1st virtual meetings on biosafety
18 june 2024

Priority themes proposed by email

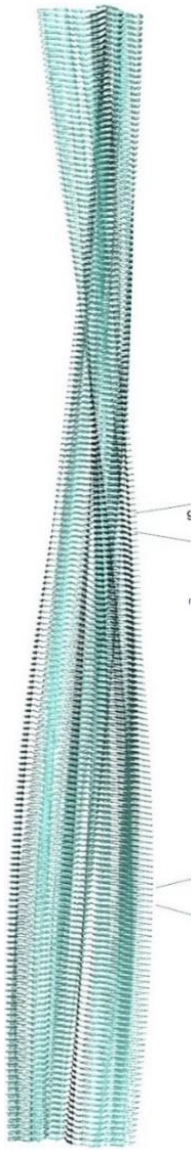
a. Risk assessment and biosafety level;
b. Biosafety issues related to a diagnostic procedure (i.e. ELISA);
c. Decontamination of lab equipment/environment;
d. Waste management and safe transportation of samples;

Identified through discussion with NRLs: **DECONTAMINATION (old equipment)**

Virtual meetings on biosafety

- short (~2 hours)
- informal, to encourage all colleagues to share their problems or solutions
- Presentations/questions for those who want to share their views or problems
- the EURL staff will share own strategies and procedures
- group discussions about the available strategies to tackle the problems that arise
- after each meeting, we will prepare a summary of the discussion and publish it on the EURL website

Intrinsic characteristics of prions



PrP^{Sc} fibril

- Prions accumulate at **very high infectious doses** in the CNS **(10^7-10^9 UI/gr)**
- Unlike viruses and many bacteria, prions have an extraordinary resistance in the environment for a long time (years or decades)
- **Easily contaminate surfaces** (bind to metals, minerals and plastics)
- **Not easily eliminated** through cleaning and washing procedures
- **Difficult decontamination:**
  - methods for viruses and bacteria that are **ineffective,**
    - Resistant to treatment with formalin,
    - Resistant to autoclave run with standard mode (121 ° C for 15 minutes or 134 ° C for 3 minutes)
    - Resistant to high doses of ionizing and ultraviolet rays
  - more **effective** methods (not 100%)
    - long-term exposure (at least 1 hour) to 1-2N NaOH or NaClO solutions with 20,000 ppm of free chlorine
    - treatment in an autoclave at 134 ° C for at least 30 min
  - **Sterilization by incineration**

When decontamination is needed:

- Personnel exposures/accidents
- Lab environment (work surfaces)
- Waste liquid and solid residues
- **Material/equipment to be re-used (non-clinical settings)**
- **Material/equipment to be disposed of**

Although most of us have procedures in place to decontaminate equipment, there still seems to be a problem with equipment being discarded.

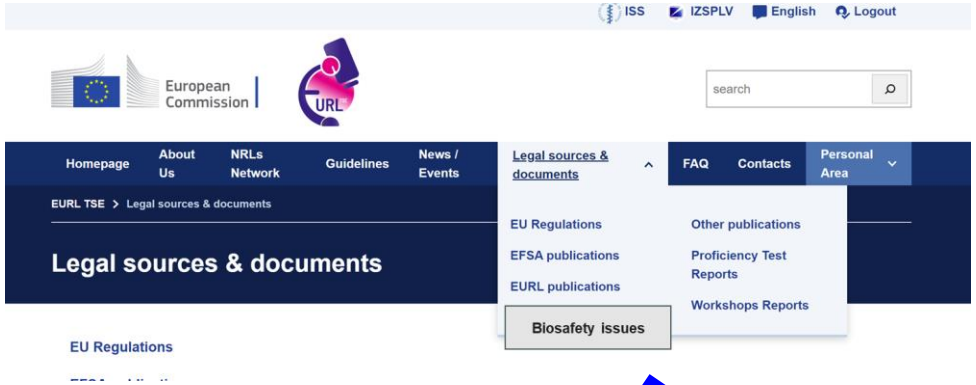
Could it depend on the type of equipment?

