



Protective measures for activities in laboratories involving TSE-associated agents



Michael Beekes, Gerhard Dobler, Christine Fast, Markus Glatzel, Peter Kämpfer, Carolyn Kästner, Gisela Martens, Susanne Modrow, Walther Schulz-Schäffer, Julia Sasse, Hans-Georg Wielinger, Inga Zerr

Rome 3.10.2023



Biosafety in TSE-Labs

Level of Biocontainment and Risk assessment

Controlled / restricted access

Biosafety protocols

- **Biosafety cabinets**
- **Specific biosafety procedures for ELISA/WB**
- **Specific biosafety procedures for pathology/IHC**
- **Specific biosafety procedures for experimental inoculation**
- **Specific procedure for safe transport**
- **Specific procedure for safe waste management**
- **Procedures to prevent the use of sharp or pointed tools**

Mitigate the risk in the event of personal exposure

Register of personal accidents

Decontamination of the lab environment incl surfaces

Decontamination of the lab environment for maintenance / disposal / accidental contamination



Federal Institute for Occupational Safety and Health (BAuA)

- Committee on Biological Agents (ABAS) -

One of the tasks of the ABAS is to provide further recommendations on how to meet the requirements of the Biological Agents Ordinance.

Other ABAS recommendations include the current evaluation of biosubstances, statements on laboratory technology and other topics, and reports and position papers on special topics. The recommendations describe the state of the art in science, technology and medicine and how the requirements of the Biological Substances Ordinance can be met.



Federal Institute for Occupational Safety and Health

- Committee -

National guideline for working with TSE agents

Ausgabe März 2011

Beschluss
603

Beschluss des Ausschusses
für Biologische Arbeitsstoffe (ABAS)

Schutzmaßnahmen bei Tätigkeiten mit
Transmissibler Spongiformer
Enzephalopathie (TSE) assoziierter
Agenzien in TSE-Laboratorien

...the current evaluation of
laboratory technology and other topics, and
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the state of the art in science, technology and medicine and how the
requirements of the Biological Substances Ordinance can be met.



Who we are

- **Dr. Michael Beekes**, Robert Koch-Institut
- **Dr. Gerhard Dobler**, University of the Bundeswehr Munich
- **Dr. Christine Fast**, Friedrich-Loeffler-Institut, Isle of Riems
- **Prof. Dr. Markus Glatzel**, UKE Hamburg
- **Peter Kämpfer**, Justus-Liebig-University, Giessen
- **Carolyn Kästner**, Federal Institute for Occupational Safety and Health
- **Gisela Martens**, Employer's Liability Insurance Association for Raw Materials and the Chemical Industry
- **Prof. Dr. Susanne Modrow**, University of Regensburg
- **Prof. Dr. Walther Schulz-Schaeffer**, Saarland University Medical Center and Saarland University Faculty of Medicine
- **Dr. Julia Sasse**, Federal Ministry of Labour and Social Affairs
Anke Stein, Federal Office of Consumer Protection and Food Safety
- **Hans-Georg Wielinger**, Fresenius Kabi
- **Prof. Dr. Inga Zerr**, University Medical Center Göttingen



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National Guideline - Overview

Full Title:

Protective measures for activities involving TSE-associated agents and proteopathic seeds of other neurodegenerative diseases in laboratories.

Overview:

- 1. Scope of application**
- 2. Definitions**
- 3. Risk assessment**
 - **Diseases associated with TSE**
 - **Specific properties of TSE-associated agents.**
 - **Proteopathic seeds**
 - **Protection levels, safety measures**
 - **Protective measures for animal experiments**
 - **Transport of samples**
- 4. Protective measures in laboratories**
- 5. Inactivation and decontamination**
- 6. Immediate measures after contact with TSE-associated agents**



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Biosecurity Level

Targeted and non-targeted activities with **TSE-associated pathogens** must be assigned to protection level 3**.

Exception: **Scrapie** (BSL 2) according to EG-RL 2000/54/EG, i(EU) 2019/1833 and (EU) 2020/739

*However, a change of host must be given special consideration in the risk assessment and may lead to measures of protection level 3**.*



Biosecurity Level

Purified recombinant prion protein (soluble, aggregated)

Infectivity of *in vitro* produced recombinant prion protein has not yet been demonstrated. Therefore work is considered non-infectious.

