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Technical Rules for Biological Agents	Protective measures for activities involving biological agents in laboratories	TRBA 100

The Technical Rules for Biological Agents (TRBA) reflect the state of the art, the state of occupational health and occupational hygiene as well as other sound work-scientific knowledge relating to activities involving biological agents.

The **Committee for Biological Agents (ABAS)** compiles or adapts the rules with the participation of the Committee for Occupational Medicine (AfAMed) and they are announced by the Federal Ministry of Labour and Social Affairs (BMAS) in the Joint Ministerial Gazette (GMBl).

Within its scope of application, TRBA 100 sets out in concrete terms the requirements of the Biological Agents Ordinance and the Ordinance on Occupational Medical Prevention. If the technical rules are adhered to, the employer can assume that the corresponding requirements under the ordinances have been fulfilled. If the employer chooses another solution, that solution must achieve at least the same level of safety and health protection for employees.

Contents

1 Scope of application

These technical rules apply to specific and non-specific activities involving biological agents in laboratories.

2 Objective

These technical rules set out in concrete terms the requirements of the Biological Agents Ordinance (BioStoffV) [1], in particular Annex II. They define the minimum requirements for structural, technical, organisational and personal protective measures in laboratories for four protection levels that are necessary for activities involving biological agents of various risk

groups. The requirements are intended to avoid and, where this is not possible, to reduce to a minimum the hazards resulting for employees from activities involving biological agents.

Note: *General protective measures for work in laboratories are regulated in TRGS 526 "Laboratories" [2].*

3 Definitions

3.1 Hazard

A hazard is the possibility of the safety and health of employees being impaired by biological-agent effects that are infectious, sensitising, toxic or otherwise harmful to health.

3.2 Classification of biological agents

Biological agents are classified into risk groups 1 to 4 according to the infection risk originating from them. The EU's legal classifications (Annex III to Directive 2000/54 EC) [3], as well as additional national classifications, can be found in the technical rules TRBA 460–468 [4–8].

3.3 Laboratories

Laboratories within the meaning of this technical rule are rooms in which activities involving biological agents are performed for research, development, teaching or investigative purposes, e.g. in human medicine, veterinary medicine, biology, biotechnology, production of biologicals, environmental analysis and quality assurance.

The term laboratories also includes functional rooms such as brooding areas, centrifuge rooms, cold-storage or freezer rooms, and rooms for inactivation of biological agents if these rooms are used for activities within the meaning of article 2 (7) of the Biological Agents Ordinance.

Notes: *TRBA 100 also applies to facilities and practices in the fields of laboratory medicine, medical microbiology and/or hygiene, and environmental medicine. This also includes laboratories in the fields of transfusion medicine and pathology, and, where applicable, laboratories in doctors' practices in the fields of e.g. dermatology, urology and internal medicine.*

For laboratory activities in doctors' practices or pharmacies and dental facilities, however, it is not strictly necessary to use TRBA 100, provided these activities are minor in type and scale, as these are covered by TRBA 250 [9]. Laboratory activities of this kind include, in particular:

- *preanalytical activities such as sample preparation and work-up for analysis (e.g. addition of reagents such as EDTA, centrifuging to extract plasma or for the urine sediment);*
- *the application of simple, rapid laboratory tests and microscopic detection methods;*
- *the application of indicative diagnostic cultivation methods in closed systems, such as dip culture media, without further diagnostics;*
- *sample storage and sample packaging for transport.*

If further diagnostic work beyond that described above takes place (in particular cultivations), this is subject to the requirements of TRBA 100. This can also apply, for example, to diagnostic investigations in veterinary practices.

The applicable TRBA in the individual case is to be determined within the framework of the risk assessment.

3.4 Protection level area

The protection level area comprises a spatial unit that is assigned to a specific protection level. In laboratories of high protection levels, the associated airlocks also form part of the protection level area.

3.5 Hygiene plan

The hygiene plan within the meaning of this technical rule is a compilation of personal and object-specific measures for reducing (microbiological) contamination, e.g. of hands, materials/objects, or surfaces by potentially hazardous biological agents. It contains information on the means to be used (concentration, exposure time, frequency of application) and states the target group that implements these measures. Employees are to be made aware of the hygiene plan in a suitable manner (e.g. with a notice in tabular form and a briefing).

3.6 Inactivation

Inactivation is the irreversible destruction of the reproductive and infectious ability of biological agents.

3.7 Sterilisation

Sterilisation is the killing of all microorganisms that are capable of multiplying, including their dormant stages, as well as the inactivation of viruses through physical or chemical processes.

