PRION BIOSAFETY: THE EURL APPROACH

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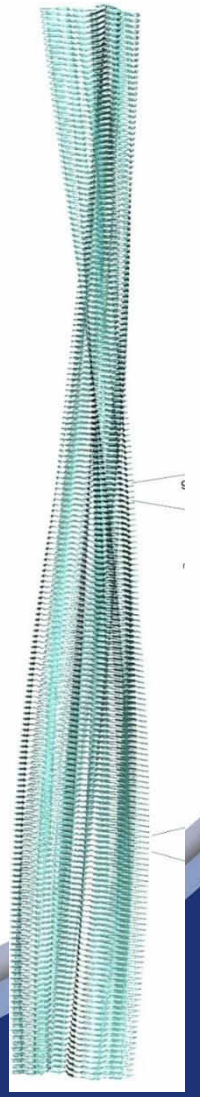
19th Annual Meeting of the TSE EURL Torino, Italy - 18th October 2022

General characteristics of Transmissible Spongiform Encephalopathies

- TSEs or prion diseases are a group of rare, invariably **progressive and fatal** neurodegenerative diseases, which affect humans and animals
- **Long course** (years or decades) and asymptomatic / silent
- They progress rapidly (months) after symptoms appear
- **Caused by prions,** pathogens **that differ from common infectious disease agents**
- TSEs are **unique in medicine** as they exist as sporadic / idiopathic, familial / hereditary or acquired forms, but **they are all transmissible**

Intrinsic characteristics of prions

- They accumulate at **very high infectious doses** in the CNS **(10^7-10^9 UI/gr)**
- **Easily contaminate surfaces** (they 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%)ç
    - long-term exposure (at least 1 hour) to 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**
- Unlike viruses and many bacteria, prions have an extraordinary resistance in the environment for a long time (years or decades)

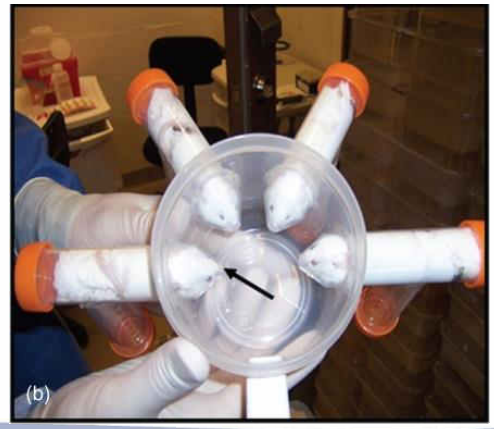


Transmission

Prions are not considered easily transmissible agents, particularly in humans
In laboratory, transmissibility is limited to specific ways of contact with infected material:

- Cuts and punctures
- Contact with not-intact skin
- Contact with mucous membranes
- Ingestion

<u>The airway has to be considered in the presence procedures that involve aerosolization or vigorous disruption of the material</u>



Denkers et al., J Gen Virol. 2010, doi:10.1099/vir.0.017335-0

1° case (?), 2016

Why is it important?

Registri e Sorveglianze

Registro nazionale della malattia di Creutzfeldt-Jakob e sindromi correlate

MALATTIA DI CREUTZFELDT-JAKOB (MCJ) E SINDROMI CORRELATE IN ITALIA

(aggiornata al 31 gennaio 2021)

SEGNALAZIONI CASI SOSPETTI		DECESSI CASI CON DIAGNOSI CERTA O PROBABILE DI MCJ							
Anno	*Segnalazioni*	*Anno*	*MCJ sporadica*	*Forme genetiche*			*MCJ iatrogena[3]*	*MCJ variante[4]*	***Totale (decessi)***
				MCJ genetica	*GSS[1]*	*FFI[2]*			
1993	51	1993	27	6	1	2	0	0	**36**
1994	62	1994	33	7	0	1	0	0	**41**
2015	280	2015	123	31	6	0	0	0	**160**
2016	244	2016	108	22	2	0	0	1	**133**
2017	305	2017	147	24	1	1	1	0	**174**
2018	295	2018	138	19	2	1	0	0	**159**
2019	306	2019	85	14	0	1	0	0	**100**
2020	275	2020	61	9	3	0	1	0	**74**
2021	15	2021	1	0	0	0	0	0	**1**
Totale (segnalazioni)	**5636**	***Totale (Casi)***	**2442**	**470**	**62**	**18**	**11**	**3**	**3006**

World Health Organization

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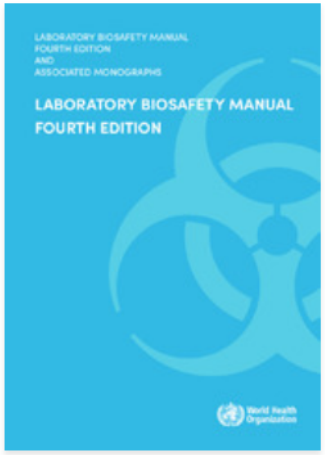
Home / Publications / Overview / Laboratory biosafety manual, 4th edition

Laboratory biosafety manual, 4th edition

21 December 2020 | Manual



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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

LABORATORY BIOSAFETY MANUAL
FOURTH EDITION
AND
ASSOCIATED MONOGRAPHS

World Health Organization

RISK ASSESSMENT

Hazard: pathogen able of causing disease

Risk: combination of the chance or probability of an accident occurring and the severity of its consequences

The risk depends on many dynamic factors (type of processing, available equipment, agent endemicity, staff / population susceptibility, staff expertise...)

https://www.who.int/publications/i/item/9789240011458

Risk assessment is an essential systematic process to defy the control measures and for ensuring biosecurity in the laboratory. The assessment takes into account different factors (pathogen-specific and local), including:

- Route (s) of transmission
- Pathogenicity and infectious dose
- Availability of prophylaxis (vaccines) or therapies
- Severity and mortality of the disease
- Contagiousness
- Endemicity
- High-risk laboratory procedures (e.g. aerosol, high titers or volume, use of cutting edges, live animals)
- Expertise of laboratory personnel

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)

istituto zooprofilattico sperimentale
del Piemonte, Liguria e Valle d'Aosta

LABORATORY BIOSAFETY MANUAL
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RISK ASSESSMENT

The manual guides you throughout the process (with practical application sheets, checklists and examples)

For example: sheets for the evaluation of residual risk

World Health Organization

STEP 4. Select and implement risk control measures (continued)

Instructions: Evaluate the residual risk that remains after risk control measures have been selected to determine if the risk is now acceptable and whether work should proceed.
Circle the residual risk of the laboratory activities after risk control measures are in place.

		Likelihood of exposure/release		
		Unlikely	Possible	Likely
Consequences of exposure/release	Severe	Medium	High	Very high
	Moderate	Low	Medium	High
	Negligible	Very low	Low	Medium

Overall **residual** risk.	☐ Very low	☐ Low	☐ Medium	☐ High	☐ Very high

If the residual risk is still unacceptable, further action is necessary such as additional risk control measures, based on the initial risk evaluated in STEP 2, redefining the scope of work such that it is acceptable with existing risk control measures in place or identifying an alternative laboratory with appropriate risk control strategies already in place that is capable of conducting the work as planned.