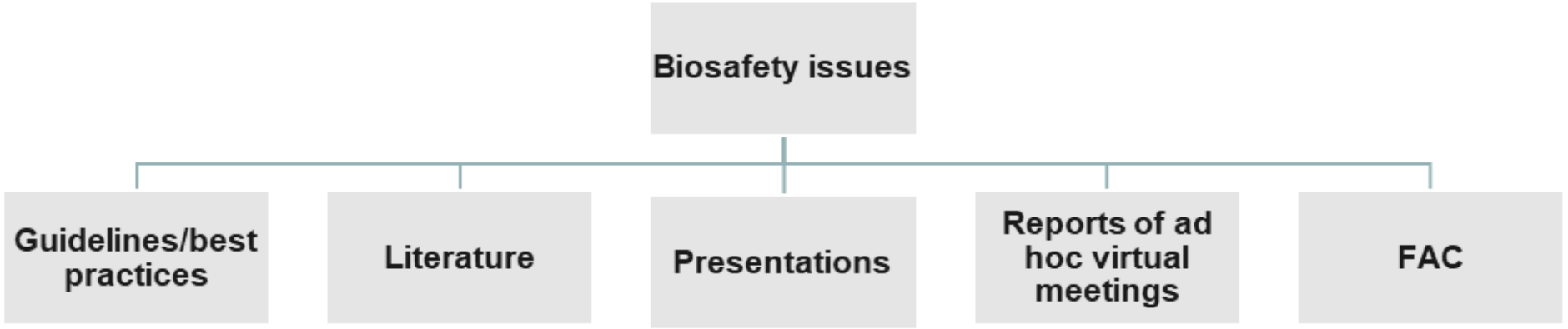
- Small equipment (pipettes, blotting tanks, thermomixers, balances..)

vs

- Large equipment (freezers, cabinets, homogenizers, microtomes..)

INTERNATIONAL GUIDELINES





https://www.eurl-tse.eu/biosafety-issues/

WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies

Report of a WHO Consultation - Geneva, Switzerland, 23 –26 March 1999

Section 6. DECONTAMINATION PROCEDURES

6.1 General considerations

- TSE agents are unusually resistant to disinfection and sterilization by most of the physical and chemical methods in common use for decontamination of infectious pathogens.
- Table 8 lists a number of commonly used chemicals and processes that cannot be depended upon for decontamination, as they have been shown to be either ineffective or only partially effective in destroying TSE infectivity.

WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies

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Table 8 Ineffective or sub-optimal disinfectants

Chemical disinfectants	Gaseous disinfectants	Physical processes
Ineffective[17] alcohol ammonia ß-propiolactone formalin hydrochloric acid hydrogen peroxide peracetic acid phenolics sodium dodecyl sulfate (SDS) (5%)	Ineffective ethylene oxide formaldehyde	Ineffective boiling dry heat (<300°C) ionising, UV or microwave radiation
Variably or partially effective chlorine dioxide glutaraldehyde guanidinium thiocyanate (4 M) iodophores sodium dichloro-isocyanurate sodium metaperiodate urea (6 M)		Variably or partially effective autoclaving at 121°C for 15 minutes boiling in 3% sodium dodecyl sulfate (SDS)

WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies

Report of a WHO Consultation - Geneva, Switzerland, 23 –26 March 1999

Annex III
Decontamination methods for Transmissible Spongiform Encephalopathies

- The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on contaminated instruments and other materials is to discard and destroy them **by incineration**.
- **In some healthcare situations**, as described in the guidance, one of the following less effective methods may be preferred. Wherever possible, instruments and other materials subject to **re-use** should be kept moist between the time of exposure to infectious materials and subsequent decontamination and cleaning. If it can be done safely, removal of adherent particles through mechanical cleaning will enhance the decontamination process.
- The following recommendations are based on the best available evidence at this time and are listed in order of more to less severe treatments. These recommendations may require revision if new data become available.

Annex III
Decontamination methods for Transmissible Spongiform Encephalopathies

All these solutions are useful for small equipment. But the equipment that is too large for incineration is often also too large for immersion and autoclaving….

1. Incineration

1. Use for all disposable instruments, materials, and wastes.
2. Preferred method for all instruments exposed to high infectivity tissues.

2. Autoclave/chemical methods for heat-resistant instruments

1. Immerse in sodium hydroxide (NaOH)[20] and heat in a gravity displacement autoclave at 121°C for 30 min; clean; rinse in water and subject to routine sterilization.
2. Immerse in NaOH or sodium hypochlorite[21] for 1 hr; transfer instruments to water; heat in a gravity displacement autoclave at 121°C for 1 hr; clean and subject to routine sterilization.
3. Immerse in NaOH or sodium hypochlorite for 1 hr.; remove and rinse in water, then transfer to open pan and heat in a gravity displacement (121°C) or porous load (134°C) autoclave for 1 hr.; clean and subject to routine sterilization.
4. Immerse in NaOH and boil for 10 min at atmospheric pressure; clean, rinse in water and subject to routine sterilization.
5. Immerse in sodium hypochlorite (preferred) or NaOH (alternative) at ambient temperature for 1 hr; clean; rinse in water and subject to routine sterilization.
6. Autoclave at 134°C for 18 minutes.[22]