3.8 Decontamination

Decontamination is the reduction of biological agents' concentration to a degree that does not present a health risk.

3.9 Disinfection

Disinfection is the targeted treatment of materials, objects or surfaces with physical or chemical processes so that they no longer present a risk of infection.

4 Risk assessment

4.1 General Remarks

(1) Before activities involving biological agents begin, the employer must conduct and document a risk assessment with professional expertise in accordance with article 4 of the Biological Agents Ordinance. A person with professional expertise is someone who is qualified to perform a task specified in this ordinance. The requirements for professional expertise depend on the respective task type and level of risk. The necessary knowledge to constitute professional expertise must be demonstrated by way of suitable vocational training and recent, relevant professional activity. Depending on the task and the level of risk, it may also be necessary for the employee to attend specific further training. If the employer does not possess the necessary expertise itself, he/she shall seek expert advice (article 4 paragraph 1 of the Biological Agents Ordinance).

Note: *TRBA 400 [10] provides general instructions for conducting a risk assessment. Requirements for professional expertise can be found in TRBA 200 "Requirements for professional expertise in accordance with the Biological Agents Ordinance" [11].*

(2) As set out in article 4 paragraph 3 of the Biological Agents Ordinance, the employer must obtain for the risk assessment information on, in particular, the identity, risk-group classification and transmission routes/uptake pathways of the biological agents used and/or that may be present, as well as the associated health hazards (effects that are infectious, sensitising, toxic, or otherwise harmful to health). In doing so, they must take into account the specific activities and workflows, as well as the possible exposure scenarios and relevant transmission routes associated with these agents. This information forms the basis for the assignment of the protection levels and the definition of the necessary protective measures.

Note: *Current decisions, statements and recommendations of the ABAS are published at www.baua.de/abas.*

(3) Classification into a corresponding risk group constitutes a definitive statement on the infection risk associated with a biological agent. The binding classifications are listed in technical rules TRBA 460–468 [4–8].

If a biological agent is not listed there, the employer must classify it according to the current state of scientific research. In the case of attenuated biological agents, one may deviate from the classification of the wild type if the attenuation of virulence is sufficiently demonstrated. This requires reliable identification and accurate knowledge of the type and extent of attenuation. If the parental strain is classified into risk group 3 or 4, a reduction is only permitted on the basis of a scientific assessment, which the ABAS can perform.

For non-listed biological agents, the BMAS can classify the agent into a risk group following consultation by the ABAS.

Note: *TRBA 450 describes criteria for the classification of biological agents into risk groups [12].*

(4) In activities involving biological agents, these agents may also produce effects that are sensitising, toxic or otherwise harmful to health separately from their infectiveness, which is used to determine their risk-group classification. These effects must be considered separately when defining suitable measures for minimising the associated health hazards (see number 4.5). Information on corresponding properties can be found in the technical rules TRBA/TRGS 406 [13], TRBA 460 [4], TRBA 464 [6] and TRBA 466 [7].

(5) In addition to the above hazards for employees due to activities involving biological agents, account must also be taken of the resulting hazards for other persons. Furthermore, other hazards must also be included, such as those associated with the environment (e.g. according to genetic engineering legislation and the Animal Pathogens Ordinance). The necessary measures in each case must be coordinated with one another.

(6) In accordance with article 4, paragraph 2 of the Biological Agents Ordinance, the risk assessment must be reviewed regularly (at least every two years) and updated as required. It must be updated immediately if this is required in the light of significant changes to the working conditions or new information (e.g. knowledge obtained through occupational healthcare check-ups), or if there are reasons to believe that the defined protective measures are not sufficiently effective.

4.2 Activity-specific hazards

(1) The possible hazards for employees in laboratories depend on the respective assigned task, the associated type and quantity of materials or biological agents used, and the specific working procedures and activities.

(2) In the assessment of activity-specific hazards, particular consideration must be made of the dangers arising through exposure, taking into account the possible transmission routes and/or uptake paths. In doing so, one must also bear in mind that, depending on the level of activity-related exposure, transmission routes other than the natural ones can play a significant role.

Uptake of biological agents can occur during laboratory activities:

- aerogenically – via the air (inhalation);
- orally – via the mouth (ingestion);
- percutaneously – through injured or uninjured skin or mucous membranes (contact infection);
- vectorially (bite or sting by carriers).

Activities with an elevated risk of exposure include, for example:

- opening sample containers,
- working on open cultures,
- pipetting,
- centrifuging,
- cell disruption,
- emptying containers,
- cutting samples,
- maintaining the functionality of automated processes (e.g. disposal of contaminated wash buffers, replacing contaminated cannulas).