Should work proceed with selected risk control measures?	Yes ☐ No ☐
Approved by (Name and title)	
Approved by (Signature)	
Date	

Protective measures

- ✓ Risk assessment (to be reviewed in case of events/accidents)
- ✓ Control strategy (equipment and procedures)
- ✓ Training and information to personnel, authorizations
- ✓ Accident registrations

- Avoid accidental exposure or injury
  - Complete PPE (shirts, gloves, shoes)
  - Goggles / mask / visor if exposed to splashes or particulates matter
  - Minimize aerosol production (if unavoidable use biological hood)
  - Use enclosed systems (homogenizers, sealed centrifuge ...)
  - Protect skin lesions in a waterproof way
  - Minimize use of glass
  - Minimize / avoid use of sharp (needles, knives, scissors)
  - Sharp / stinging procedures (armoured glove and more)
  - Disposable materials
  - Minimize accumulations of residual infectivity on instruments and surfaces (decontamination)
- Special cases:
  - Pathology: formalin and glutaraldehyde do not decontaminate (use formalin + **formic acid**); disposable blades, cut resistant gloves, face shield
  - Animals: **inoculation** and sampling (sedation), autopsies

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 {next slide}**
- **DECONTAMINATION PROCEDURES (INSTRUMENTS and SURFACES)**
- **EXPOSURE MANAGEMENT**
- **DESIGN and TECHNICAL CHARACTERISTICS of the LABORATORIES**
- **WORKING PROCEDURES**
- **CLEANING and DISINFECTION**

Biosecurity Guidelines *1/2*

Law 18/12/2020 n. 176 Update

Misure di contenimento	Livello di contenimento ∗2	
	2	3
1. Il luogo di lavoro deve essere separato da qualsiasi altra attività svolta nello stesso edificio	No	Raccomandato
2. Il luogo di lavoro deve essere sigillabile in modo da consentire la fumigazione	No	Raccomandato
3. Il materiale infetto, compreso qualsiasi animale, deve essere manipolato in cabine di sicurezza o in condizioni di isolamento o di adeguato contenimento	Se del caso	Sì, in caso di infezione trasmessa per via aerea
4. L'aria in entrata e in uscita dal luogo di lavoro deve essere filtrata con un sistema di filtrazione HEPA(1) o simile	No	Sì, per l'aria in entrata e in uscita
5. Superfici impermeabili all'acqua e facili da pulire	Sì, per bancone e pavimento	Sì, per bancone, pavimento e altre superfici determinate nella valutazione
6. Il luogo di lavoro deve essere mantenuto a una pressione negativa rispetto alla pressione atmosferica	No	Raccomandato
7. Superfici resistenti ad acidi, alcali, solventi e disinfettanti	Raccomandato	Sì

New Table (Attached XLVII)

The choice of the level of Biosafety for handling activities of potentially prion-infected materials depends on the nature of the agent, the samples to be handled and the activities that are carried out.

VS

	2	3
1. La zona di lavoro deve essere separata da qualsiasi altra attività nello stesso edificio	No	Raccomandato
2. L'aria immessa nella zona di lavoro e l'aria estratta devono essere filtrate attraverso un ultrafiltro (HEPA) o un filtro simile	NO	SI, sull'aria estratta
3. L'accesso deve essere limitato alle persone autorizzate	Raccomandato	Si
4. La zona di lavoro deve poter essere chiusa a tenuta per consentire la disinfezione	No	Raccomandato
5. Specifiche procedure di disinfezione	Si	Si
6. La zona di lavoro deve essere mantenuta ad una pressione negativa rispetto a quella atmosferica	No	Raccomandato
7. Controllo efficace dei vettori, ad esempio, roditori ed insetti	Raccomandato	Si

(Old Table, ex attach XLVII)

D. Lgs 81/2008 and subsequent amendments: XLVI TSE agents are classified as risk group 2 (scrapie) and risk group 3 ** (BSE and other associated animal TSEs).

The agents classified in group 3 and indicated with a double asterisk (**) may entail a limited risk of infection because they are normally not carried by air, so the containment levels indicated in the boxes may be apply.

∗2 Containment level 4 is omitted (not of interest / relevance)

Biosecurity Guidelines 2/2

Law 18/12/2020 n. 176 Update

8. L'accesso deve essere limitato soltanto agli operatori addetti	Raccomandato	Sì
9. Controllo efficace dei vettori, per esempio roditori e insetti	Raccomandato	Sì
10. Procedure specifiche di disinfezione	Sì	Sì
11. Stoccaggio in condizioni di sicurezza dell'agente biologico	Sì	Sì
12. Il personale deve fare una doccia prima di uscire dall'area di contenimento	No	Raccomandato
13. Processo di inattivazione convalidato per lo smaltimento sicuro delle carcasse di animali	Raccomandato	Sì, sul sito o fuori sito
14. Il laboratorio deve contenere la propria attrezzatura	No	Raccomandato
15. Presenza di una finestra di osservazione, o di una soluzione alternativa, che consenta di vedere gli occupanti	Raccomandato	Raccomanda

Nella tabella, «*raccomandato*» significa che le misure do… …licate… a meno che i risultati della valutazione del rischio non indich…

(1) HEPA: filtro anti-particolato ad alta efficienza
* ALLEGATO XLVII del D.lgs. n. 81/2008 come…
§ per gli agenti appartenenti alla classe 3**, sulla b…
può essere sufficiente assicurare livelli di contenimento pr…

New table (Attached XLVII)

RISK ASSESSMENT

VS

8. Superfici idrorepellenti e di f…		
9. Superfici resistenti agli acidi, agli alcali, ai solventi, ai disinfettanti	R…	
10. Deposito sicuro per agenti biologici	Si	
11. Finestra d'ispezione o altro dispositivo che permetta di vederne gli occupanti	Raccomandato	Rac… …ato
12. I laboratori devono contenere l'attrezzatura a loro necessaria	No	Racc… …ndato
13. I materiali infetti, compresi gli animali, devono essere manipolati in cabine di sicurezza, isolatori o altri adeguati contenitori	Ove opportuno	Si, quando l'infezione è veicolata dall'aria
14. Inceneritori per l'eliminazione delle carcasse degli animali	Raccomandato	Si (disponibile)
15. Mezzi e procedure per il trattamento dei rifiuti	Si	Si
16. Trattamento delle acque reflue	No	Facoltativo

(old tab… ex Att. XLVII)

The main risks related to rapid diagnostic activities for the detection of PrPRES are represented by cuts, inoculation and accidental ingestion.

The personnel working in the laboratories must strictly comply with ***Good Laboratory Practices.***

…xperimental studies indicate a …ossible transmission of some prion …inalation. As a precaution, …omogenization of brain tissues …ially or certainly contaminated) …out in sealed systems …laced inside suitable biosecurity …ods in order to contain any …duction of drops and / or aerosols.