Annex III
Decontamination methods for Transmissible Spongiform Encephalopathies

3. Chemical methods for surfaces and heat sensitive instruments

1. Flood with 2N NaOH or undiluted sodium hypochlorite; let stand for 1 hr.; mop up and rinse with water.
2. Where surfaces cannot tolerate NaOH or hypochlorite, thorough cleaning will remove most infectivity by dilution and some additional benefit may be derived from the use of one or another of the partially effective methods listed in Section 5.1 (Table 8).

4. Autoclave/chemical methods for dry goods

1. Small dry goods that can withstand either NaOH or sodium hypochlorite should first be immersed in one or the other solution (as described above) and then heated in a porous load autoclave at ≥ 121°C for 1 hr.
2. Bulky dry goods or dry goods of any size that cannot withstand exposure to NaOH or sodium hypochlorite should be heated in a porous load autoclave at 134°C for 1 hr.

Annex III
Decontamination methods for Transmissible Spongiform Encephalopathies

5. Notes about autoclaving and chemicals

Gravity displacement autoclaves: Air is displaced by steam through a port in the bottom of the chamber. Gravity displacement autoclaves are designed for general decontamination and sterilization of solutions and instruments.

Porous load autoclaves: Air is exhausted by vacuum and replaced by steam. Porous load autoclaves are optimized for sterilization of clean instruments, gowns, drapes, towelling, and other dry materials required for surgery. They are not suitable for liquid sterilization.

Sodium Hydroxide (NaOH, or soda lye): Be familiar with and observe safety guidelines for working with NaOH. 1N NaOH is a solution of 40 g NaOH in 1 litre of water. 1 N NaOH readily reacts with CO_2 in air to form carbonates that neutralize NaOH and diminish its disinfective properties. 10 N NaOH solutions do not absorb CO_2, therefore, 1N NaOH working solutions should be prepared fresh for each use either from solid NaOH pellets, or by dilution of 10 N NaOH stock solutions.

Sodium hypochlorite (NaOCl solution, or bleach): Be familiar with and observe safety guidelines for working with sodium hypochlorite. Household or industrial strength bleach is sold at different concentrations in different countries, so that a standard dilution cannot be specified. Efficacy depends upon the concentration of available chlorine and should be 20 000 ppm available chlorine. One common commercial formulation is 5.25% bleach, which contains 25 000 ppm chlorine. Therefore. undiluted commercial bleach can be safely used. If solid precursors of hypochloric acid is available, than stock solution and working solutions can be prepared fresh for each use.

WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies

Report of a WHO Consultation - Geneva, Switzerland, 23 –26 March 1999

6.5 Personal protection during decontamination procedures

- Persons involved in the disinfection and decontamination of instruments or surfaces exposed to the tissues of persons with TSE should wear single-use protective clothing, gloves, mask and visor or goggles, as noted in Section 5.1, Table 6.
- All individuals involved with disinfection and decontamination procedures should be familiar with these basic protective measures and precautions. Handling of contaminated instruments during transfers and cleaning should be kept to a minimum.

Annex III
Decontamination methods for Transmissible Spongiform Encephalopathies

6. Cautions regarding hazardous materials

In all cases, hazardous materials guidelines must be consulted.

1. Personnel

NaOH is caustic but relatively slow acting at room temperature, and can be removed from skin or clothing by thorough rinsing with water. Hot NaOH is aggressively caustic, and should not be handled until cool. The hazard posed by hot NaOH explains the need to limit boiling to 10 minutes, the shortest time known to be effective.
Hypochlorite solutions continuously evolve chlorine and so must be kept tightly sealed and away from light. The amount of chlorine released during inactivation may be sufficient to create a potential respiratory hazard unless the process is carried out in a well-ventilated or isolated location.

