



Updated guidelines for the conduction of autopsies in cases of suspected Creutzfeldt-Jakob-Disease

Autopsies of patients suffering from prion diseases are intrinsically hazardous and mandate special precautions to minimize the risk of infection. While prions may be less contagious than many other human pathogens, prion infections are inexorably lethal, and neither effective treatment nor any proven post-exposure prophylaxis is currently available (Aguzzi and Polymenidou, 2004). Of further concern is the fact that prions are transmissible by a variety of pathways (Table 1) potentially relevant to the autopsy situation (Bell and Ironside, 1993; Ironside and Bell, 1996) with an incubation time of 20 years or more in humans (Martinez-Lage et al., 1994).

Universal precautions, the basic level of prevention that suffices for most infectious autopsies, are insufficient in the setting of suspected prion disease. Accordingly, it is essential to implement stringent measures to prevent the transmission of prions to personnel performing autopsies and downstream analytical activities, such as histotechnical and biochemical procedures.

Recent heightened institutional concern over biosafety has renewed interest in optimizing the risk management of infectious diseases in the hospital environment, and particularly in anatomical pathology. New procedures for performing autopsies and handling tissue specimens from patients with suspected prion diseases have been introduced that offer more effective protection of medical personnel than those published 16 years ago (Bell and Ironside, 1993; Budka et al., 1995). The following guidelines provide an updated step by step approach to the autopsy of patients with suspected prion diseases.

General Guidelines

Autopsy room requirements

Biosafety Level 3 (BSL3) containment conditions are highly recommended, but rarely available. If a BSL3 environment is unavailable, prion autopsies should be performed under environmental containment conditions of at least BSL2.

Recommended procedures

Measures to minimize contamination of the autopsy suite

- The body should be kept within a body bag lined with absorbent sponges during the autopsy to avoid spillage of body fluids onto the autopsy table.
- Water should never be used for rinsing during the autopsy.
- Disposable instruments should be used for dissection, whenever possible. Alternatively, a set of instruments dedicated for CJD autopsies can be decontaminated after each use.

Personal Protective Equipment

- Personal Protective Equipment worn by persons performing the post-mortem examination should consist of a disposable full body suit (Microgard 2000 Plus),



plastic shoe covers, 3 pairs of gloves (taped to the sleeves of the full body suit), surgical cap, face mask and goggles or face shield. Chainmail gloves worn underneath the latex gloves are required to protect the personnel performing the autopsy from penetrating injuries. A helmet with a transparent face shield provides additional protection from splashes.

Post mortem examination

A limited post mortem examination minimizes the risk of infection. Body cavities should be opened for inspection of the inner organs. Minimal in-situ-dissection should be performed to determine the cause of death and major relevant illnesses.

Brain removal

The head should be opened with a stainless-steel handsaw to avoid aerosol formation. An electrically-powered saw contained within a plastic bag may be used as an alternative. Standard procedure is recommended to remove the brain from the cranium.

- The first step is separate the skull cap from the skull bases by sawing circumferentially through the cranial bone, using the frontal tuberosities and occipital protuberance as anatomical landmarks.
- Incise the temporal dura mater on both sides without removing it from the inner table of the skull cap.
- After tilting the head slightly backwards, gently separate the olfactory bulbs from the skull base, then sever the optic nerves, pituitary stalk, and internal carotid arteries at their entry points into the cranial cavity.
- While using one hand to dissect, gently support the cerebral hemispheres with the other hand to avoid stretching the midbrain.
- Section the tentorium on both sides along the sphenoid bone as far posterior as possible. Identify the vertebral arteries and cut with scissors.
- As the last step, insert a scalpel into the spinal canal through the foramen magnum to transect the cervical spinal cord at the lowest possible point. The brain can now be lifted out of the cranium and placed on a washable plastic board for inspection and initial sampling.

Initial Sampling

The following areas of the central nervous system should be sampled at the time of autopsy, then frozen in liquid nitrogen and stored at -80°C in clearly labelled, leak-proof containers:

- Cortex frontal
- Cortex parietal
- Cortex occipital
- Cerebellum

Tissue from the following body sites should be sampled whenever possible:

- Skeletal muscle
- Lymph node
- Palatine tonsil
- Spleen
- Peripheral nerve



The remaining CNS tissue should be fixed in 4% formalin (without added phenol, which interferes with formic acid used for decontamination) for at least 10 days before further sectioning and processing using the same cautionary measures as described above.

Brain dissection

In order to limit dispersal of contaminated fluids onto work surfaces, dissection should be performed in a shallow tray. After the standard inspection of the cerebral convexities, base of the brain, brainstem, and cerebellum, the brainstem and cerebellum can be separated from the diencephalon and hemispheres by inserting a scalpel into the interpeduncular fossa and cutting through the midbrain transversely. The cerebral hemispheres are sectioned in the coronal plane into slices of approximately 1-2 cm thickness, whereas the brainstem and cerebellum should be sectioned in the transverse plane.