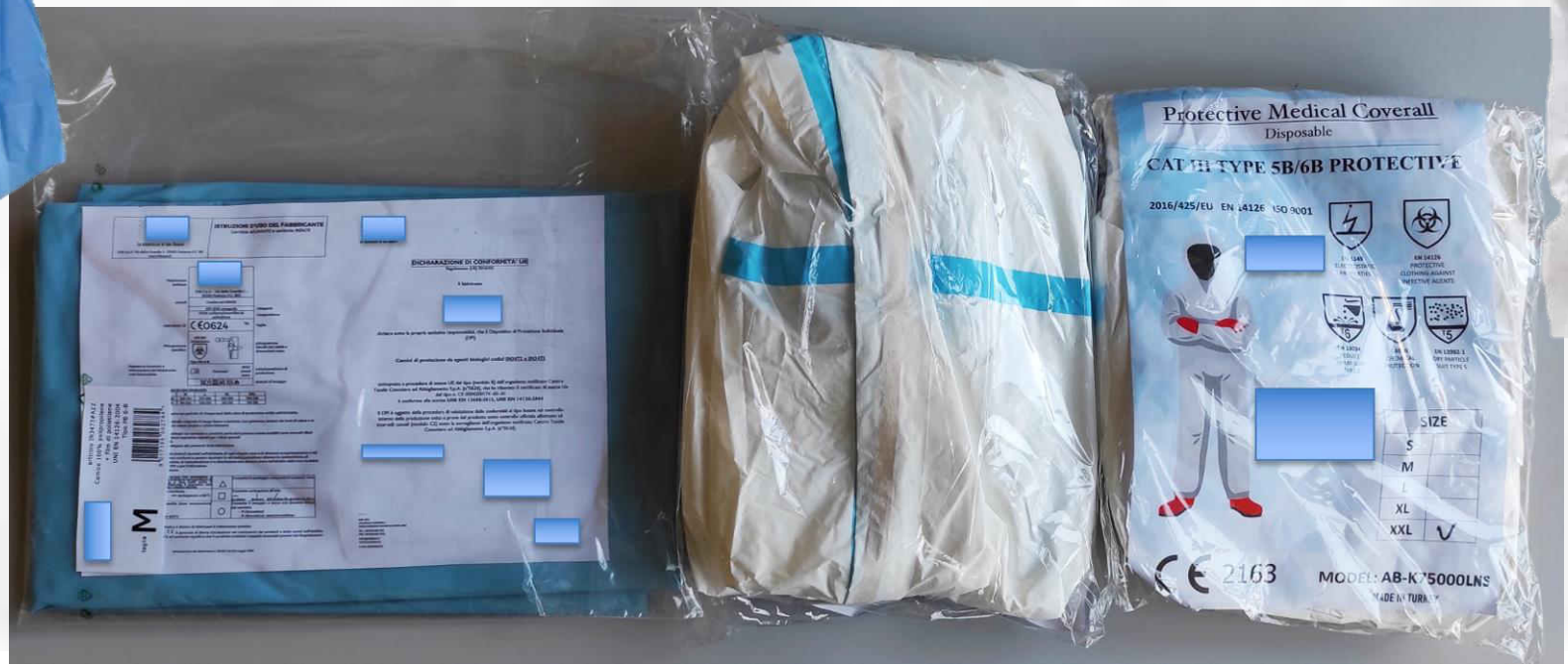
Brain sampling


1
2

Personal protective equipment

Polyethylene-Coated Polypropylene Isolation Gowns

High density polyethylene suit



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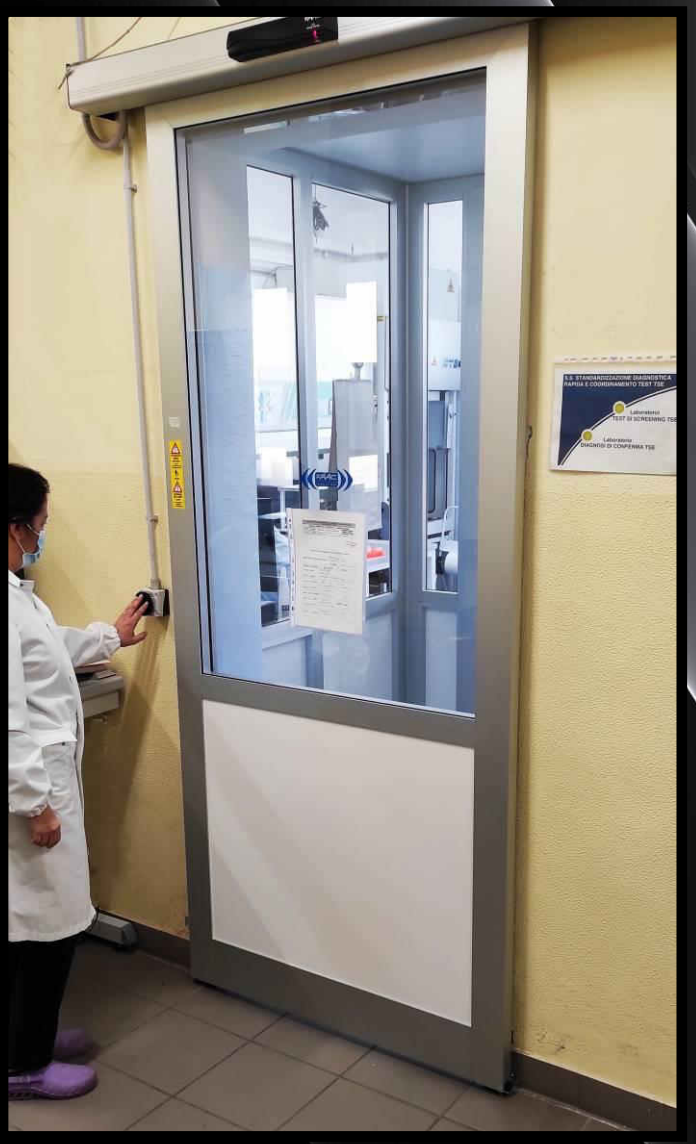
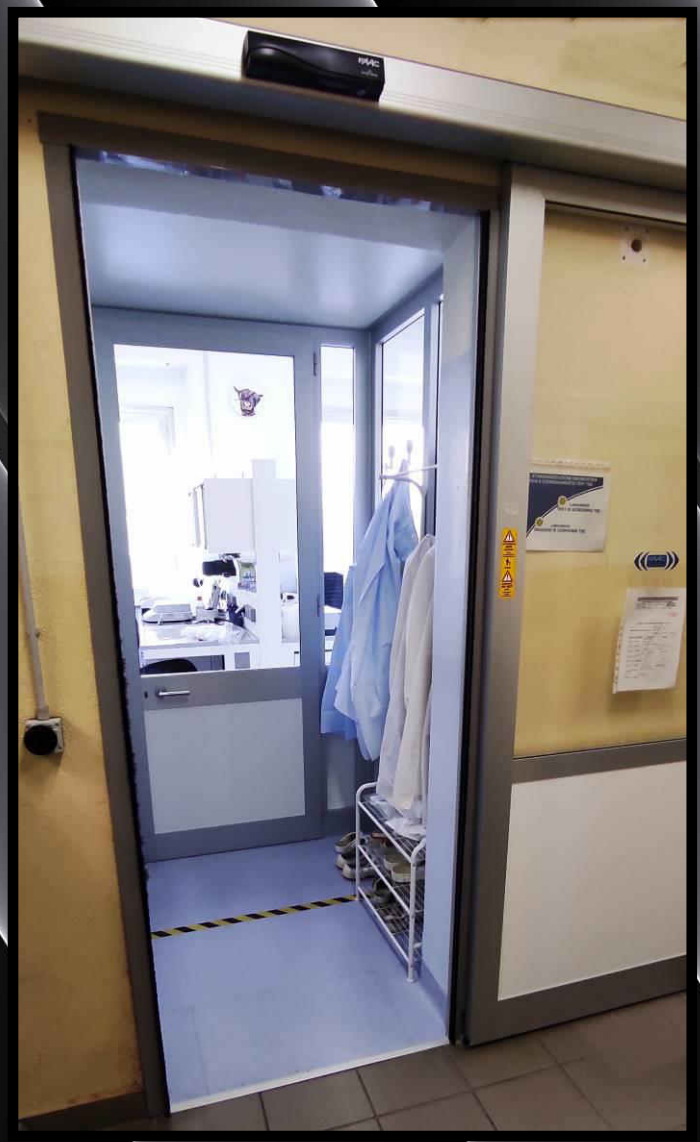
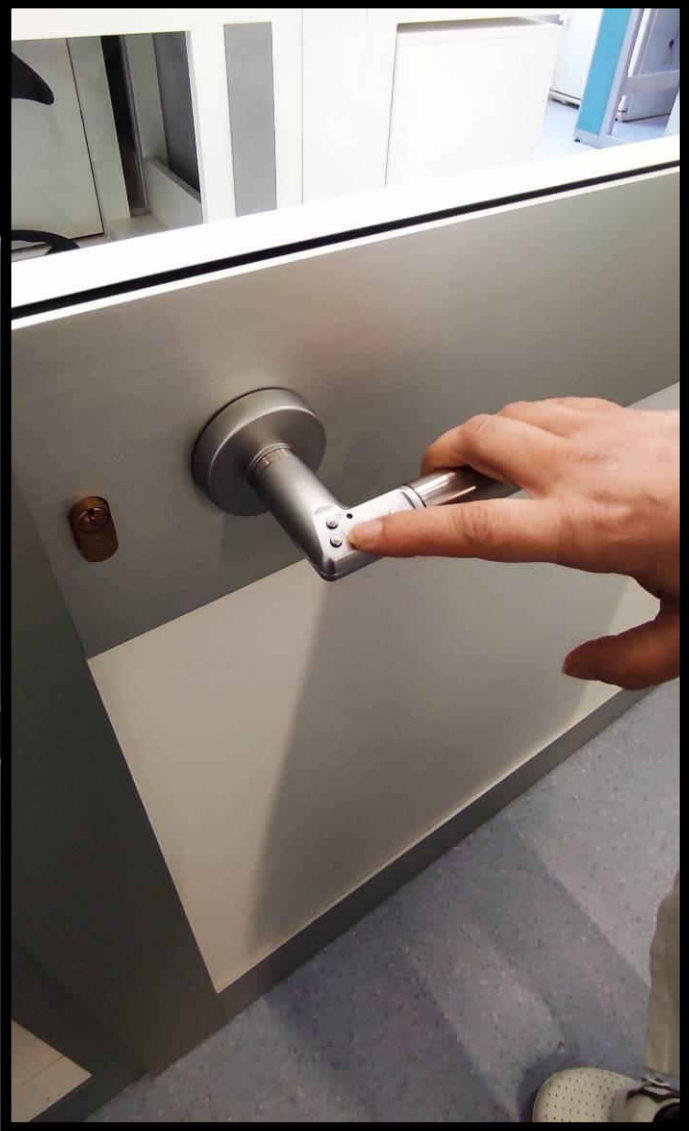
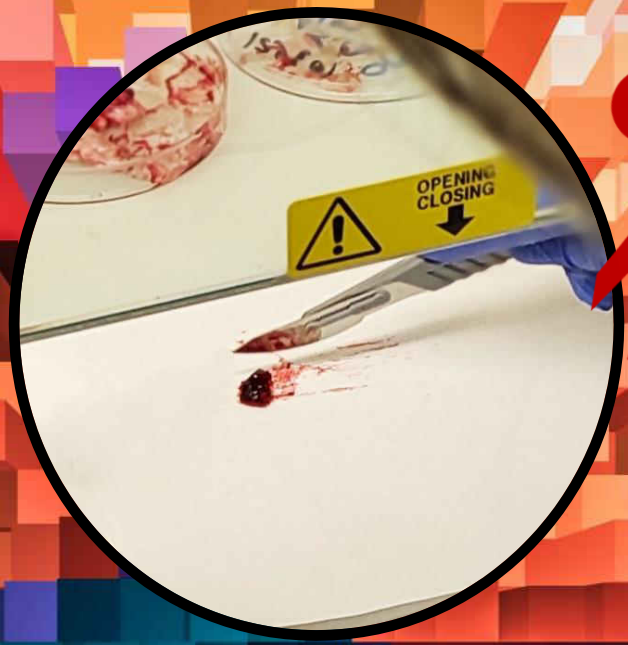
G. Caporale