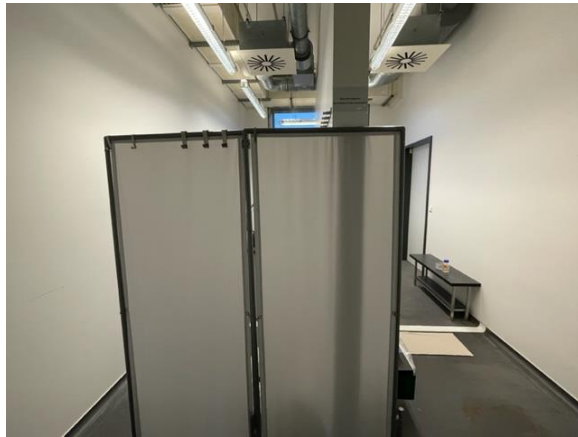
However, laboratory work with recombinant prion protein that aims at or enables the enhancement of infectivity or involves the handling of infectious preparations is assigned to the respective infectious TSE agent in terms of its protection level, but at least to protection level 2.



TSE-Lab / general principles

TSE laboratory must be physically separated from other laboratories.

- In justified exceptional cases (reasons must be documented in the risk assessment), strictly separated work areas must be designed within the lab.
Such an exception is not possible in the case of BSE.



Access to BSE-lab
with lock system





TSE-Lab / general principles

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Access is to be restricted to authorized persons who must be instructed accordingly





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The TSE laboratory shall be marked with the protection level and the "biohazard" symbol.





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The TSE laboratory shall be marked with the protection level and the "biohazard" symbol.

The TSE laboratory should have its own equipment

The devices used for TSE tests must not be touched with contaminated gloves. This also applies, to switches, buttons and membrane keypads.

Disposable plastic material should be used as much as possible.

- *Disposable work surface pads should be used (i.e. PE sheets, absorbent pads)*

Personal protective equipment must be worn and removed when leaving the lab.



TSE-Lab / Personal Protection Equipment

- Laboratory coat closed at the back (autoclavable or disposable)
- Overshoes or a separate pair of shoes (closed), which remain in the TSE-lab
- Double disposable protective gloves must be worn for all work in the laboratory where there is a possibility of contact with infectious material.
 - *Gloves required resistance to the chemicals used to inactivate TSE-agents (AQL $\leq 0,65$ nach DIN EN 374-2).*





TSE-Lab / Personal Protection Equipment





TSE-Lab / Personal Protection Equipment





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TSE-Lab / Personal Protection Equipment





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Ready for work



TSE-Lab / Personal Protection Equipment

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Double disposable protective gloves must be worn for all work in the laboratory where there is a possibility of contact with infectious material.

- *Gloves required resistance to the chemicals used to inactivate TSE-agents (AQL $\leq 0,65$ nach DIN EN 374-2).*

Safety goggles for all activities with a concrete eye hazard, i.e.:

- *the application of sodium hydroxide solution,*
- *the decontamination of instruments*

Gloves out of cut-resistant fiber (latex or nitrile gloves above and below) must be worn at all times where there is a risk of cuts.

- *Problem: cannulas / pointed tweezers!*
safety versus handling



TSE-Lab / Microbiological Safety Cabinet

All work with contaminated (and potentially contaminated) samples and agents must be performed under a microbiological safety cabinet. This applies in particular the work with potentially high-titer material.

- *ELISA: all work before the first washing of the ELISA plate using an automatic plate washer.*
- *Western blot: all work before loading the gels.*

If work is performed in a closed system, this step can also be performed outside the safety cabinet, i.e. homogenization of samples



TSE-Lab / Microbiological Safety Cabinet



Biosafety procedures for processing TSE agents at the microbiological safety cabinet

- *Disposable sleeve protectors as well as two pairs of disposable protective gloves on top of each other.*
- *The transition from the lower glove to the gown sleeve should be covered with disposable sleeve protectors*
- *The upper pair of gloves is changed after completion of a work process, but at the latest when leaving the microbiological safety cabinet.*



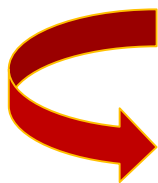
TSE-Lab / Pathology and Histology

For histological processing, the following steps must be performed in a laboratory of protection level 2 (scrapie) or 3** (BSE, CWD):

- *Necropsy*
- *Cutting of tissues*
- *Fixation of tissue samples in formalin or other fixatives,*
- *Cutting of tissue blocks to a maximum of 5 mm.*

Formic acid (98%) treatment:

- *1h treatment of 5 mm tissue preparations (standard embedding cassette)*
- *15-minute pretreatment of unstained, but already rehydrated paraffine sections (1 - 10µm)*



No risk to personnel from prion infectivity can be assumed for all subsequent work steps

Appropriately treated sections can therefore be further processed in a protection level 1 laboratory



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For tissue samples **not** decontaminated with formic acid