2. Material

In principle, NaOH does not corrode stainless steel, but in practice some formulations of stainless steel can be damaged (including some used for surgical instruments). It is advisable to test a sample or consult with the manufacturer before dedicating a large number of instruments to decontamination procedures. NaOH is known to be corrosive to glass and aluminum. Hypochlorite does not corrode glass or aluminum and has also been shown to be an effective sterilizing agent; it is, however, corrosive both to stainless steel and to autoclaves and (unlike NaOH) cannot be used as an instrument bath in the autoclave. If

WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies

Report of a WHO Consultation - Geneva, Switzerland, 23 –26 March 1999

- **6.3 Decontamination of work surfaces**
- Because TSE infectivity persists for long periods on work surfaces, it is important to use disposable cover sheets whenever possible to avoid environmental contamination, even though transmission to humans has never been recognized to have occurred from environmental exposure. It is also important to mechanically clean and disinfect equipment and surfaces that are subject to potential contamination, to prevent environmental build-ups. Surfaces contaminated by TSE agents can be disinfected by flooding, for one hour, with NaOH or sodium hypochlorite, followed by water rinses (see Annex III for detailed instructions). Surfaces that cannot be treated in this manner should be thoroughly cleaned; consider use of a partially effective method as listed in Table 8.
- Cleaning materials treated as potentially contaminated (see Section 6.4).

Advisory Committee on Dangerous Pathogens (ACDP)

Health and Safety Executive (UK)

Part 3 – updated 2021

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 ≥134°C for ≥20 minutes (7).

Exposure to high concentrations of ionic detergents in aqueous solution at elevated temperature, eg >2% w/v SDS at >45°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]

Prion and Prion-like Protein Guidance

Inactivation of prions

Most effective treatments include; incineration, enzymatic treatments with SDS, vaporized hydrogen peroxide, 4% SDS in 1% acetic acid at 65-134 degrees C, or mildly acidic hypochlorous acid[14].

Disposable instruments, materials & waste	Incineration
Biological Safety Cabinet	1 N NaOH or 50% v/v of 5.25% sodium hypochlorite bleach, and rinsed with water
Reusable instruments and surfaces	1 N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for 1 hour followed by water rinse. 1 N NaOH equals 40 grams of NaOH per liter of water. Solution should be prepared daily
Routine staining	Slides are decontaminated by soaking them for 10–60 min in 2 N NaOH or sodium hypochlorite (20,000 ppm) followed by distilled water
Fixation of small tissue samples	96% absolute formic acid for 30 minutes, followed by 45 hours in fresh 10% formalin
Formalin-fixed and paraffin-embedded tissues remain infectious	Immerse for 30 minutes in 96% absolute formic acid or phenol before histopathologic processing

Italian guidelines (Ministry of Health)

TSE diagnosis: «*Principles of biosafety applicable to Rapid Test laboratories involved in the epidemiological surveillance program of transmissible spongiform encephalopathies. Guidelines update* ».

Nota 0006558-15/03/2021-DGSAF-MDS-P

- REGULATORY UPDATES
- **DECONTAMINATION PROCEDURES (INSTRUMENTS and SURFACES)**
- EXPOSURE MANAGEMENT
- DESIGN and TECHNICAL CHARACTERISTICS of the LABORATORIES
- WORKING PROCEDURES
- CLEANING and DISINFECTION

Italian guidelines (Ministry of Health)

Equipment

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 for at least one hour; remove the instruments from the solution and place them in a gravity-replacement or steam-injection 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.

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

- How to decide which to use?
  - Feasibility
  - Risk assessment

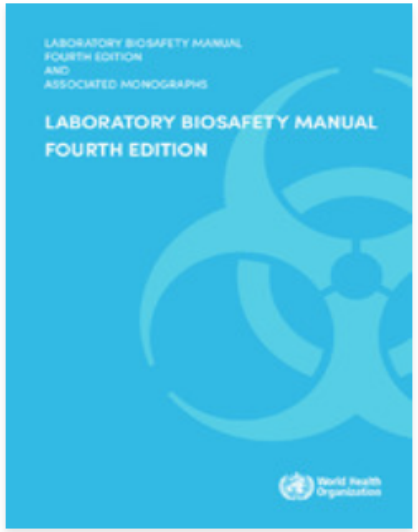
World Health Organization

Health Topics | Countries | Newsroom | Emergencies | Data

Home / Publications / Overview / Laboratory biosafety manual, 4th edition

Laboratory biosafety manual, 4th edition

21 December 2020 | Manual



Download (3 MB)

Overview

The WHO Laboratory Biosafety Manual (LBM) has been in broad use at all levels of clinical and public health laboratories, and other biomedical sectors globally, serving as a de facto global standard that presents best practices and sets trends in biosafety.

LBM encouraged countries to accept and implement basic concepts in biological safety and to develop national codes of practice for the safe handling of biological agents in laboratories within their geographical borders.

This fourth edition of the manual builds on the risk assessment framework introduced in the third edition. A thorough, evidence-based and transparent assessment of the risks allows safety measures to be balanced with the actual risk of working with biological agents on a case-by-case basis.