Specific hazards can arise through accidental contamination, e.g. through spillage, breakage or leakage, through injury by pointed, sharp instruments (such as syringes and cannulas), or through operating errors.

(3) If activities involving endoparasites are planned, account must be taken in the risk assessment of the life-cycle stages of parasites that can infect humans and that are relevant to the activity.

Note: *For more information on parasites, please refer to annex 1 to this technical rule and the corresponding information in TRBA 464 [6]. In the case of activities involving ectoparasites, it must be taken into account that these can act as carriers (vectors) for biological agents that are pathogenic to humans.*

4.3 Assignment of protection levels

Assignment to specific or non-specific activities is of key importance for the process of defining the necessary protection level (see article 5 paragraph 1 of the Biological Agents Ordinance). Typical laboratory workflows frequently include both types of activities.

Specific activities are directly based on a specific biological agent whose species/subspecies is known, and where the degree of exposure of the employee in intended operation is sufficiently known or can be estimated. For example, a specific activity is the propagation of bacteria as a pure culture or the propagation of a defined virus species using cell cultures.

Non-specific activities are activities where none of the above criteria for specific activities are met. A non-specific activity is, for example, the examination of human samples (e.g. blood, smears, tissue samples) within the framework of microbiological, clinical/chemical or other special diagnostics. This also applies to samples originating from a donor in whom infection is clearly suspected or has been found, provided this is not based on the corresponding biological agent. Cytological or histological examinations of materials that have not been inactivated also constitute non-specific activities.

4.3.1 Assignment of protection levels for specific activities

With specific activities, the necessary protection level corresponds to the risk group of the biological agent used. For specific activities involving biological agents of different risk groups, the protection level is assigned based on the classification of the biological agent of the highest risk group.

4.3.2 Assignment of protection levels for non-specific activities

For non-specific activities involving biological agents, one must determine the spectrum of biological agents to be expected or that might be present. The risk groups and properties of the biological agents must be taken into account when estimating the possible infection risk. An **activity-specific overall assessment** must be conducted based on the individual assessments. Here, the assignment to a protection level need not necessarily be determined by the biological agent with the highest risk group, but rather by the overall risk identified through assessment of the exposure situation.

The following factors, in particular, can determine how the overall risk is assessed:

- specific **properties** relevant to classification,
- the **infectious dose**,
- **stages** of specific infection risks,
- **probabilities of occurrence** (e.g. incidence, prevalence),
- **concentrations** and **culture volumes**,
- activities involving **aerosol formation**,
- type and proportion of **manual** working steps,
- activities involving **a risk of injury.**

Examples of assigned protection levels for non-specific activities are listed in section 4.4.

4.3.3 Distinction of non-specific and specific activities

(1) In various areas of work, e.g. medical diagnostics or microbiological research, a transition can arise from non-specific activities to specific activities within the framework of the investigations. This is the case, for example, if the biological agent identified in initial diagnosis is then specifically propagated for the purpose of further characterisation.

This can occur, among others, in the following situations:

- further characterisation of isolates,
- subtyping,
- determination of chemotherapeutic resistance.

Since these are specific activities, the protection level is based on the risk group of the relevant biological agent (see number 4.3.1).

(2) If defined control strains are used in diagnostic detection processes, these are also specific activities. If attenuated laboratory strains are available, these must be reverted to under the substitution requirement set out in article 8 paragraph 4 number 1 of the Biological Agents Ordinance.

Note: *If necessary, it is permissible to deviate from the classification of the wild type in accordance with article 3 paragraph 4 of the Biological Agents Ordinance. See TRBA 450 [12].*

(3) Non-specific activities also include the storage and (within the framework of waste disposal) inactivation of the sample or the isolated biological agent following successful identification and/or diagnosis, provided this is not followed by further specific activities.

4.4 Example assignment of protection levels for non-specific activities

4.4.1 Medical/veterinary laboratories

(1) Human samples (body fluids, tissue, cell cultures, etc.) whose infection status has not been further characterised must be considered as **potentially infectious**. For this reason, corresponding activities must generally be conducted under the conditions of **protection level 2**.

(2) If the infection status of the sample is not known and an infection with HIV, HBV or HCV is present, it must be checked whether the protective measures of protection level 2 are sufficient for the specific activity according to the criteria stated in number 4.3.2. This is the case, for example, if the sample is inactivated rapidly or if a largely automated process is used.

Otherwise, the protective measures of **protection level 3** are to be applied according to number 5.4.1.