The following areas of the brain should be sampled:

1. Cortex frontal
2. Cortex temporal
3. Cortex parietal
4. Cortex occipital
5. Hippocampus
6. Basal Ganglia
7. Thalamus
8. Mesencephalon
9. Pons
10. Cerebellum
11. Medulla oblongata
12. Cervical Spinal Cord

Tissue samples should be placed into tissue cassettes and stored in specialized, tightly sealed, leak-proof containers with 4% formalin until the time of formic acid treatment. All containers containing potentially infectious material should be clearly labelled as "*hazardous material*".

Decontamination of tissue blocks for histology, processing

Although the exact procedure may vary slightly, formic acid treatment consists of placing small fragments of fixed tissue, no more than 5 mm thick, into 50 to 100 ml of 96 - 98% formic acid for an hour, followed by washing in H₂O for 2 hours, then fixation in fresh formalin for an additional 48hrs. Further processing of the blocks is conducted according to routine histologic techniques.

Caution: the 4% formalin solution in which tissue is fixed is considered as infectious as brain tissue.

All the sequential steps from processing the blocks from formalin into paraffin, sectioning, processing mounted paraffin sections back into aqueous staining solutions, should be carried out manually or in an automatic processor dedicated to TSE tissues. Similarly, it is advisable to dedicate a microtome for sectioning tissue blocks that were not inactivated with formic acid, since there is no practical way to disinfect the instrument.



Formic acid-treated sections can be cut on a standard microtome (if possible, using a disposable knife or dedicated blade) and processed as usual.

Histology slides made from sections which have been treated with formic acid are considered non-infectious.

Decontamination of the autopsy suite, including work surfaces and instruments, and waste disposal

Decontamination of work surfaces and surgical instruments to be re-used can be achieved by any one of the following procedures:

- Porous load steam autoclaving at 134°C for 18 min or 6 cycles at 134°C for 3 min
- Gravity displacement steam autoclaving at 134°C for 60 min
- 2 N NaOH for 1 hour, with constant rewetting of surfaces
- 96% formic acid for 1 hour
- NaClO (sodium hypochlorite) solution containing 20'000 p.p.m. chlorine

Note that formic acid and NaClO solutions are corrosive and may not be suitable for decontamination of metal instruments or surfaces.

The recommended procedure for instrument decontamination performed at the Swiss National Reference Center for Prion Disease consists of placing instruments in 1N NaOH for 2 hours (or 2N NaOH for 1 hour), then rinsing with water and autoclaving at 134°C for 1 hour.

All waste material from the autopsy, brain dissection and histological processing is infectious and should be deposited in biohazard containers that must be incinerated. This applies to solutions used for fixation and processing of tissues (i.e., formalin, xylene) as well as disposable blades used for cutting tissue. Fluids may be absorbed with sawdust before incineration.

Emergency procedures

Once a suspected exposure to prion infected material has occurred, a mandated plan of action should be implemented. The supervisor should be immediately notified and the person should seek medical evaluation to document the event. Although there is no validated prospective study assessing the efficacy of postexposure prophylaxis, recommendations include skin decontamination with 1 mol/L NaOH or 20% sodium hypochlorite followed by copious rinsing. For accidents with penetrating injuries and/or contamination of open skin wounds, one source suggests surgical excision of the site of inoculation, followed by oral prednisolone 60 mg daily for 7 days, then 45 mg daily for a further 7 days accompanied by gastric protection with an H2 antagonist (or a proton pump blocker) and antibiotics according to the nature of the inoculation (Aguzzi and Collinge, 1997). The rationale for this post-exposure prophylaxis treatment lies in the experimental finding that immunosuppression antagonizes the neuroinvasiveness of extraneurally administered prions (Klein et al., 1997).

Summary

Extensive requirements in terms of specialized autopsy facilities must be in place to ensure adequate protection from prion infected tissue.



References

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Table 1: Potential routes of prion transmission during autopsies

Route of transmission	Typical incident	Reference
Parenteral	Knives, needles, sharps	Lancet. 1977 Feb 26;1(8009): 478-9. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. Bernoulli C, Siegfried J, Baumgartner G, Regli F, Rabinowicz T, Gajdusek DC, Gibbs CJ Jr.
Conjunctival	Spray	Vet Microbiol. 1993 Apr;34(4):305-9. Conjunctival instillation of scrapie in mice can produce disease. Scott JR, Foster JD, Fraser H.
Aerosol	Liquid handling	Haybaeck, 2011
Gastroenteric	Food, chewing gum	Aguzzi and Calella, 2009
Intracerebral	Highly efficient, but irrelevant in the autopsy context	