This novel evidence- and risk-based approach will allow optimised resource use and sustainable laboratory biosafety and biosecurity policies and practices that are relevant to their individual circumstances and priorities, enabling equitable access to clinical and public health laboratory tests and biomedical research opportunities without compromising safety.

https://www.who.int/publications/i/item/9789240011311

Risk assessment

Composition of the manual: how to use LBM4

LBM4 suite consists of the following:

- LBM4 core document
- Subject-specific monographs
  - Risk assessment
  - Laboratory design and maintenance
  - Biological safety cabinets and other primary containment devices
  - Personal protective equipment
  - Decontamination and waste management
  - Biosafety programme management
  - Outbreak preparedness and resilience

Readers may wish to start with reading through the LBM4 core document that provides general remarks and overarching concepts that are essential to understand the evidence- and risk-based approach. The risk assessment monograph may be of particular help, especially for those who are not familiar with it, given that a proper risk assessment should always be performed before undertaking any activities and inform risk control measures.

Other monographs were developed in order to accommodate diverse interests and requests for learning more specific details, supplementing the core document. Readers are encouraged to learn the subject explained in each monograph accordingly.

https://www.who.int/publications/i/item/9789240011311

Risk assessment in laboratories handling prions

ACDP guidelines (UK)

Factors that need to be considered in the biosecurity risk assessment include:

- ✓ The type of processing;
- ✓ **The quantity and type of material to be handled;**
- ✓ The procedures and equipment in place, evaluating the potential of:
  - risk of injury
  - dispersion of the agent
  - contamination of personnel
  - contamination of instruments and surfaces
  - adhesion of prions to metals (consider disposable)

Hazard groupings for animal and laboratory prion strains
(based on zoonotic risk being known/suspected, or still uncharacterised)

Animal prion diseases	HG
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*

Laboratory strains of prion diseases	HG
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*

Sensitive questions to answer:

- How much high risk material has been handled?
- How much Class 3 high risk material has been handled?
- Is equipment routinely cleaned and decontaminated?
- Are there records of incidents (e.g. loss of free material in a freezer)?
- …….

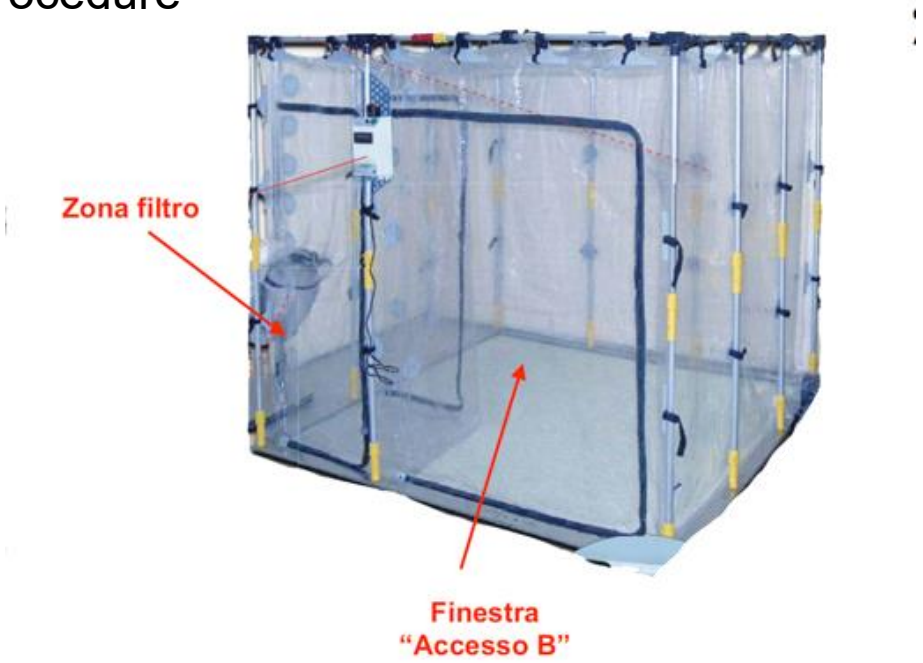
https://www.gov.uk/government/publications/guidance-from-the-acdp-tse-risk-management-subgroup-formerly-tse-working-group

In summary, what we do at EURL (large quantity of high-risk tissues, class 3 agents…..):