(3) If the sample material's infection status is known, if biological agents of risk group 3 are present, and if the activities do not focus on these agents, a check must be performed according to the criteria stated in number 4.3.2 within the framework of the risk assessment to establish whether the protective measures of protection level 2 are sufficient (if necessary with individual protective measures that are to be additionally defined).

If this is not the case, the activities must be carried out under the conditions of **protection level 3** according to number 5.4.2.

(4) If an infection with a biological agent of risk group 4 is suspected, all indicative examinations of the primary sample with material that has not been inactivated must be conducted at least under the conditions of **protection level 3**.

(5) If no pathogens of risk group 2 and higher are present according to the characterisation of human sample materials from clinically normal donors, the conditions of **protection level 1** are sufficient. This is the case, for example, if the samples are HIV-, HBV- and HCV-negative. One can then proceed on the basis that, although a risk of infection by other pathogens cannot be ruled out, it is negligible if the general hygiene measures are complied with.

(6) The conditions of **protection level 2** are sufficient for carrying out activities within the framework of diagnosing tuberculosis, e.g. direct microscopic examination to detect acid-fast bacilli, culturing in liquid and solid culture media (based on primary material), and sample treatment and inactivation for the purpose of conducting molecular biological techniques (PCR).

The examination of mycobacteria of risk group 3 that are present as a pure culture or highly enriched cultures, e.g. within the framework of final identification using physiological tests or for testing sensitivity to antituberculosis medicines, is a **specific** activity. This must be carried out under the conditions of **protection level 3** according to number 5.4.2.

(7) Activities within the framework of anthrax diagnosis are to be assigned to **protection level 2** in the case of indicative diagnostic examinations of:

- samples of human or animal origin such as smears, blood, etc.;
- environmental samples, such as soil samples, that might contain anthrax pathogens.

Indicative diagnostic examination includes the production and assessment of microscopic preparations, the creation and assessment of cultures, and, where applicable, direct serological and molecular biological examinations of the specimen.

Until the inactivation is complete, the requirements of **protection level 3** must be observed during further diagnostics, i.e. the final differentiation (ruling out or confirming anthrax pathogens) of the suspected bacteria as propagated in the primary culture using microbiological, biochemical and molecular biological techniques, as well as during diagnostic animal testing.

Note: *Annex 3 to TRBA 130 [14] applies to the analysis of suspicious samples in biohazardous situations.*

(8) **Decision 603 of the ABAS** “Protective measures for activities involving Transmissible Spongiform Encephalopathy (TSE) associated agents in TSE laboratories” [15] applies to activities involving materials that contain or may contain TSE-associated agents.

(9) Laboratories in which activities involving animal samples from vertebrates (excluding primates) are carried out are to be assigned to **protection level 1**, provided that the donor animals show no symptoms of disease. In general, one can then proceed on the basis that, although a risk of infection by other pathogens cannot be ruled out, it is nevertheless negligible if the general hygiene measures are complied with. If there is reasonable suspicion that an infection with a zoonotic pathogen is present, the protective measures of at least **protection level 2** must be observed.

(10) Activities involving uncharacterised materials from primates are to be assigned to **protection level 2**. If in the case of a disease in the donor animal or other indications, pathogens of a higher risk group are to be expected (e.g. in samples from wild animals) the protection level must be specified within the framework of the risk assessment.

(11) Activities involving samples from animals used for experimental purposes that are known to carry or to have been infected with biological agents that are pathogenic to humans are to be assigned to a protection level appropriate to the biological agent's risk group. Deviations from this are permissible in some circumstances if it is ascertained in the risk assessment that the risk of infection has decreased significantly (see TRBA 120 number 3.4) [16].

4.4.2 Other microbiological laboratories, environmental-analysis laboratories

(1) As a general rule, the majority of environmental samples (water, soil, sediments, air, etc.) are to be considered as non-infectious, even if they might contain biological agents of risk group 2 to a certain extent. For this reason, activities involving these materials can generally be carried out under the conditions of **protection level 1**. If particular contamination of the environmental habitats by biological agents that are pathogenic to humans is suspected,

it must be defined within the framework of the risk assessment whether the activities are to be carried out under the conditions of **protection level 2**.

Activities involving enriched microbial fractions that are produced through specific purification or selective propagation, for example, are to be carried out under the conditions of **protection level 2** if a concentration of biological agents of risk group 2 or higher can be assumed. It is to be defined in the risk assessment in the individual case whether an even higher protection level is necessary.

(2) Waste water (polluted water) and sewage sludge contain biological agents that are pathogenic to humans and whose composition and concentration can vary widely depending on their origin and the respective process. Small-scale activities taking place occasionally, e.g. occasional turbidity measurements can be carried out under the conditions of protection level 1. Regular and more-extensive activities involving corresponding samples are to be carried out under the conditions of **protection level 2**.

