Biosafety principles applicable to Rapid Test Laboratories included in the TSE epidemiological surveillance program

Transmissible Spongiform Encephalopathies (TSEs) are neurodegenerative diseases caused by prions with fatal outcome in humans and animals (Table 1). Prions are infectious agents with unconventional characteristics that are particularly resistant to the usual biological inactivation procedures. All forms of TSEs are experimentally transmissible. No effective therapeutic or prophylactic treatments are available.

Tissues infected with TSEs contain an abnormal protein, pathological prion protein (PrP^{RES}) which is derived from an altered three-dimensional structure of cellular prion protein (PrP^{C}). The altered conformation would give PrP^{RES} the infectious and resistance characteristics.

Prions spread in the body reaching relevant titers in peripheral tissues and especially in the central nervous system where they cause the formation of PrP^{RES} and the typical neuropathological lesions, then the onset of symptoms, clinical signs, and death.

Since animal prions (BSE) have been able to infect humans (variant Creutzfeldt-Jakob disease), rapid diagnostic activities of animal TSEs must be conducted in facilities that ensure the protection of human health and the environment. The BSE agent is listed as a Class 3** biological agent in international classifications of pathogenic organisms. European Directive 2000/54/EC (and the subsequent [in the Italian legal system] Legislative Decree no. 81 of April 9th, 2008) considers Class 3** infectious agents to be of limited risk of infection by air for workers. The same Directive considers the agent of Scrapie to be class 2, recommending less stringent containment procedures.

Regardless of the specific classification of prions in risk Class 3** (BSE) or 2 (Scrapie), safety measures should be identified based on a careful risk analysis that takes into account the particular nature of the pathogen, the possible manipulation, the structural, organizational, and procedural characteristics of the testing laboratory. With special reference to the level of containment, the minimum requirements are established by current regulations. In particular, for agents of risk group 3**, in relation to the type of operation carried out and the quantities used, it may be sufficient, in order to implement the measures set out in points 3 and 4 of ANNEX XLVII of Legislative Decree no. 81/2008 as amended by Legislative Decree no. 149 of November 9th, 2020 (“Ristori-bis Decree” – Italian legislation) (Table 2), to ensure the levels of containment provided for agents of group 2.

The main risks related to rapid diagnostic activities for the detection of PrP^{RES} are stab wounds, accidental inoculation and ingestion. Laboratory personnel should strictly adhere to Good Laboratory Practices. Recent experimental studies indicate a possible transmissibility by inhalation of some prion diseases. Therefore, as a precautionary measure, the homogenization of brain tissues (potentially or definitely contaminated) must be performed in sealed systems placed inside suitable biosafety cabinets to contain the possible production of drops and/or aerosols. Table 3 shows the minimum safety requirements for TSE rapid test laboratories.

Cleaning and disinfection procedures as well as waste management assume a strategic importance, considering the resistance of prions to common inactivation treatments.

The possibility of persistence in the laboratory environment of the pathological prion protein is high because of the particular biological properties that make it infectious for a long time in adhesion to metal surfaces and other materials. Although a containment level of 3** combined with strict working and behavioural procedures appear sufficient to reduce

the possibility of environmental spread or exposure to prions, it is necessary, during risk analysis and development of procedures, to take into account the low infectious dose characteristic of TSEs, the impossibility of achieving complete decontamination with standard inactivation methods, and the absence of analytical methods capable of detecting traces of prion protein in the working environment. Where possible, disposable equipment discarded by incineration should be used. Any non-disposable equipment should be dedicated and properly decontaminated after use (see "Decontamination Procedures").

Incineration remains the safest method for disposal of prion-contaminated biological materials and waste.

Decontamination Procedures

Standard chemical and physical inactivation and decontamination treatments cannot ensure complete inactivation of the TSE agent. Infectivity persists after treatment with formalin, autoclaving with standard mode (121 °C for 15 min) and high doses of ionizing and ultraviolet radiation.

Instruments

The best precaution is to use disposable instruments to be disposed of by incineration. If this precaution is not applicable, the instruments should be subjected to one of the decontamination procedures below in descending order of efficiency:

1. Immerse the instruments in a solution of 1N NaOH (40 grams per liter of water)[1] or NaClO with 20,000 ppm free chlorine[2] for at least one hour; remove the instruments from the solution and place them in a gravity-replacement[3] or steam-injection[4] autoclave at 134 °C for at least 30 minutes.
2. Immerse the instruments in a 1N NaOH solution and boil for 5-10 min at atmospheric pressure. Wash the instruments thoroughly in water.
3. Immerse the instruments in a 2N NaOH or NaClO solution with 20,000 ppm free chlorine for at least one hour. Wash the instruments thoroughly in water. This procedure is easily applied during a normal diagnostic routine.