	ISS	IZSPLV
Lab solid waste	Incineration	Incineration
Lab liquid waste	NaClO solution with 20,000 ppm of free chlorine for at least 1 hr and then incineration	1N NaOH for at least 2 hr and then incineration
Surfaces and environment	Pre-cleaning (water + detergents); 2N NaOH for at least 1 hr (using soaked paper towels); rinse with water	Pre-cleaning, then NaClO (20,000 ppm of free chlorine), let stand for 1 hour; clean and rinse with water
Equipment for repair	On site repair	Technical service performed in laboratory. If not possible, agreement with companies: cleaning with NaClO (20,000 ppm of free chlorine) for 1 hr, clean and rinse with water. Packaging and pickup by the technical expert service
Small equipment to be disposed of	Incineration	Incineration, or Packaging and pickup by the supplier company (in this case: NaClO 20,000 ppm of free chlorine for 1 hr, clean and rinse with water). This facility is part of our agreement with companies in the equipment purchase contract
Large equipment to be disposed of	Pre-cleaning; 2N NaOH for at least 1-2 hr using soaked paper towels; rinse with water (treatment of all accessible parts)	Pre-cleaning; NaClO (20,000 ppm of free chlorine for 1 hr); clean and rinse with water (treatment of all accessible parts)
Special cases (heavily contaminated equipment..)	See next slide	

“Special” procedure for heavily contaminated equipment (ISS)

- Intended to substitute “immersion” in decontaminating solutions with “spraying” for equipment that cannot undergo incineration
- In the BSL3 lab, the equipment is pre-cleaned, partially disassembled when possible, wrapped up with plastic film and put in a polypropylene bag
- Then it is moved to a biosafety plastic structure to allow to spray chemicals (2N NaOH) with a pump so to reach any part of the equipment for the desired time (1 hr) (chemical risk to prevent during spraying!)
- Very complex, impractical, time-consuming and costly (we did it only once for old safety cabinets, fridges and freezers): not a “good” procedure



Prion 7:5, 420–433; September/October 2013; © 2013 Landes Bioscience

Laboratory activities involving transmissible spongiform encephalopathy causing agents

Risk assessment and biosafety recommendations in Belgium

Amaya Leunda[1,*], Bernadette Van Vaerenbergh[1], Aline Baldo[1], Stefan Roels[2], and Philippe Herman[1]

[1]Biosafety and Biotechnology Unit; Institut Scientifique de Santé Publique; Brussels, Belgium; [2]Orientation and Veterinary Support; National Reference Laboratory for TSE (Belgium & Luxemburg); Veterinary and Agrochemical Research Center; Brussels, Belgium