Note: *Annex 2 to TRBA 220 contains an overview of biological agents occurring in waste water [17].*

(3) Biological agents of risk groups 1 and 2 are usually present in sample materials from waste, compost and rotting materials. If infectious biological agents could be enriched or propagated during the examination of such samples, these activities are generally to be carried under conditions of **protection level 2**.

Note: *Samples of this kind generally also contain biological agents with sensitising and toxic effects (see number 4.5).*

4.4.3 Microbiological quality assurance/sterility tests

(1) Laboratories in which **sterility tests**, determinations of colony count and other biological quality-assurance work is carried out that does not serve to specifically detect biological agents of risk group 2 and higher can be carried out under the conditions of **protection level 1**. This includes, for example, samples from food production, medical products, medicines, biologics or cosmetics.

If, during the course of the activities, biological agents of risk group 2 or 3 are selectively propagated or enriched, the activities must be carried out under the conditions of at least **protection level 2**.

(2) Samples from the manufacture of biologics, e.g. plasma proteins, recombinant proteins or other products created from biological materials, are examined with regard to contamination with bacteria, viruses and other microorganisms. Since these intermediate and final products originate from tested source materials, these analyses can be carried out under the conditions of **protection level 1**.

4.5 Determination of protective measures based on biological-agent effects that are sensitising, toxic, or otherwise harmful to health

(1) Biological agents with sensitising or toxic effects can play a significant role, in particular:

- in research laboratories with corresponding investigative focuses;
- in environmental analysis laboratories;
- in microbiological quality assurance, e.g. in the food production industry.

(2) If biological agents of risk group 1 have sensitising or toxic effects, further suitable protective measures must be defined in addition to the general hygiene measures of protection level 1. In general, these are also measures that serve to minimise or prevent contact with

biological agents and/or the formation of bioaerosols, e.g. the use of a microbiological safety workbench (MSW) (see number 5.2.2).

(3) In protection level 2, it can be assumed that the release of biological agents with effects that are sensitising, toxic and otherwise harmful to health is sufficiently minimised if the required structural, technical and organisational measures are implemented.

(4) It must be taken into account that biological agents with sensitising or toxic effects may retain their sensitising or toxic potential even after inactivation. In such cases, therefore, the corresponding protective measures must be implemented within the framework of the risk assessment even after these biological agents have been inactivated.

5 Protective measures

5.1 General

(1) Before using biological agents that are harmful to health, the employer must check whether these can be replaced by less harmful alternatives (substitution requirement). This is possible in individual cases for specific activities, e.g. if a less pathogenic strain is available and the experimental aim can equally be achieved with this strain or the corresponding wild-type strain. For non-specific activities, the employer generally cannot comply with the substitution requirement. In the field of medical research, however, it is possible in individual cases to revert to characterised specimens (HIV-, HBV- and HCV-negative). In such cases, the substitution requirement must be complied with.

(2) Working procedures and working equipment must be designed so that biological agents cannot be released at the workplace. If this is not possible, exposure of employees must be reduced to a minimum through suitable technical protective measures and organisational measures. These fundamentally take priority over individual protective measures. Suitable Personal Protective Equipment (PPE) need only be worn if technical and organisational measures alone are insufficient to achieve the protection objective. This must be defined within the framework of the risk assessment (see number 4).

(3) Taking into account the state of the art and of scientific knowledge, working procedures are to be preferred:

- that are performed in a largely automated fashion;
- in which only a few manual steps with the least possible volumes are necessary;
- in which aerosol formation is minimised;
- in which the material is inactivated quickly;
- in which the devices used can be decontaminated.

If the state of process technology has developed and therefore occupational safety has improved significantly, this technology must be introduced if the activity allows.

(4) Safety-relevant devices and systems such as microbiological safety workbenches (MSW), laboratory centrifuges subject to compulsory inspection, autoclaves and air-conditioning systems must be maintained. This requires regular checking of their correct operation or operating safety and, if necessary, their repair.

(5) For activities involving biological agents in laboratories, the necessary hygiene rules must always be observed. These also include the prohibition on storing and consuming food, beverages and tobacco in the corresponding protection-level areas. The employer must provide suitable, easily accessible areas for this.

The operational hygiene measures must be recorded in a hygiene plan for activities involving sensitising or toxic biological agents, as well as for activities in protection level 2 and higher. Here, the special cleaning and decontamination procedures must be specified. The hygiene

plan must be publicised in a suitable manner (see paragraphs 6 and 7). Compliance with the plan must be monitored.