Make sure that the instruments can withstand these treatments by consulting the manufacturer's instructions. Aluminum instruments cannot withstand the NaOH decontamination procedure.

Decontamination of surfaces (worktables and counters, hoods, etc.)

Use 2N NaOH solution (80 grams per liter of water) for at least one hour or, alternatively, a NaClO solution with 20,000 ppm free chlorine for at least one hour. However, it is always advisable to precautionarily protect surfaces with absorbent, impermeable material to limit contamination.

Exposure Management

In case of accidental contact with infected material, wash the affected part thoroughly with warm soapy water, avoiding the use of abrasive substances. In case of a wound, facilitate bleeding and clean the affected part with a 1N NaOH solution and rinse with water.

In case of contamination of the mucous membranes (conjunctival, nasal, oral), wash thoroughly with physiological solution. Such exposures must be formalized and reported to the *Prevention and Protection Service*.

[1]1N NaOH solution at room temperature is caustic but acts relatively slowly and can be removed from skin or clothing with water. Warm NaOH 1N solution is strongly caustic and should not be handled until it has cooled. 1N NaOH solution reacts rapidly with carbon dioxide rendering it inactive. Therefore, it must be prepared at the time of use from either solid NaOH or by diluting a 10N NaOH solution (the latter is not inactivated by carbon dioxide and is therefore stable). NaOH solutions at any temperature should be treated as hazardous chemicals and transported only in closed, leak-proof containers.

[2]Sodium hypochlorite (NaClO – bleach): efficiency depends on the concentration of free chlorine. Commercially available are usually 5.25% bleach solutions that must be diluted 2.5 times (one part bleach plus 1.5 parts water). Bleach in easily inactivated in air and therefore it is important to prepare solutions only at the time of use and from new or hermetically sealed containers. Bleach should be kept away from light. It is advisable to conduct decontamination procedures in a well-ventilated room, as the amount of chlorine released during inactivation can pose a health risk.

[3]In these autoclaves, air is replaced by steam from the base of the chamber. These autoclaves are used for decontamination and sterilization of solutions and instruments.

[4]These autoclaves produce a vacuum before the steam is introduced. They are not suitable for sterilization of liquids.

Table 1. Prion diseases of humans and animals and their probable etiology

Host species	**Disease**	**Probable etiology**
Human	Sporadic Creutzfeldt-Jakob disease Sporadic Fatal Insomnia (extremely rare) Variably protease-sensitive prionopathy	Unknown (spontaneous?)
	Iatrogenic Creutzfeldt-Jakob disease	Medical-surgical procedures
	Variant Creutzfeldt-Jakob disease	BSE agent through consumption of infected foods
	Iatrogenic variant Creutzfeldt-Jakob disease	Transfusion of infected blood. Plasma-derived therapy (?)
	Genetic Creutzfeldt-Jakob disease Gerstmann-Sträussler-Scheinker syndrome Fatal Familial Insomnia PrP Cerebral Amyloid Angiopathy	Genetic (*PRNP* gene mutation)
Sheep and Goat	Scrapie	Infectious (Scrapie agent)
	Atypical Scrapie	Unknown (spontaneous?)
	BSE	Infectious (BSE agent)
Bovine	Transmissible Spongiform Encephalopathy (C-type BSE)	Infectious (food-borne BSE agent)
	Atypical forms (H-type and L-type BSE or BASE)	Unknown (spontaneous?)
Mink	Transmissible Mink Encephalopathy (TME)	Infectious (food-borne with infectious agent of uncertain origin)
Cervids	Chronic Wasting Disease (CWD)	Infectious (food borne CWD agent, contagion, environmental contamination)
Feline	Feline Spongiform Encephalopathy (FSE)	Infectious (BSE agent?)