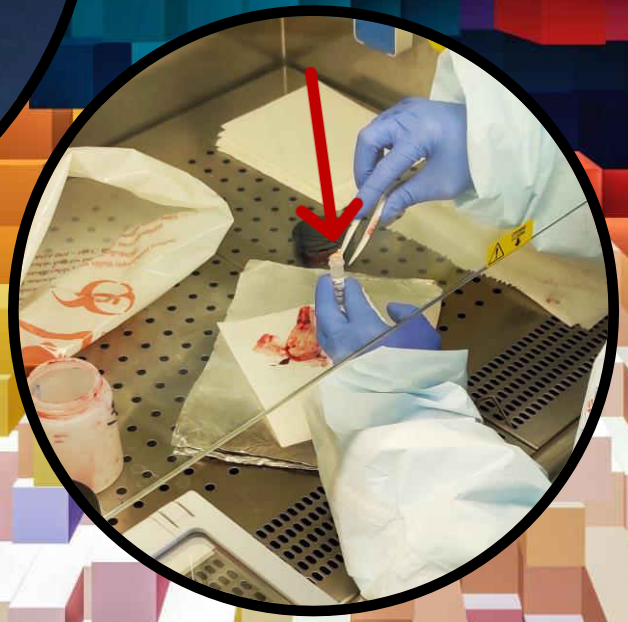


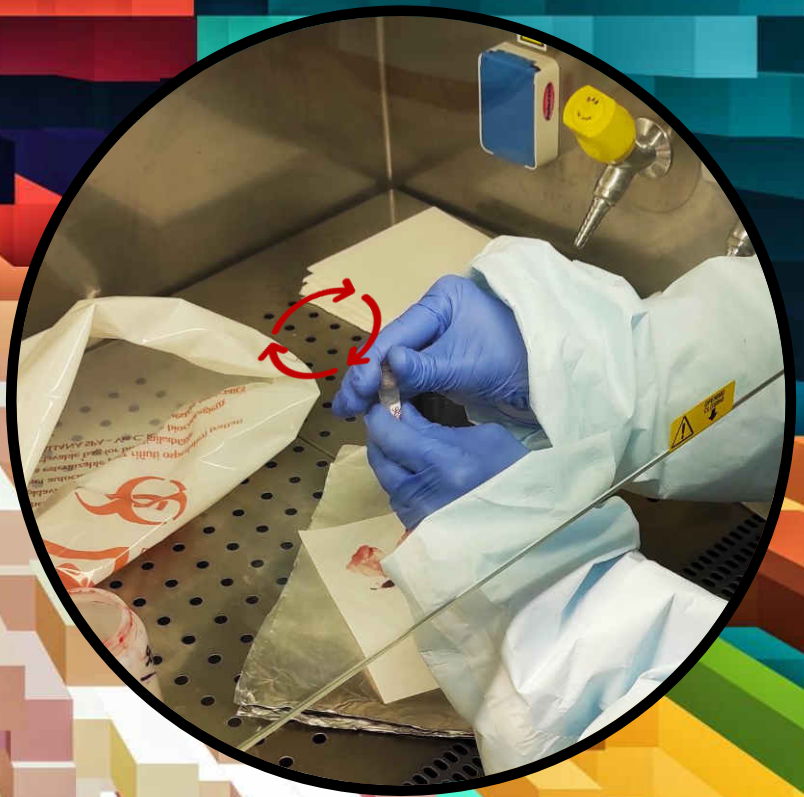




Rapid Test







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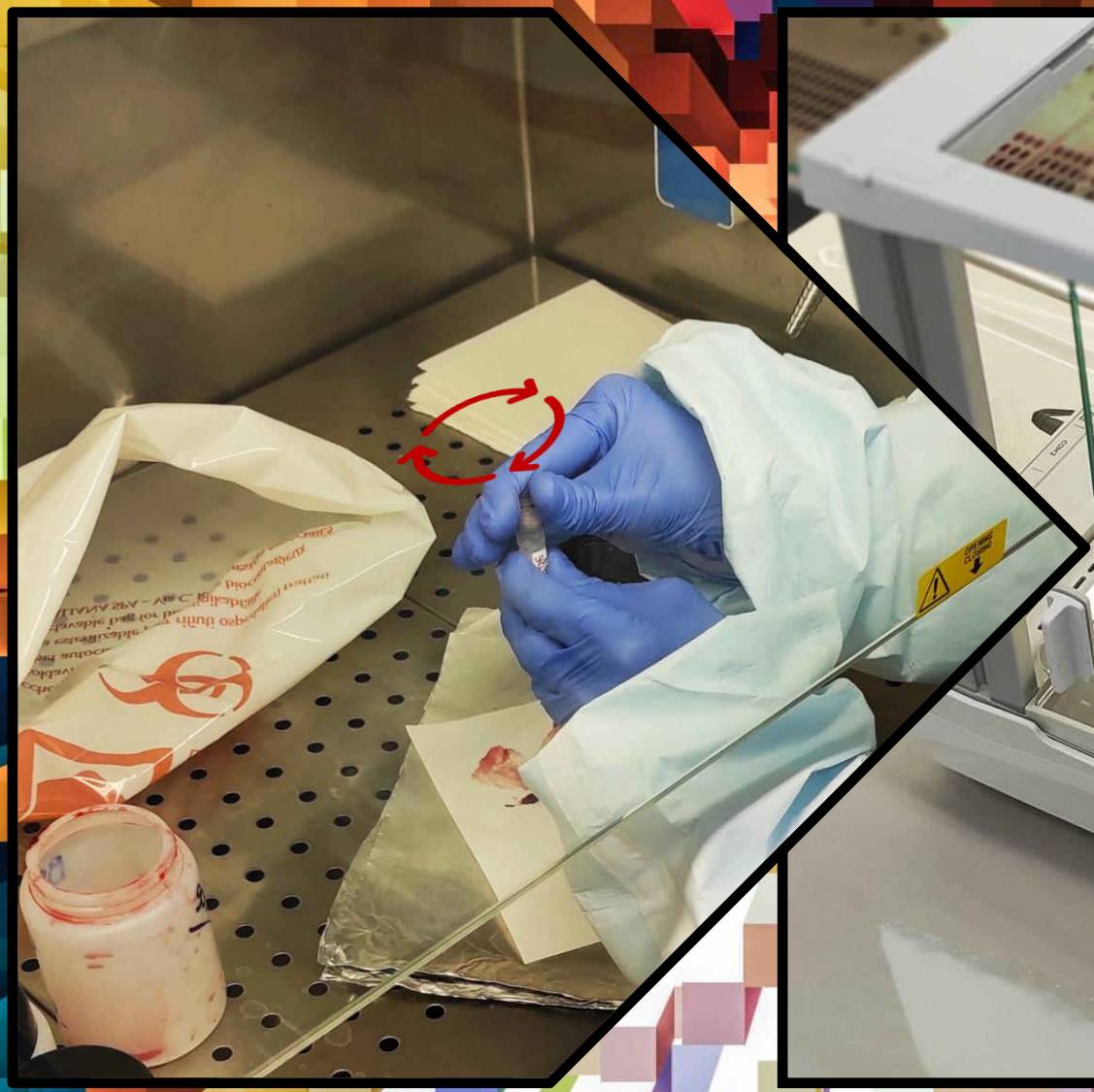
