

Guidelines for Prion-like proteins

Introduction

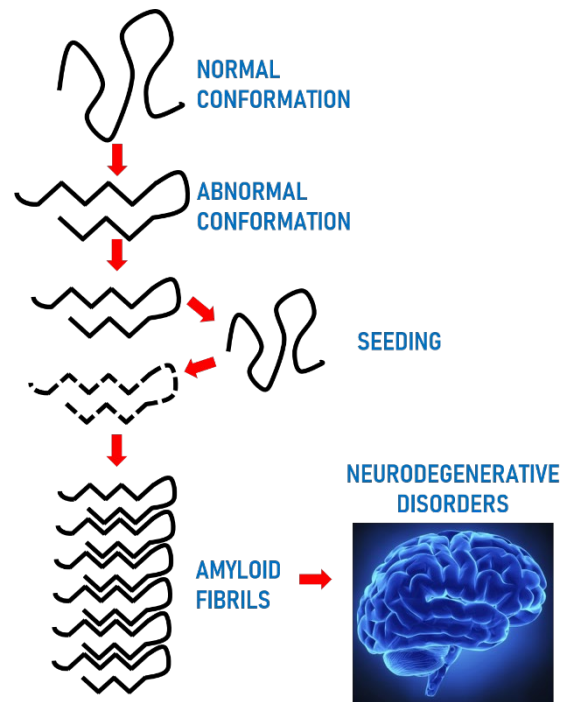
Recent studies in experimental models demonstrate that certain misfolded proteins associated with neurodegenerative diseases can induce misfolding of cognate native proteins and propagate across neural systems, thus displaying some of the properties of prions.

Contrarily to prions, infectivity of these misfolded proteins (named prion-like proteins) has not been demonstrated in humans so far. However, this issue has not been deeply explored and there is a series of critical questions to be addressed to assess the real risk of transmission/ infectivity.

Due to their proteopathic seeding ability, a precautionary approach must be adopted when manipulating prion-like containing samples, and prion-like proteins are classified as **risk group 2** pathogens.

In the context of occupational exposure in laboratory setting, major risks would be accidental parenteral inoculation, and aerosol inhalation. Mucous membrane contamination should also be considered as a potential route of transmission.

Guidelines for biosafety level 2 (BSL-2) environment should thus be followed (see Biosafety Card for BSL2). Additional indications are addressed in this document.

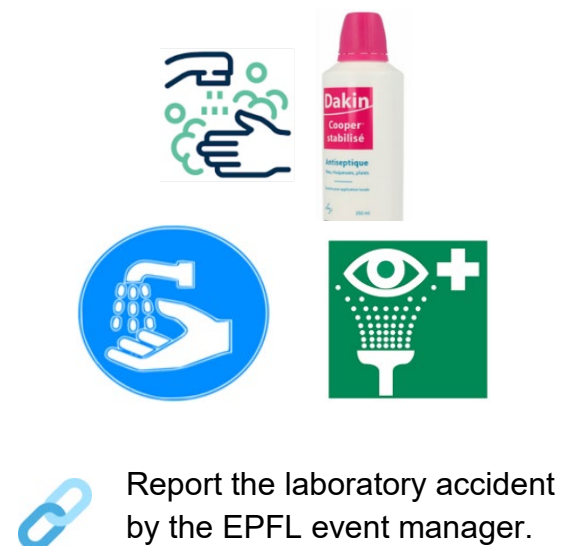


Post exposure management

- Contamination of **unbroken skin** with samples containing prion-like proteins: wash with detergent and abundant quantities of warm water (avoid scrubbing), rinse, and dry. Brief exposure (1 minute) to Dakin's solution (sodium hypochlorite 0.4% - 0.5%) can be considered for maximum safety.

- **Needle sticks or lacerations**: gently wash under running tap water with warm soapy water (avoid scrubbing), rinse, dry. Brief exposure (1 minute) to Dakin's solution (sodium hypochlorite 0.4% - 0.5%) can be considered for maximum safety. Then cover with a waterproof dressing.

- **Splashes into the eyes, mouth, or nose**: irrigate with either saline (eye) or tap water (mouth and nose) for 15-20 minutes.



Sharp Objects

The use of sharp material (e.g. needles) should be minimized. Blunt-ended forceps and needles should be used whenever possible.



Histopathological examination of tissues

Cryostat

Non-fixed samples (group 2 samples) have to be processed with a cryostat which is placed in a BSL-2 laboratory. Since tissue sectioning cannot be performed in a biosafety cabinet, users must wear the standard PPE for a BSL-2 laboratory and an FFP2 mask when cutting not fixed samples.

Fixed samples (group 1) can be processed in a BSL1 laboratory.

The sample should be clamped BEFORE clamping the blade. The blade should be covered with the knife guard and the handwheel locked before changing the sample and prior to any other manipulation and in case of breakdown or malfunction. The blade for the microtome should be disposed after each use.

The internal part of the cryostat will need to be cleaned with an alcohol-based disinfection wipe. All external surfaces potentially contaminated must be treated with a wipe soaked in a solution of Hellmanex III and rinsed just after with an alcohol-based disinfection wipe.

Slides staining and mounting

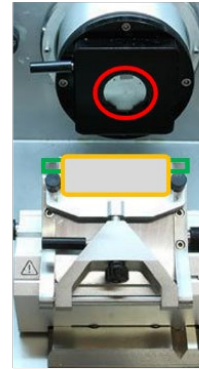
Fixation of slides containing fresh frozen tissues must be performed in a BSL2 laboratory. Once fixed, slides can be manipulated in BSL1 laboratories.

Because of conflicting results between different studies from the literature, the ability of formalin to completely inactivate prion-like proteins aggregates is still debated. Even if the risk is very low, formalin fixed samples should be handled with precautions following good microbiological practices. Operators must: 1) always wear a lab coat, gloves, and safety goggles; 2) decontaminate surfaces at the end of the working session.

Storage

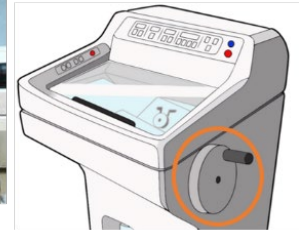
Slides with **non-fixed** tissues must be stored as BSL2 samples (double envelop principle).

Whenever possible slides with fixed tissues should be stored in a sealed container.

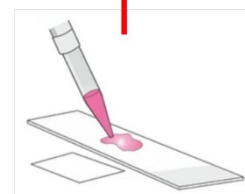
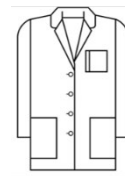


1. CLAMP THE SAMPLE

2. CLAMP THE BLADE



COVER THE BLADE WITH THE KNIFE GUARD AND LOCK THE HAND WHEEL BEFORE MANIPULATIONS



Waste

Material	Treatment	Packaging
Solid (including sharp objects) and semi-solid waste; animal carcasses	No specific inactivation in house, waste is transported to TRIDEL for incineration	30 L or 60 L blue boxes certified UN 3291 with OMoD code 18 01 03
Liquid waste	NaOH 1M	Plastic containers suitable for liquid waste with OMoD code 18 01 06
Surfaces / objects	1% Hellmanex III* (Z805939, Sigma Aldrich), 2% SDS solution *	

*Trash the tissues used to clean the surfaces and objects in a blue box