- *Cutting the paraffin blocks*





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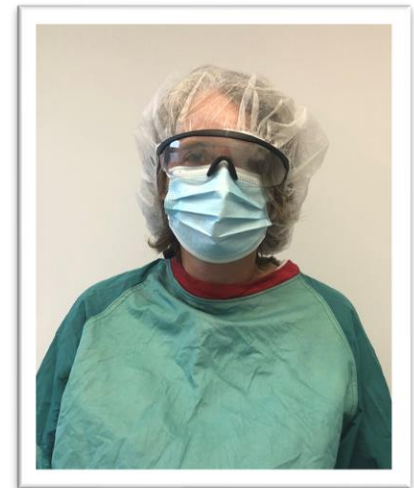
For tissue samples **not** decontaminated with formic acid

- *Cutting the paraffin blocks*



Additional personal protective equipment

- *Laboratory safety goggles (with side shields and, if possible, covering the upper eye area)*
- *disposable non-woven hood*
- *at least mouth/nose protection according to DIN14283 (as contact protection) or optionally FFP2 mask*
- *two pairs of disposable protective gloves worn on top of each other*
- *Cut-resistant fiber gloves*





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Additional personal protective equipment

- *Laboratory safety goggles (with side shields and, if necessary, covering the upper eye area)*
- *disposable gloves*

Paraffin residues must be collected and inactivated. Vacuum cleaners used for cleaning must be equipped with HEPA filters (category H13).
According to DIN14283 (as contact cleaner) or optionally FFP2 mask

- *two pairs of disposable protective gloves worn on top of each other*
- *Cut-resistant fiber gloves*



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For tissue samples not decontaminated with formic acid

- *Cutting the paraffin blocks*
- *Staining (i.e. H&E, IHC)*



All fixatives, staining solutions, wash buffers, etc. that come into contact with the sections must be considered potentially infectious and must be decontaminated before disposal



TSE-Lab / Histology

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For tissue samples **not** decontaminated with formic acid

- *Cutting the paraffin blocks*
- *Staining (i.e. H&E, IHC)*
- *Covering the stained slides*



After decontamination of the covered slides,
evaluated in a BSL 1 lab.

Tissues/sections that have **not** been pretreated are still considered infectious and must be disposed of in the TSE laboratory.
The residues of the NaOH for 5 minutes and subsequently, the residues of the NaOH needs to be washed off with water.



TSE-Lab / Cleaning & Decontamination

- Liquid and solid waste containing pathogens must be inactivated before disposal.**
- Used autoclavable protective clothing must be autoclaved before cleaning**
 - *134° C (1 h, 3 bar)*
- The Microbiological safety cabinet must be decontaminated with a suitable disinfectant (and cleaned with water) in following scheme:**
 - *Work surface daily*
 - *Other potentially contaminated surfaces inside the workbench after 5-10 working days*
 - *In case of splashes of (potentially) infectious material, the contaminated area of the workbench must be decontaminated immediately*
- The work surfaces must be cleaned with water every working day and disinfected after at least 5-10 working days.**
- The decontamination of the floors shall be carried out in accordance with the hygiene plan => at FLI every 20th working day**



TSE-Lab / Cleaning & Decontamination

TSE-associated pathogens are largely tolerant to a variety of disinfectants and to standard heat or steam sterilization procedures

Thermal processing

- *Combustion at sufficiently high temperatures ($\geq 850^{\circ} \text{C}$ for ≥ 2 seconds or $\geq 1000^{\circ} \text{C}$ for ≥ 1 second with $< 7\%$ carbon content in the ash)*
- *Autoclave in a steam sterilizer (with aerosol filters) at 134°C , 3 bar absolute, $\geq 1\text{h}$ (for layer thicknesses $< 5 \text{ cm}$).*



TSE-Lab / Cleaning & Decontamination

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Chemical processing

- ***Final conc. of at least 1 M NaOH or 2.5% sodium hypochlorite for $\geq 1h$***
 - *for liquid inactivation, this is achieved by adding an equal volume of 2 M NaOH or 5% sodium hypochlorite.*
 - *The duration should be increased up to 24 hours depending on the type of waste and pathogen load.*



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- **Treatment with 0.2% (w/v) sodium dodecyl sulfate (SDS)/0.3% (w/v) NaOH for 10 minutes at room temperature**
 - *proven effective against rodent-adapted 263K scrapie and human vCJD prions, **not to be used for BSE.***



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- **Treatment with 6 M (15 min), 4 M (1h) or 3 M (24h) guanidinium thiocyanate**
- **Treatment with first 4% (w/v) SDS at 65° C (30 min) followed by 4% (w/v) SDS/ 1% (v/v) acetic acid at 65° C (18h)**
 - *proven for single scrapie agent*



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Many thanks for your attention!