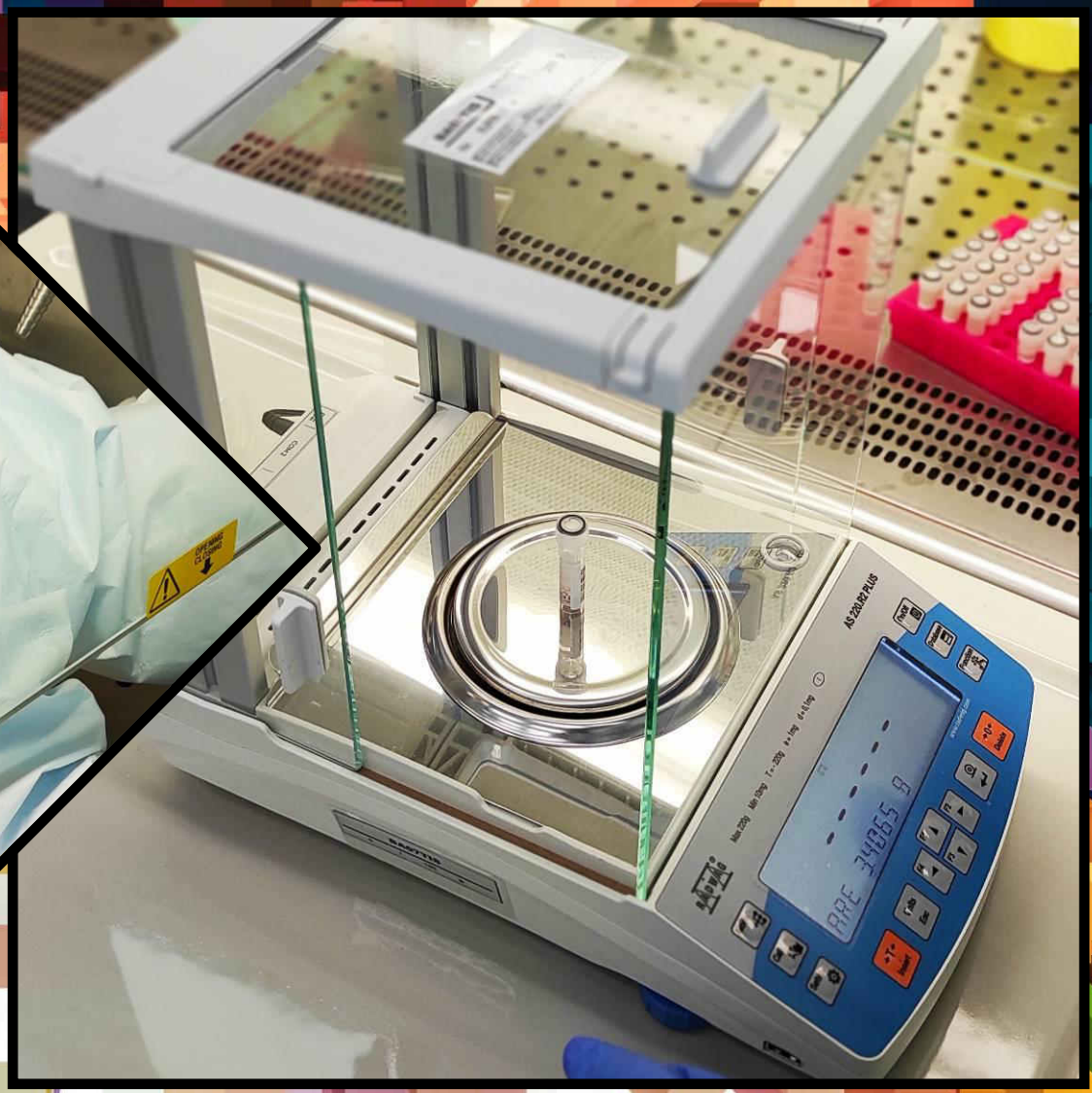
Rapid Test



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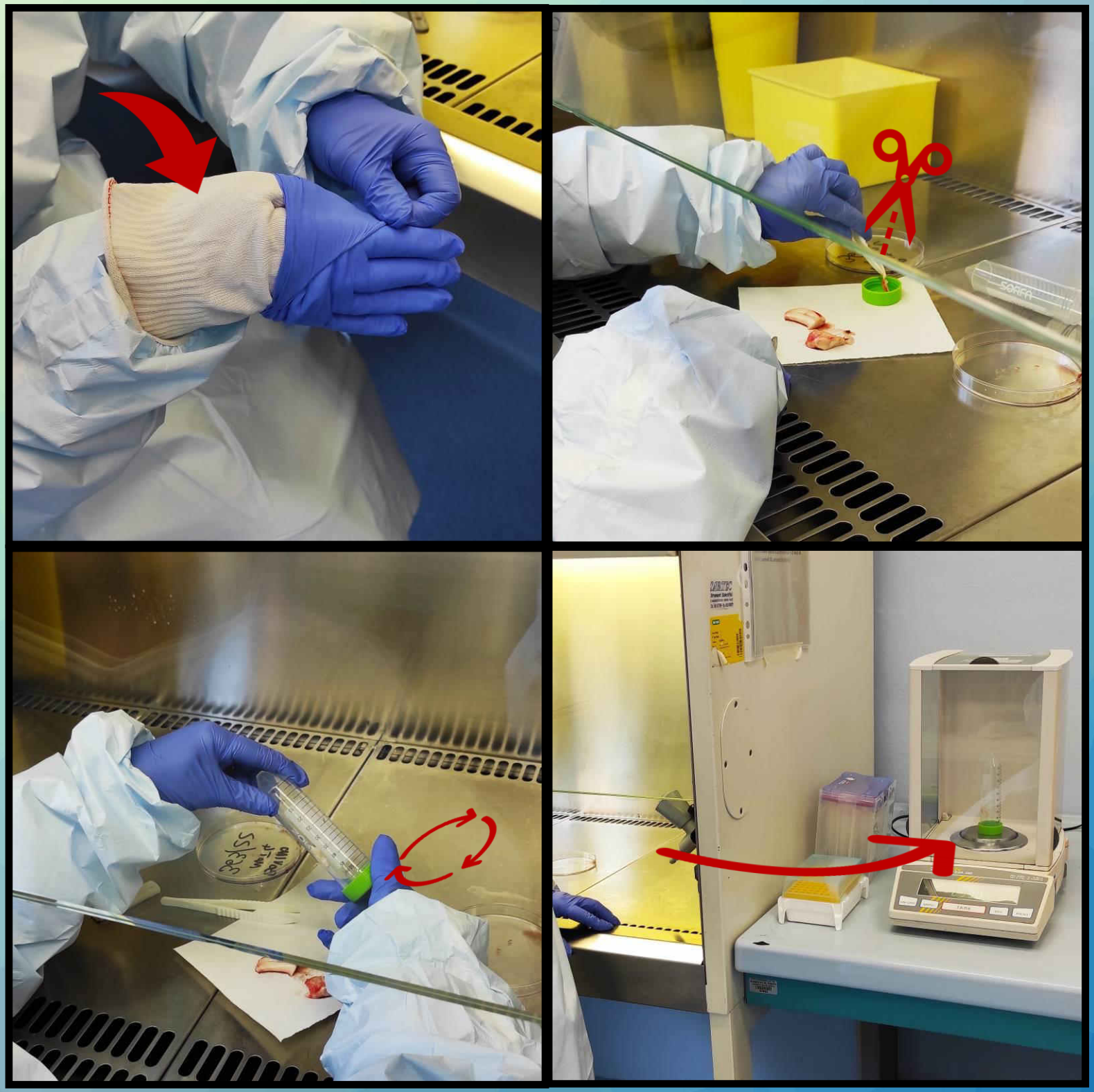
G. Alleva