Note: *A sample hygiene plan can be found in BGI 629 [18].*

(6) In accordance with article 14 paragraph 1 of the Biological Agents Ordinance, operating instructions must be prepared and updated as required. This is not necessary if only activities are performed that involve biological agents of risk group 1 without sensitising or toxic effects.

In particular, the operating instructions must include the following items:

- The hazards arising during the course of the activities, and especially:
  - the biological agents used or that might possibly occur, along with their risk groups;
  - the relevant transmission routes or uptake pathways.
- Protective measures and code of conduct:
  - exposure-prevention measures;
  - internal hygiene measures, referring to the hygiene plan where applicable;
  - wearing, application and taking off of personal protective equipment.
- Conduct in an emergency and in the event of accidents and breakdowns.
- First-aid measures, with information on post-exposure prophylaxis (PEP) where applicable.
- Measures for disposing of contaminated solid and liquid waste.

Note: *Examples of sample operating instructions can be found in TRBA 500 [19], TRBA 250 [9] and BGI/GUV-I 853 "Operating instructions as specified in the Biological Agents Ordinance" [20].*

(7) All employees working in laboratories, including the employees of external companies and other persons (e.g. interns), must be briefed on the hazards arising in their activities involving biological agents and the necessary protective measures. This must take place before the activities begin or when there are significant changes to the activities and then at regular intervals, but at least annually, in verbal form and in a manner specific to the workplace.

The briefing is carried out on the basis of the operating instructions and the company's hygiene measures (hygiene plan). The content and scheduling of the briefings must be recorded in writing and signed by the instructed persons by way of confirmation.

The briefing should be designed so that it improves the employees' awareness of safety. The implementation of the briefing's contents must be monitored.

A general occupational-health consultation should be conducted as part of the briefing (see number 6.1).

Notes: *If work is being carried out by employees of different employers (e.g. cleaning, construction and maintenance companies), the duty of coordination according to article 8 of the Occupational Safety and Health Act [21] must be observed. The activity-specific protective measures of these technical rules, including the responsibilities, as well as the implementation and contents of the briefing, are to be agreed between the employers involved. The agreement must be made in writing and is binding.*

(8) The number of employees carrying out activities involving biological agents of risk group 2 or higher must be restricted to the necessary number. The same applies to activities involving biological agents with effects that are sensitising, toxic or otherwise harmful to health.

(9) In the case of specific activities, the identity of the biological agents used must be checked and documented regularly, provided this is necessary for assessing the hazard po-

tential. This is not necessary if it can be ensured through other procedures that the identity is preserved, e.g. by reverting to master cultures.

(10) In implementing the measures set out in this technical rule, it is necessary to consider the individual circumstances in the workplace and the type of activity. One may deviate from a measure set out in this technical rule if the results of the risk assessment permit this or if a measure is taken that affords comparable protection to employees. The equivalence must be demonstrated at the authorities' request.

The following sections, numbered 5.2 to 5.5, respectively contain all specific protective measures for the corresponding protection levels. Protection level 3, for activities involving biological agents of risk group 3 that are labelled with (**) (see number 5.4.1), builds on the protective measures described for protection level 2 (see number 5.3).

5.2 Protection level 1

5.2.1 Activities of protection level 1 without hazards due to sensitising or toxic effects

In these activities, a risk of infection for employees is unlikely. It is therefore sufficient to ensure that the laboratory operates in accordance with the requirements, and in compliance with the basic rules of good microbiological technology (GMT).

Structural and technical protective measures

(1) Laboratories of protection level 1 are to consist of restricted spaces of sufficient size. Depending on the activity, an adequate work surface must be ensured for each employee.

(2) Surfaces (work surfaces, floors) are to be easy to clean and must be resistant to the substances and cleaning agents used.

(3) Depending on the laboratory's use, the doors are to open in the direction of the escape route and are to be fitted with a viewing window for reasons of personal safety.

Note: *This applies, in principle, to laboratories that come within the scope of TRGS 526 [2].*

(4) A wash basin with dispensers for hand detergent and disposable towels must be present in the working area.

Organisational protective measures

(5) Windows and doors are to remain closed during work.

(6) Working areas are to be kept tidy and clean. Only the work equipment that is actually needed may be present on the work surfaces.

(7) Pipetting aids must be used.

(8) Cannulas and pointed and sharp objects are only to be used if absolutely necessary. Used cannulas and pointed and sharp instruments must be collected and disposed of in puncture-resistant waste containers that can be sealed firmly. Cannulas must not be reinserted into the needle cover.

