



## BIOSAFETY IN HANDLING PRIONS FOR DIAGNOSTIC PURPOSES IN ANIMAL HEALTH

NI-LCV-25 Rev. 01

### PURPOSE

The purpose of this document is to develop the minimum biosafety conditions necessary for handling prions for diagnostic purposes in animal health. It is a non-binding document that is issued in the form of recommendations in accordance with current regulations and scientific literature.

The National Reference Laboratory (NRL) for Transmissible Spongiform Encephalopathies (TSEs) together with the Official Animal Health Laboratories authorized by the autonomous communities for the same disease have participated in its preparation:

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## 1 Biological risk characterization

According to the Surveillance, Control and Eradication Programs for Bovine Spongiform Encephalopathy (BSE) and small ruminant spongiform encephalopathies (scrape or scrapie), the types of prions that are handled in the Network of Official Authorized Animal Health Laboratories (RESALAB) for diagnostic purposes are the agents of these two diseases.



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The etiological agent of scrapie is the pathogenic isoform of a prion (PrP<sup>sc</sup>) known in Europe since the 18th century and which affects small domestic ruminants, in other words, sheep and goats. Two forms of this disease are known: classical and atypical scrapie, although today different strains are described that cause classical scrapie. In any case, the handling of this agent must be carried out under type 2 biosafety conditions since it does not represent a danger to people's health. In Spain, as in the rest of the countries around us, the situation is endemic, which is why there is a National Surveillance, Control and Eradication Program.

For its part, the etiological agent of BSE (Wells, 1987), which was first described and is now known as classical BSE, typically affects cattle, although it is known to cause variant Creutzfeldt-Jakob disease (vCJD) in people (Bruce, 1997), described in the United Kingdom for the first time in 1995 and associated with the ingestion of contaminated tissue from cattle. Therefore, given its zoonotic nature and the absence of an effective treatment, the manipulation in the laboratory of tissues that contain or are suspected of containing this agent must be carried out with a biosafety level 3. This level can be relaxed to what is called as a security level 3 derogated considering that the transmission of this agent can only occur by parenteral or digestive route.

Parenteral infection in people has been described at the laboratory level on two occasions due to occupational accidents related to cutting during the handling of samples containing the BSE agent. In both cases, the accidents took place in prion research laboratories in France and on both occasions, after an incubation period between 7 and 17 years, the result was fatal (Brandel, 2020; Science, press release 2021).

Furthermore, in addition to the classical form, cattle can also develop two atypical forms of BSE. Type H-BSE and type L-BSE (Biacabe, 2004; Casalone, 2004), which must also be treated under a derogated biosafety level 3. It is important to note that experimental studies have shown that these two strains are transmitted in wild-type and transgenic mouse models for sheep, cattle and humans, as well as non-human primates (type L), reaching higher lethality than classical BSE ( Beringue, 2008).

In relation to the epidemiological situation of BSE in cattle, it should be mentioned that at the 84th General Session of the OIE World Assembly held in May 2016, the World Organization for Animal Health officially recognized Spain as a country whose risk of BSE is insignificant because since 2014 Spain has not confirmed any case of classical BSE. Since that year, Spain has only confirmed cases of atypical BSE.



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Likewise, classical BSE can also affect sheep and goats, as has been shown experimentally (Foster, 1993), although its real risk is very limited since its description at the field level is anecdotal (European Commission, press release 2005).

### **2 Minimum biosafety conditions for handling prions for diagnostic purposes in animal health**

In general, in Spain, the protection of workers against biological risks is regulated by *Real Decreto 664/1997*, of May 12, on the protection of workers against risks related to exposure to biological agents during work.

This standard establishes the minimum conditions that must be met by facilities with different levels of biocontainment (2, 3 and 4) and classifies biological agents according to the risk they represent (1-4). However, at present, the classification of biological agents is subjected to a risk analysis that takes into account factors specific to the agent (form of presentation, concentration of work), type of activity to be carried out on it, risk presence of the pathogen in the samples handled or characteristics of the facilities where it is to be handled, as stated in the fourth edition of the WHO Laboratory Biosafety Manual (WHO, 2020) and in the 2021 edition of the OIE Manual (OIE, 2021).

#### **2.1 Facilities**

The manipulation of prions for the diagnosis of Scrapie or BSE must be carried out in an exclusive laboratory with a biological safety level 2 or 3, whose differentiating characteristics are included in *Real Decreto 664/1997*.

Access to these facilities will be restricted, and it is necessary to have a record of entries and exits of both authorized personnel and visitors. These records must be kept for a minimum period of 10 years.



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### 2.2 Work clothes and PPE

The handling of samples in a prion laboratory must always be carried out with work clothes for exclusive use and double gloves, changing the outer pair of gloves as many times as necessary in the event of contamination.

#### 2.2.1 Disposable coverall

For the handling of suspicious samples, disposable overalls should be used. This suit can be reused as long as its integrity is intact and there have been no splashes or contaminations of any kind.

Each laboratory will estimate the frequency of change according to the volume of work but, in general, it will be changed weekly unless splashes occur, when it must be discarded immediately.