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Table 2. Containment Measures and Levels*§

Containment Measures	Containment Levels	
	2	**3**
1. The workplace must be separate from any other activity conducted in the same building	No	Recommended
2. The workplace must be sealable to allow for fumigation	No	Recommended
3. Infected material, including any animal, must be handled in secure booths or under conditions of isolation or proper containment	If applicable	Yes, in case of airborne infection
4. Air entering and leaving the workplace must be filtered with a HEPA[(1)] or similar filtration system	No	Yes, for inlet and outlet air
5. Water-proof and easy to clean surfaces	Yes, for counter and floor	Yes, for counter, floor, and other surfaces determined in the evaluation
6. The workplace must be maintained at a negative pressure relative to atmospheric pressure	No	Recommended
7. Surfaces resistant to acids, alkalis, solvents, and disinfectants	Recommended	Yes
8. Access must be restricted to designated operators only	Recommended	Yes
9. Effective vector control, e.g., rodents and insects	Recommended	Yes
10. Specific disinfection procedures	Yes	Yes
11. Safe storage of the biological agent	Yes	Yes
12. Personnel must shower before exiting the containment area	No	Recommended
13. Validated inactivation process for safe disposal of animal carcasses	Recommended	Yes, on-site, or off-site
14. The laboratory must contain its own equipment	No	Recommended
15. Presence of an observation window, or alternative solution, that allows occupants to be seen	Recommended	Recommended

In the table, «*recommended*» means that the measures should be applied in principle unless the results of the risk assessment indicate the opposite.

[(1)]HEPA: High Efficiency Particulate Air filter.

*Legislative Decree no. 81/2008, ANNEX XLVII as amended by Legislative Decree no. 149 of November 9, 2020 – Specifications on containment measures.

[§]For class 3** agents based on the risk analysis, it may be sufficient for the measures in 3 and 4 (in italics) to provide containment levels for group 2 agents.

Table 3. Minimum design requirements, technical features, safety equipment, work practices, and waste management (disposal) required in TSE rapid test laboratories

Design, technical characteristics of laboratories
Laboratory physically separated from other areas of the building or located in stand-alone building. Activities performed exclusively dedicated to the handling of TSEs
Entry via a filter zone 0 a BSL2 laboratory
Automatically locking front door
Furniture suitable for the application of cleaning and disinfection procedures
Presence of inspection window
Presence of hands-free sink
Floor and counters easy to clean, water-proof and resistant to acids, alkalis, organic solvents, disinfectants and chemicals used for decontamination
Presence of telephone or other system of communication with the outside world
Presence of at least one Class I or Class II biological safety cabinet. Handling of potentially infectious aerosol sources performed under a hood with guaranteed and formalized functionality
Work procedures
Personnel properly trained in the specific hazards as well as in safety and protective measures applicable to their work in biocontainment environments 3**
Controlled access restricted to authorized personnel
The following must be displayed on the laboratory entrance door: - biohazard symbol; - containment level; - contact details of the person in charge; - nature of the biohazard; - list of authorized personnel; - procedures required for laboratory access
In the presence of obvious contamination with biological liquids, remove the bag containing the sample holder under biosafety cabinet
Use disposable instruments for cutting the sample in compliance with the specific regulations in force. For other cases follow the indications reported in the paragraph “decontamination procedures”
When operating with sharp tools, wear cut-resistant gloves
Equipment and relative constituent elements dedicated exclusively to TSE analytical activities. Regarding the instruments and/or reference materials necessary to verify the performance of the equipment, it is possible to introduce them into the laboratory either before the start of the work activity or at the end of the – previous – activity, in the latter case only after having carried out the appropriate reclamation and disinfection foreseen. If these procedures are not compatible with the instruments and/or reference materials used, it is advisable for the laboratory to purchase them for dedicated use
In case of storage of infected material, maintain traceability: controlled access to refrigerators, loading and unloading log of material (including quantities). Biohazard symbol applied to refrigerators and freezers dedicated to TSEs
Sample homogenization: under a suitable biological cabinet to avoid operator exposure to aerosols. In case of suspected aerosol production due to crushing tube breakage, wait at least 30 minutes before proceeding with any king of operation on the homogenizer, wear necessary PPE
Incubator and microplate washer: equip the incubating plate with a cover or film. Such covers are to be removed under a biosafety cabinet. Use eye and respiratory protection PPE when placing plate washer waste in the collection container
In case of accidental spillage, use absorbent material and clean up the spillage by wearing appropriate PPE
Procedures to be taken in the event of an incident must be clearly displayed in the laboratory. All incidents must be formalized and reported to the Prevention and Protection Service

Disposal of waste by means of suitable, leak-proof containers, closed, and labeled before leaving the laboratory
Waste sent for incineration through specialized and accredited companies. Waste liquids previously reclaimed by an appropriate and validated method (2N NaOH for at least one hour)
Cleaning and Disinfection
Instructions for the proper use of disinfectants must be available to staff
If cleaning and disinfection operations are entrusted to an external company: provide and formalize a detailed training with specific operating instructions and a period of tutoring concerning the implementation of operation, concentration of products to be used, management of materials dedicated to activities