ISTITUTO ZOOPROFILATTICO SPERIMENTALE del Piemonte Liguria e Valle d'Aosta

WB



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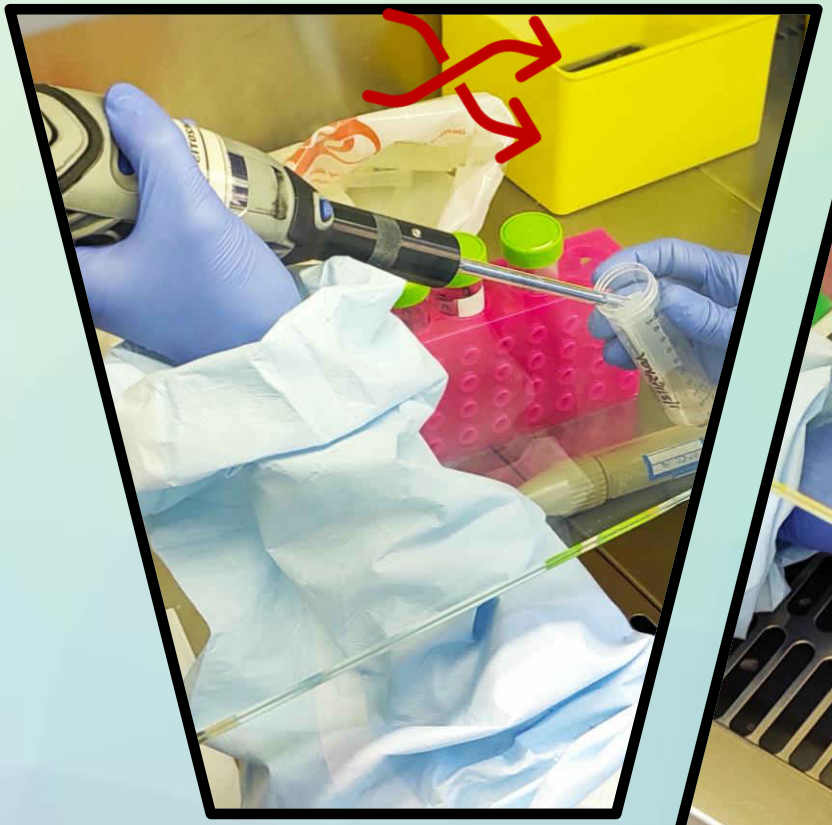
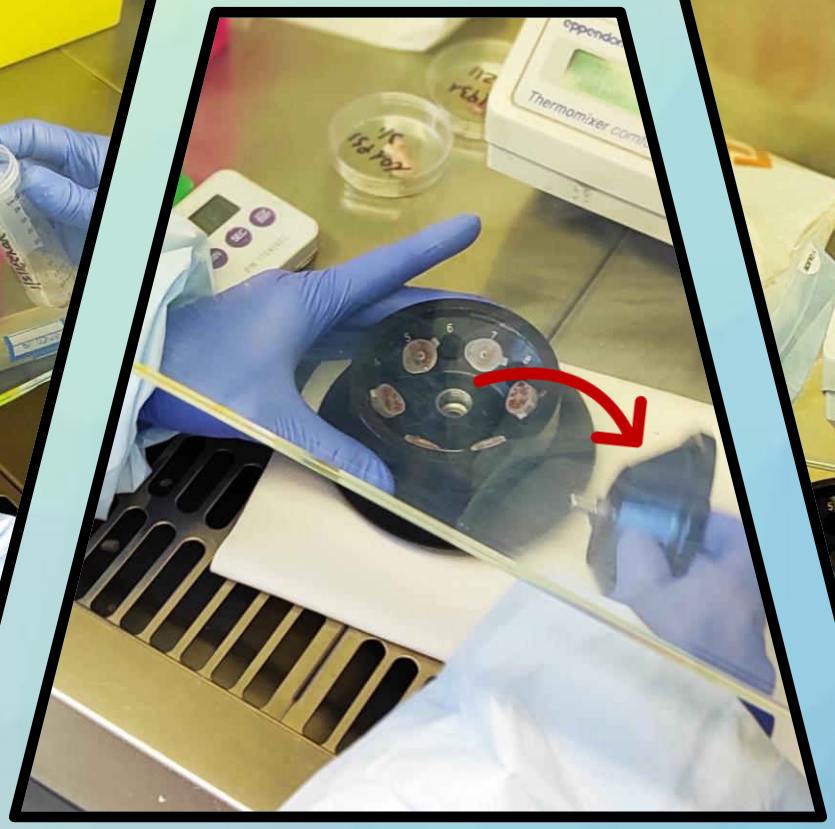
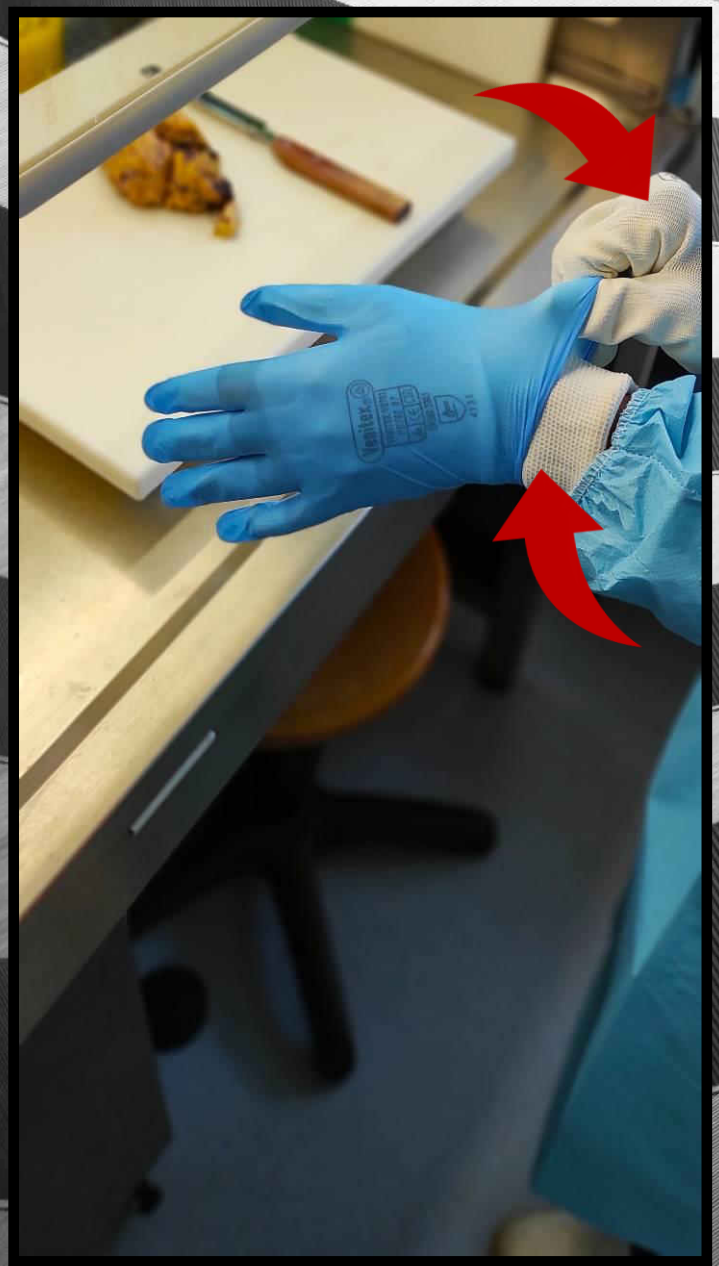
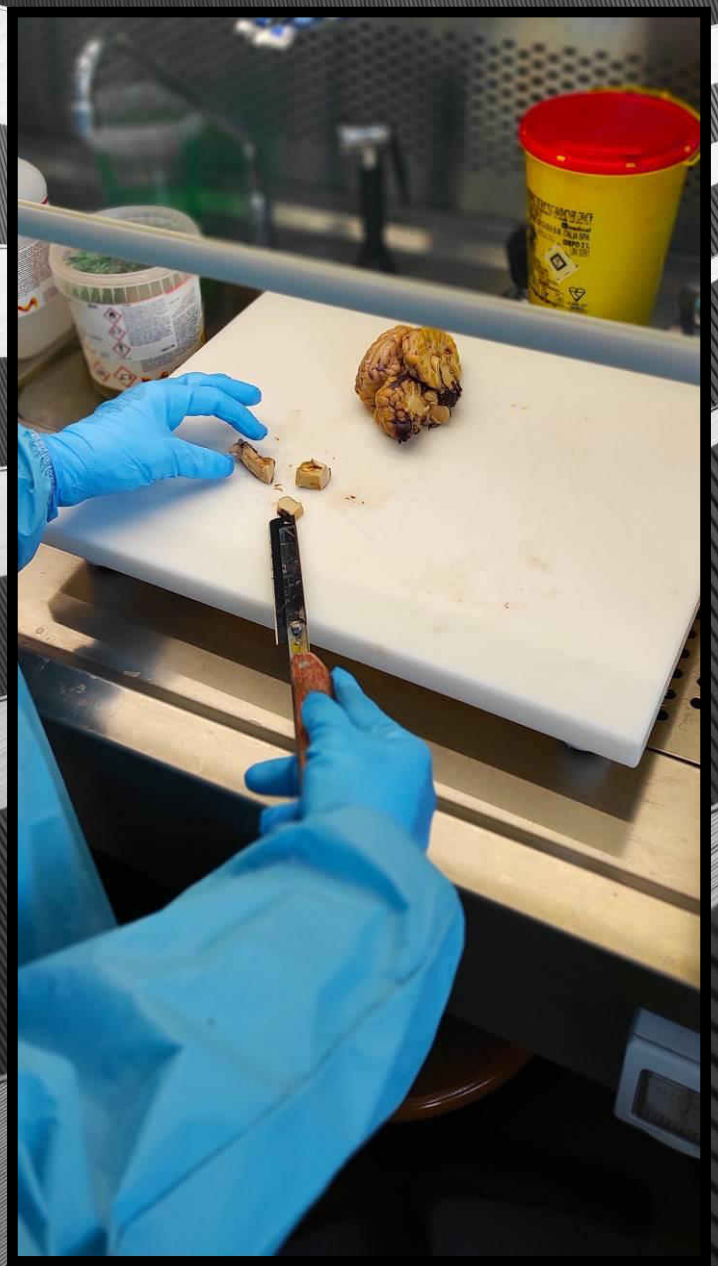