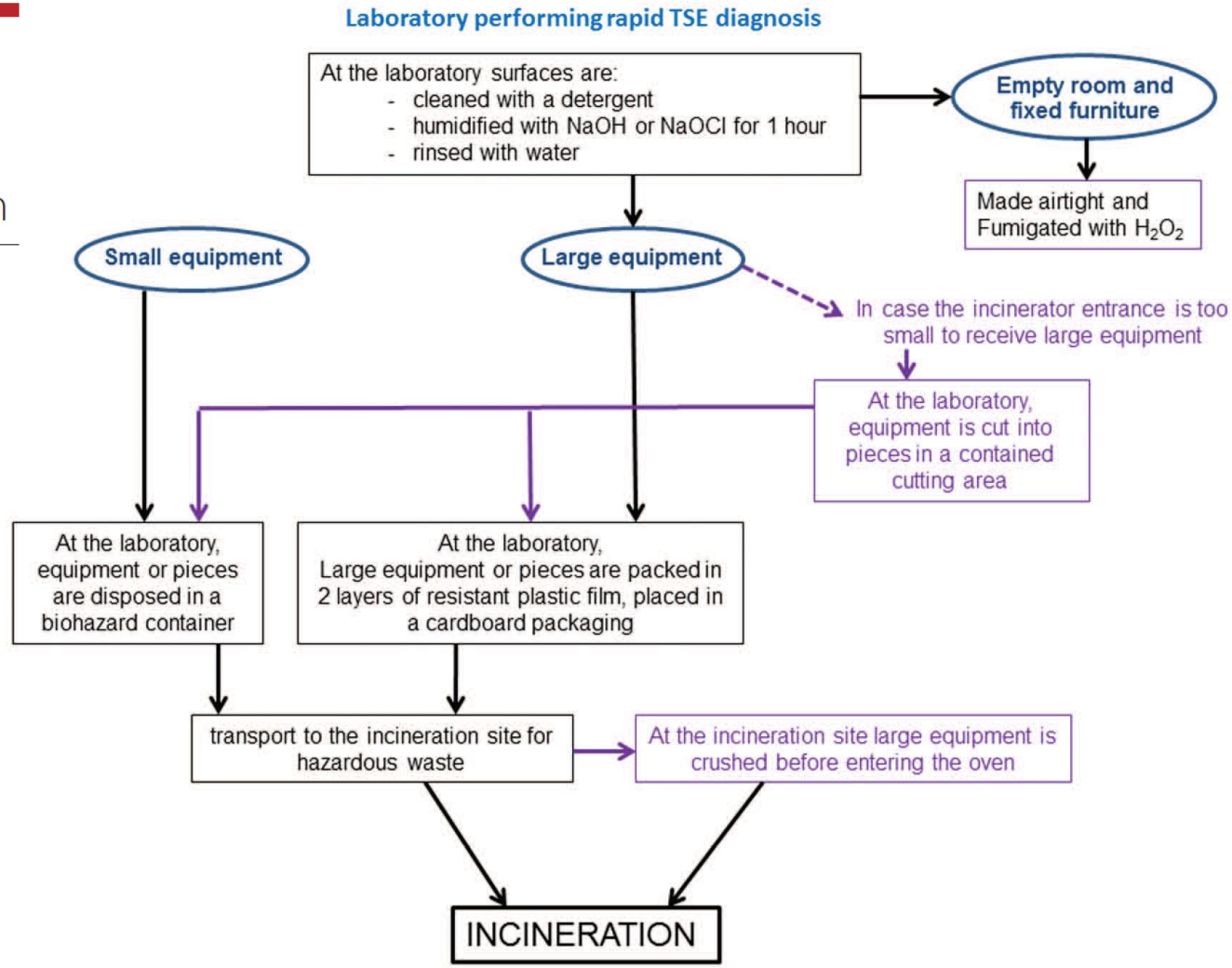


Figure 1. Procedure for decontamination and dismantlement of laboratories performing rapid detection of Transmissible Spongiform Encephalopathy. Dismantlement starts with equipment and furniture in the room and finishes with decontamination of the emptied room. Small equipment is disposed in a biohazard container without prior decontamination and transported to the incinerator. Large equipment, walls, floor and fixed furniture are cleaned with a detergent and then vaporized (humidified) with NaOH 2N or NaOCl 20 000 ppm for one hour. Surfaces are then rinsed with water. Large equipment is packed in 2 layers of resistant plastic film, placed in cardboard packaging and transported to the incinerator. The room is made airtight and fumigated with hydrogen peroxide. In case of "small" incinerator oven entrance, two solutions are proposed: large equipment is cut in the laboratory into pieces small enough to enter the oven, or large equipment is crushed just before entering the oven at the incineration site. Small and large equipment are incinerated in a specialized incinerator for hazardous waste.

Lucien van Keulen, NRL the Netherlands

….I do have a proposal for a specific topic: during the latest Iberian prion conference in May, I ran into a poster which described vaporized hydrogen peroxide (VHP) decontamination of prions using equipment from the following supplier:

https://devea-environnement.com/en/product/phileas-genius/

it would be nice to know if any NRL lab in Europe has experience with this kind of apparatus/ VHP decontamination and if so if they would be willing to share their experience …….

https://devea-environnement.com/en/product/phileas-genius/

https://devea-environnement.com/en/our-areas-of-activity/research-laboratory/

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Disinfection is thus a crucial process in **research laboratories**. Airborne Surface Disinfection (ASD) is a terminal disinfection procedure. The purpose of ASD in research laboratories is to protect your work environment and all of your employees.

5. Prion-induced seeding is inactivated by incineration, but not vaporous hydrogen peroxide

Allen Herbst[1], Harrison Lamb[1], Emily Smith[2], Grace Hareid[2], Lauren Gritzmacher[2], Hannah Faber[2]

[1]*U.S. Geological Survey, National Wildlife Health Center, Madison, WI 53711, USA.*
[2]*Akima Systems Engineering, Herndon, VA, 20171, USA, Contractor to the U.S. Geological Survey.*

Prions are widely regarded as the most resistant pathogen to chemical and physical inactivation. The aim of this work was to evaluate the capability of the real-time quaking-induced conversion (RT-QuIC) assay to monitor chronic wasting disease (CWD) prion inactivation after combustion in an incinerator or after exposure to vaporous hydrogen peroxide (VHP). Both incineration and VHP-exposure are recognized as effective for prion disinfection. White-tailed deer heads were tested for CWD, and batches of negative or positive heads were incinerated at a primary combustion temperature of about 1400°F. The resultant incinerator ash from each batch was collected and assayed by RT-QuIC. CWD positive brain tissue homogenate was dried onto plastic surfaces and treated in an encapsulated biological safety cabinet with vaporized hydrogen peroxide generated using a spinning disk. The seeding activity of the CWD-contaminated VHP-treated surfaces was compared to replica plated controls that were not exposed. Similarly, residual prion protein immunoreactivity from the CWD-contaminated VHP-treated surfaces was measured by western blotting and compared with replica plated non-exposed controls.

