diseases. Preparations might be made for any of a number of purposes: structural characterisation, biochemistry, Western blot, or transmission experiments. Techniques for concentration might include filtration, affinity purification or centrifugation (pelleting and resuspension), with the end result of an increased concentration of proteopathic seed relative to the starting material.
<b>Hazard Groups 3 and 3*</b>	Most prion agents are classified as HG3. However, they are in the Approved List of biological agents within HG3 (denoted 3*) identified as presenting a more limited risk of infection for workers because they are not normally infectious by the airborne route. Those working with these agents may not necessarily need to use all the containment measures normally required at CL3, based on a risk

assessment. Dispensing with some of the control measures from CL3 (termed CL3\*) does not imply that the work can be carried out at CL2, it simply allows certain physical containment requirements (particularly those aimed at controlling airborne infection, and the ability to fumigate an area, which are not relevant measures for prions) normally expected at CL3 to be dispensed with. All other aspects of the work, in particular supervision and training, should reflect the high standards expected at CL3.