IHC/ Histology

IHC/ Histology





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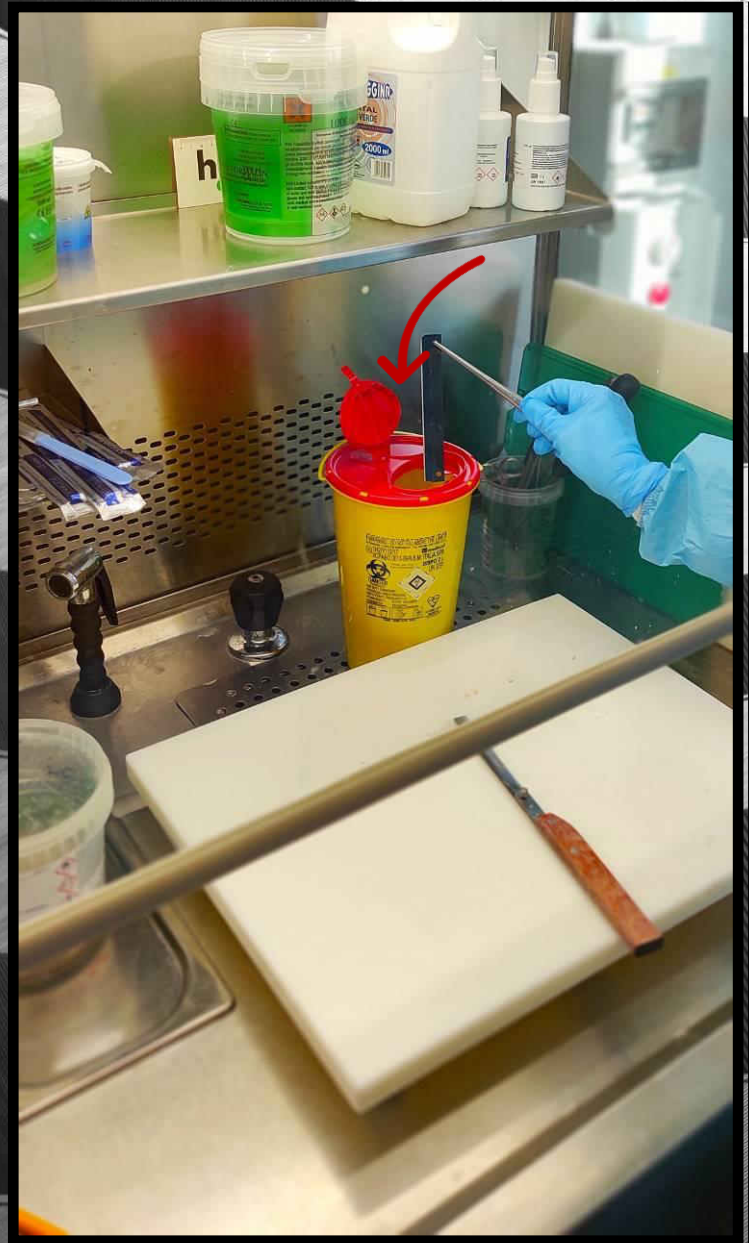