We were unable to detect seeding activity in incinerator ash samples generated by the combustion of either CWD+ or CWD- deer heads. By contrast, when ash from CWD- heads was spiked with CWD+ brain homogenate, we were able to detect seeding down to 10^-6 dilution.

VHP treatment was ineffective at destroying RT-QuIC seeding activity. Seeding was observed in VHP treated wells with even low concentrations of CWD prions. Western blotting data indicate that VHP treatment sensitized CWD prions to proteinase K. Incineration is effective at decontaminating and disinfecting prions, and RT-QuIC is a suitable method to evaluate residual CWD contamination in incinerator ash. Published bioassay data indicate that VHP treatment is effective at inactivating prions, and this is confirmed by our western blot data. VHP treatment is effective at disinfecting prions, but not decontaminating prions.

Severine Matthijs, NRL Belgium

1. In the EURL or in other labs, is all biological material from the lab first autoclaved before it leaves the lab?
   - **NO (autoclaving can be done before disposing of for incineration)**
2. Or is it disposed of in yellow biohazard bins (to be burned)?
   - **YES (In our labs all waste and biological material is discarded in biohazard bins and sent for incineration)**
3. What about tissues in formaldehyde or certain buffers from the ELISA kits?
   - **Separate liquid from solid and send both to incineration. The buffers of ELISA kits (often very small volumes – e.g. 1-2 ml) are discarded in the vials and sent for incineration**
4. How is chemical waste treated in the EURL (and empty bottles of chemical products) that cannot be autoclaved?
   - **Incineration (unless bottles were not in contact with biological samples or contaminated surfaces)**
5. What is done with old equipment from the BSE lab (laminar flows/safety cabinets, fridges and freezers, ELISA machines)?
   - **Ideally, they should be incinerated, but this is not always feasible. Refrigerators and freezers can be decontaminated by spraying all accessible parts (inside and outside) with NaClO or NaOH solutions, leaving for at least 1-2 hours and then rinsing with water. The same procedure is used for cabinets, but the filters are treated beforehand by fumigation, carried out by specialised companies.**
   - **Avoid reuse**
6. Does the EURL or other labs sometimes disinfect the lab rooms with fumigation?
   - **NO, but see previous slides**

Kristina Tekavec, NRL Slovenia

1. Is it possible to decontaminate the old equipment or equipment for tests that we no longer perform to such an extent that it can be used in another laboratory?
    - **In principle, it should be avoided. But it depends a lot on the equipment, the type (class 2 *vs* 3) and the degree of contamination: risk assessment! (e.g. homogenizer *vs* plate reader)**
2. Initially, all work with prions was carried out in a small laboratory that was specifically used for only this purpose. Due to the need for a greater capacity, the main laboratory was then relocated. The small laboratory remained as a backup, which is still quite well equipped and is used for the preparation of suspect samples for histology before decontamination with formic acid. As the small laboratory is rarely needed (recently we have had one atypical scrapie case per year) and is conveniently located next to the necropsy room, our superiors would like us to clean and decontaminate it, since our institute is short of space and we would like to use it for other purposes. We would be grateful if you could share your expertise on the matter and advise us on how we could decontaminate the small laboratory appropriately.
    - **In principle, prion labs should be exclusively dedicated to TSE activities**
    - **Risk assessment (type of material, spills/incidents…)**
    - **If permitted, consider: removal of highly contaminated material; decontamination of the environment; establishment of procedures for spatial and temporal separation of different activities and equipment; information and training of personnel; decontamination procedures (effective for prions)**
3. We still keep some tissues of positive cases in formalin in the small laboratory (some are more than 20 years old). Does this material still pose a safety risk, should we decontaminate it before sending it for destruction (I assume yes), and how can we decontaminate it?
    - **Formalin is ineffective against prions. Decontamination is needed (separate liquid from solid and send both to incineration)**

The plan is to have periodical virtual meetings on biosafety

Also depends on how useful and effective NRLs find them

Suggestions for the organisation of future meetings?

Suggestions for future topics?