Notes: *Number 4.2.5 paragraph 6 of TRBA 250 [9] describes the requirements for puncture-resistant waste containers.*

Annex 2 to TRBA 120 [16] must be taken into consideration if the use of pointed and sharp instruments is planned within the framework of animal experiments of protection level 1.

(9) Liquid and solid waste containing biological agents must be collected and disposed of properly. The waste may be disposed of without pretreatment provided this does not violate other regulations (e.g. water, waste or genetic engineering legislation).

(10) After the activity is completed or after contamination by biological agents, the hands must be cleaned carefully and cared for in accordance with the skin-protection plan.

Note: *In activities requiring disinfection of the hands, no jewellery, watches or wedding rings may be worn on the hands and forearms. Fingernails are to be cut short.*

Personal protective equipment/protective measures

(11) Lab coats or other protective clothing must be worn in the protection level area. Used lab coats must be stored separately from street clothes.

5.2.2 Activities of protection level 1 with hazards due to sensitising or toxic effects

For activities involving biological agents of risk group 1 that exhibit sensitising or toxic effects, a health risk to employees is possible. For this reason, further protective measures that minimise employees' exposure must be defined within the framework of the risk assessment **in addition** to the measures stated in number 5.2.1.

In particular, the following measures can come into question:

Structural and technical protective measures

(1) Activities in which airborne biological agents (e.g. in spore-forming development phases of fungi or actinomycetes) can be released or other bioaerosols can be formed must be carried out in a microbiological safety workbench (MSW) or in a facility providing comparable personal protection (e.g. fume cupboard with high-efficiency particulate absorption filter).

Notes: *For protective measures in the case of respiratory sensitisers, see TRBA/ TRGS 406 [13].*
For information on working safely with microbiological safety workbenches, see BGI 863 [22].

Organisational protective measures

(2) Depending on the specific properties of the biological agents used, effective inactivation and cleaning measures must be specified in the hygiene plan in accordance with number 5.1 paragraph 6.

Personal protective equipment/protective measures

(3) Where applicable, additional personal protective equipment may be necessary, e.g. protective gloves or respiratory protection.

5.3 Protection level 2

The protective measures of protection level 2 are intended to prevent exposure of employees to biological agents that can give rise to an infectious disease in humans.

The requirements described below must be observed for employees' protection.

Notes: *Measures of protection level 2, which protect against the infectious effects of the biological agents present, can also provide adequate protection from effects that are sensitising, toxic, or otherwise harmful to health. This does not apply to the inactivation measures, where applicable. Sufficient protection must be specified for the individual case within the framework of the risk assessment.*

The relevant authority must be given at least 30 days' notice of the commencement, for the first time, of specific activities involving biological agents of risk group 2 (article 16 (1) number 1 and article 16 (3) of the Biological Agents Ordinance).

Structural and technical protective measures

(1) Laboratories must be made up of sufficiently large spaces that are structurally separated from other spaces and usable areas in which no activities involving biological agents are carried out.

(2) The doors of the protection level area must be fitted with a viewing window and must open in the direction of the escape route.

(3) Surfaces (work surfaces and adjacent wall surfaces, floors, surfaces on devices and apparatus that comes into contact with biological agents) must be easy to clean and resistant to the disinfectants used. A seamless connection must be ensured between the wall and floor.

(4) For disinfecting and cleaning the hands, a wash basin, preferably a separate hand-wash basin, must be present, along with dispensers for disinfectant, hand detergent and disposable towels. As a matter of priority, water fittings and disinfectant dispensers must be set up in a manner that allows them to be operated without using the hands. The installations must be installed so that they are easily accessible and preferably near the laboratory door. Eye-washing facilities must be present.

Note: *In laboratories for the propagation of cell cultures, the wash basin may also be located in an adjacent area for product-protection reasons. A disinfectant dispenser must be kept available in the laboratory.*

(5) Activities in which a hazard due to bioaerosols is to be expected must be carried out in a microbiological safety workbench (MSW) or in a facility providing comparable personal protection (e.g. fume cupboard with high-efficiency particulate absorption filter).

Note: *For information on working safely with microbiological safety workbenches, see BGI 863 [22].*

(6) In general, devices are to be used that do not release bioaerosols (e.g. centrifuges with sealed rotors or centrifuge tubes).

(7) An autoclave of sufficient size and that is suitable for the requirements of inactivation, or a similar facility (e.g. thermal disinfection equipment), is to be available in the same building.

Notes: *Inactivation in a central facility within the company premises or proper contract disposal may be carried out if the same protection objective is achieved. Transport outside of the company premises is subject to the dangerous goods regulations for class 6.2 "Infectious substances".*

(8) Contaminated process exhaust air must not be emitted into the working area without treatment. It must be decontaminated through suitable processes such as filtration or thermal post-treatment.