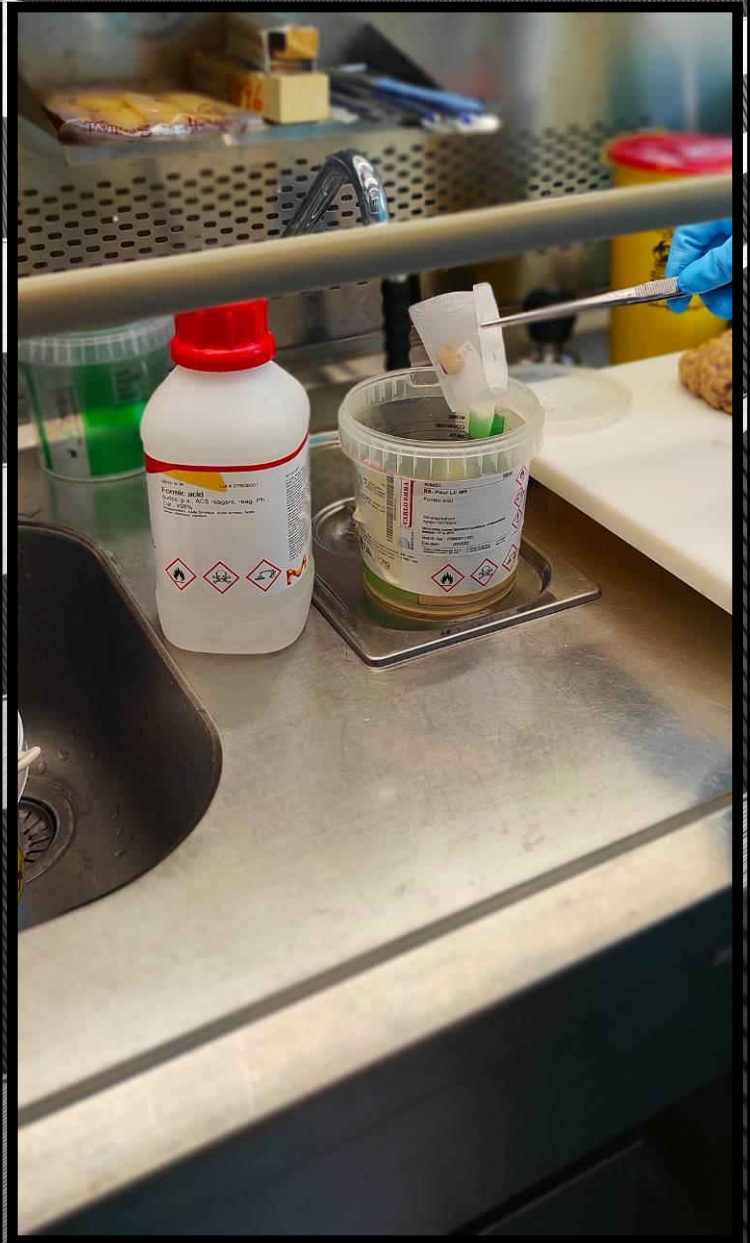

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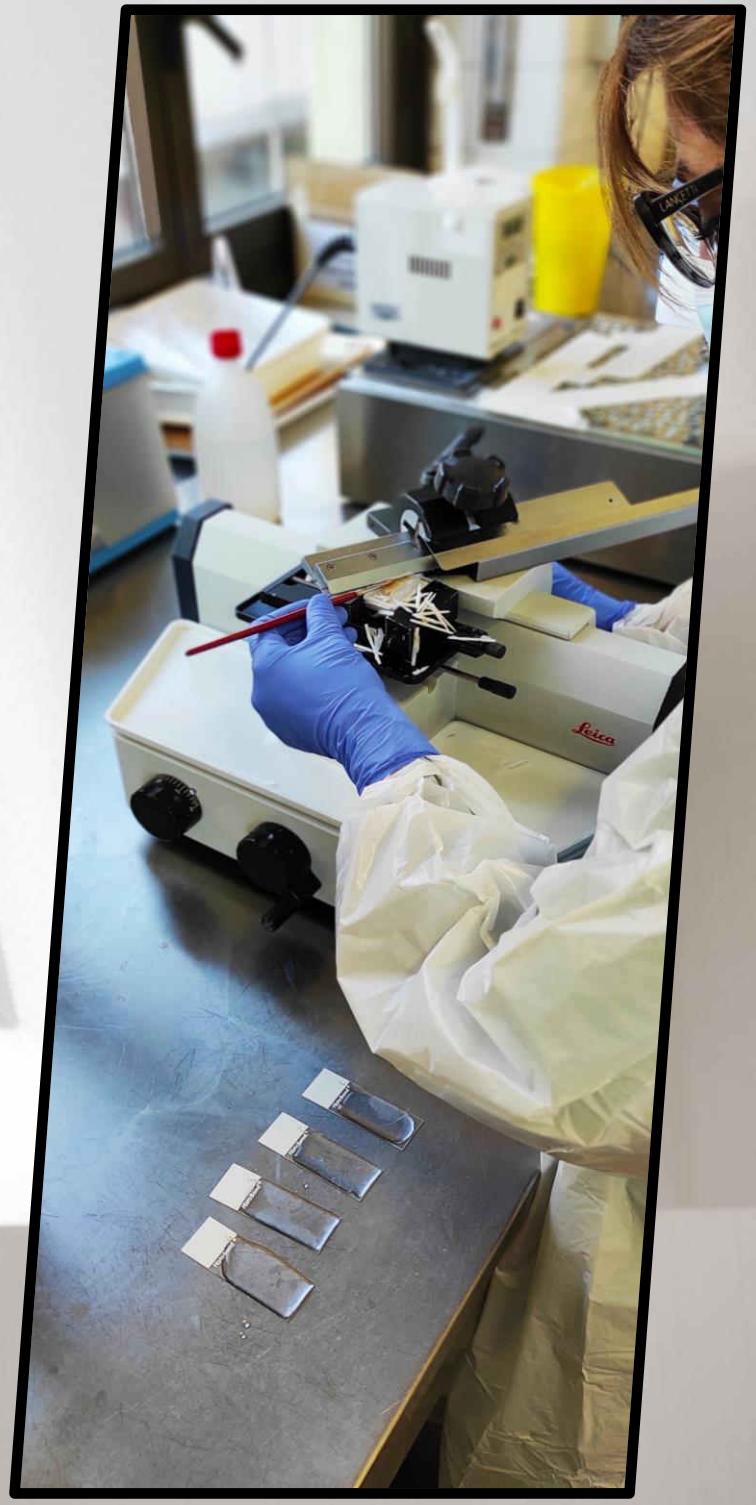

Transport of samples

Sample, place of sampling –> Analysis Laboratory (external transport)/internal transport (intra-mural): importance both for the **safety of the transport operators** and for the **recipients of the samples**, but also for the **conservation of the samples** themselves (and therefore for the quality of the analytical result).

BIOPROTECTION: extension to the normal approach to biosecurity aimed at defending, from a One Health perspective, laboratories, the population, livestock, agriculture and the environment from acts of sabotage.

Perfect **traceability** is first and foremost necessary.

Strict national and international regulations (in particular ADR) which aim above all to reduce the probability of **damage to containers**.

CLASSIFICATION of INFECTIOUS SUBSTANCES:

- **CATEGORY A** (UN2814/UN 2900);
- **CATEGORY B** (UN3373).

Transport of samples

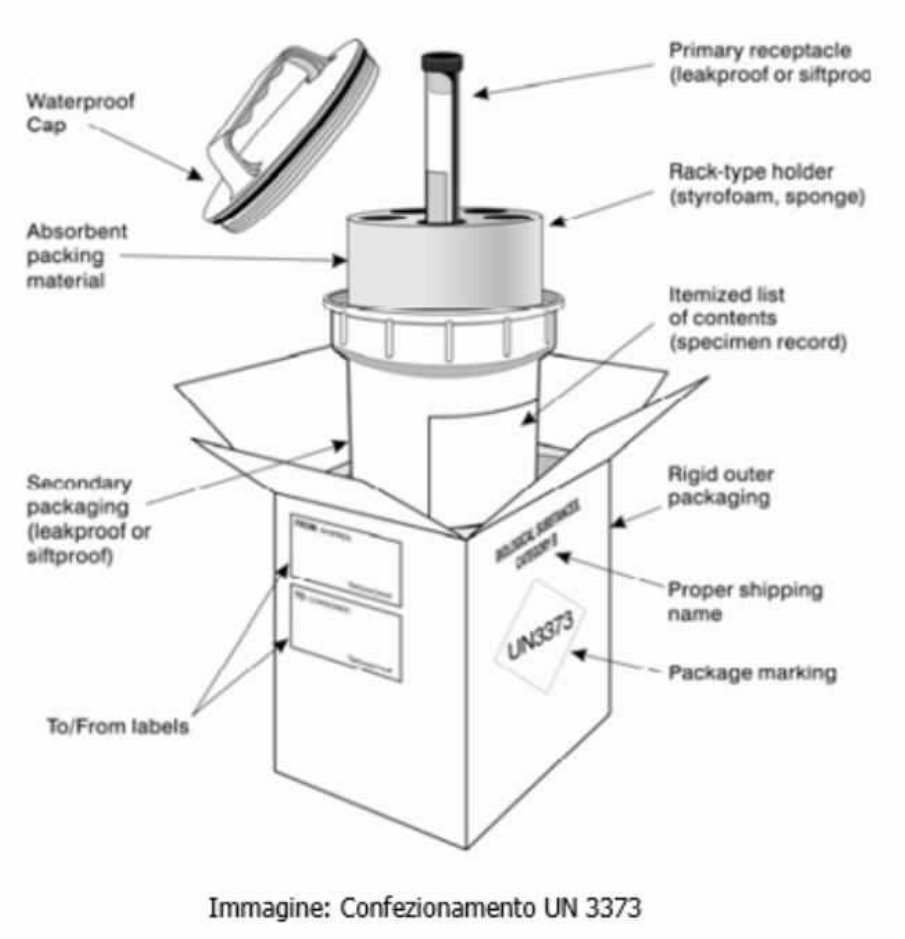

Immagine: Confezionamento UN 3373

Triple container :

- **Primary casing:** watertight and equipped with a descriptive label of the contents**;**
- **Intermediate (secondary) casing:** used to close and protect the first packaging; also watertight, it can accommodate one or more primary casings;
- **External (tertiary) casing:** physical protection from damage.

+

Specific accompanying documentation (which certifies the information on the nature of the transported sample, its identification as well as the sender and recipient details).

Waste management

Waste from diagnostic activity on prions:

- **SOLID waste: incineration** is useful when it is necessary to eliminate animal carcasses, anatomical parts and other laboratory waste, with or without previous decontamination. The incineration of infected materials is an alternative to the autoclave and is operated by specialized companies**;**

- **LIQUID waste:** infected liquid waste contaminated with prions must be treated for 1 h with sodium hypochlorite containing free chlorine at the final concentration of 20 g / l (2%) or with 2M sodium hydroxide.

More information are reported in:
Nota 0006558-15/03/2021-DGSAF-MDS-P


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