To reduce the frequency of changing overalls, a waterproof reusable apron can be used, after disinfection with bleach, for cutting samples.

#### 2.2.2 Specific footwear

Specific footwear for prion laboratories should also be available or, failing that, use disposable leggings.

#### 2.2.3 Anti-cut gloves

In the case of using scalpels or other sharp material to cut fresh samples, each laboratory will assess the use of anti-cut gloves to reduce the risk associated with an accident of this type.

The use of these anti-cut gloves will be carried out by placing them between the two pairs of mandatory gloves and their use is especially recommended on the left hand, when the personnel is right-handed, or vice versa. In this way, the hand holding the scalpel is freed and the risk of cutting the hand holding the sample is considerably reduced.



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### 2.2.4 Sleeves

In the same way, each laboratory will assess the use of disposable and waterproof sleeves during the cutting of samples in order to avoid splashes in the region of the wrists that could be exposed.

### 2.2.5 Mask

In order to avoid the risk of exposure due to splashes on the oral mucosa during the analysis of samples, each laboratory will assess the use of masks. Since the risk is associated with splashes and not with inhalation of the agent, surgical-type masks will be sufficient, although the use of another type (FFP2 or FFP3) may be considered.

### 2.2.6 Goggles

Depending on the type of activity and the volume of samples, each laboratory will assess the risk associated with conjunctival exposure due to splashes and may establish the use of protective goggles or screens.

## 2.3 Waste management

In relation to waste management, it must be taken into account that prions are resistant to conventional decontamination methods. Therefore, as defined by the World Health Organization in the WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies (WHO, 2000), the inactivation of prions is effective under the following methods:

- Incineration.
- Reach a final concentration of 20,000 ppm of free chlorine or remain in NaOH 1N for one hour in combination with an autoclave treatment at 121°C for one hour.
- Autoclave treatment with a specific cycle for prions: 134°C for 18 minutes.
- For sensitive reusable instruments, soak in undiluted bleach or 2N NaOH for one hour.



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Taking these data into account, the management of waste generated in TSE diagnostic activities in the field of animal health must be carried out following a method that guarantees the inactivation of prions either at source or at the management company which have contracted this service with.

Consequently, if the management company carries out an effective decontamination method for prions, that is, incineration or autoclaving at, at least, 134°C for 18 min, it is not necessary to carry out any decontamination treatment in the laboratory that produces these wastes, although it is recommended to carry out an on-site decontamination in order to reduce the risk associated with transport.

In the event that the treatment carried out by the waste management company is not effective, it is necessary to autoclave the waste with a specific prion cycle (134°C, 18 min). If the necessary equipment for this operation is not available, decontamination with diluted bleach at a final free chlorine concentration of 20,000 ppm or 1N NaOH for one hour is recommended, which, in combination with the autoclaving treatment that has been verified to perform the management company, would be enough to inactivate prions.

However, in order to reduce the volume of waste to be decontaminated, each laboratory will assess the possibility of segregating the waste generated from positive or negative samples. The waste from the analysis of samples that have been negative may be managed like the rest of the laboratory's biosanitary waste.

### 2.4 Action in case of accidents

TSEs are a set of degenerative diseases, neurological type and fatal course. The seriousness of this type of disease, for which no effective treatment has been described, together with the resistance to conventional decontamination methods, makes it necessary to clearly define the guidelines to follow in the event of accidents during the analysis of suspicious samples, especially those that may contain the BSE agent, of zoonotic nature.



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### 2.4.1 Spills

First of all, in case of spills of considerable volumes (> 5 ml), apply undiluted bleach or 2N NaOH and leave to act for one hour. After that time, remove the liquid using adsorbent material and dry with paper.

### 2.4.2 Splashes

For its part, when splashes occur, if they occur on PPE (personal protective equipment), it is necessary to discard the affected PPE and change it for a new one immediately.

If the splash occurs on any mucosa (oral, nasal or conjunctival), it is recommended to apply running water for at least 10 minutes.

### 2.4.3 Cuts

The occupational accident that presents the greatest risk of prion transmission is cuts, as occurred in the two registered laboratory accidents with a fatal outcome (Brandel, 2020; Science, press release 2021). For this reason, in the event of a cut that causes bleeding, the wound must be bled and immersed in a container of undiluted bleach for at least 10 minutes. In addition, depending on the affected surface, it is convenient to assess a resection around it with the health personnel to avoid a possible expansion of the agent.

## 2.5 Other aspects

Other important aspects to take into account to guarantee a high level of biosafety in the handling of prions in animal health are homogenizers and biological safety cabinets.

### 2.5.1 Homogenizers

Today, the use of closed homogenizers is quite widespread. In this type of homogenizers, the risk of splashes is very low, so it is not considered necessary to use any means of protection additional to those that are generally used. However, if open type homogenizers are used, it is recommended to do so inside a biological safety cabinet and, if considered appropriate and especially if samples with the BSE agent are handled, use protective masks and goggles that limit possible splashes on mucous membranes.





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### 2.5.2 Biological safety cabinets

Lastly, it is advisable to have a type II biological safety cabinet exclusively for cutting samples, as well as periodically planning their in-depth cleaning.

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