Note: *This applies, for example, to the exhaust air from autoclaves, pumps or bioreactors. Please refer to ABAS statement [23] for more information on the treatment of exhaust air from autoclaves.*

Organisational protective measures

(9) The access door to the protection level area must be permanently labelled with the protection level and the "biohazard symbol" (annex 1, Biological Agents Ordinance) so that it is clearly visible from the outside.

Note: *Requirements for the symbol can be found under warning sign W 009 "Warning; Biological hazard" in accordance with annex 1 to ASR A 1.3 [24].*

(10) Windows and doors are to be kept closed during activities involving biological agents.

(11) The number of persons who are allowed to gain access must be limited to designated employees. Other persons must only access the protection level area with permission from the person responsible.

Controlled access (e.g. by way of electronic access control) to the protection level area is necessary if activities are carried out involving biological agents that are pathogenic to humans and listed in Regulation (EC) No. 388/2012 [25] for the control of export of dual-use items.

(12) Biological agents of risk group 2 must be kept safely in tightly sealed containers. If these are biological agents that are pathogenic to humans, as listed in Regulation (EC) No. 388/2012, they must be kept under lock and key.

(13) Working areas must be kept tidy and clean. Only the work equipment that is actually needed may be present on the work surfaces. The laboratory must be cleaned regularly. Work surfaces must be decontaminated and cleaned once the activity is completed, and contaminated equipment must be decontaminated and cleaned after use. Any accidental contamination must be eliminated immediately.

(14) Pipetting aids must be used.

(15) Contaminated liquid and solid waste (e.g. cultures, tissue, samples containing bodily fluids) must be collected safely in suitable, sealable containers and inactivated in a suitable process for waste of this kind (see paragraph 7). Physical or chemical processes that are proven to be effective in relation to the specific pathogen are to be used for the inactivation.

Notes: *Suitable thermal processes include autoclaving, for which the nature of the infectious waste must be considered, and incineration in an approved incineration plant (proper contract disposal). Approved transport containers must be used for transport outside of the company premises.*

For more information on treatment of carcasses, see TRBA 120 [16].

(16) After work is completed, the hands must be disinfected even if protective gloves were worn and must be cared for in accordance with the skin-protection plan. Skin-protection and skin-care products must be provided in containers, e.g. tubes, that are protected from contamination.

Notes: *TRGS 401 "Risks resulting from skin contact – identification, assessment, measures" must be observed [26].*

(17) In activities requiring disinfection of the hands or the wearing of gloves, no jewellery, watches or wedding rings may be worn on the hands and forearms. Fingernails must be cut short.

(18) Activities involving needles, syringes and other pointed and sharp instruments and objects must be limited to the extent that is absolutely necessary and may only be carried out under the application of corresponding safety precautions. It must be checked whether alternative processes exist and to what extent the danger due to punctures and cuts can be reduced, e.g. by using equipment with a safety mechanism. As far as technically possible, safe working equipment must be used in preference. After use, pointed and sharp instruments, including instruments that have been secured, must be collected and disposed of in puncture- and break-resistant containers.

Notes: *Number 4.2.5 of TRBA 250 [9] describes the requirements for puncture-resistant waste containers. If blood samples are to be taken from patients in diagnostic facilities, the requirements of TRBA 250 number 4.2.5 for the use of safety equipment shall apply.*

In order to minimise the risk of injury by cutting, a sled microtome, for example, must be provided with a knife guard in pathological/histological laboratories and the knives must be changed using blade boxes.

(19) The transport of biological agents or materials that contain or might contain biological agents outside of the protection level area must be carried out in closed, rigid, break-proof and liquid-tight vessels that can be disinfected from the outside and can be marked and labelled in a permanent fashion. It must not be possible for external influences to open these vessels accidentally.

(20) If contamination of the secondary packaging and the request forms is determined in the sample-delivery area, these must be disinfected and, where applicable, relabelled. Sample vessels must allow safe opening.

(21) Before maintenance work is carried out, the laboratory personnel must disinfect or arrange for the disinfection of working areas, including the devices and facilities to be maintained. This also applies to devices/equipment that are transferred for repair.

If disinfection is not possible, suitable personal protective equipment must be provided for the repair staff. The additional protective measures required specifically for the activity must be stipulated in writing in working instructions. The employees must be given workplace- and activity-specific briefings (see number 5.1 paragraph 7). The person responsible must issue a written work authorisation for the repair work.

(22) If sample vessels containing specimens are kept unsealed for a while, e.g. during parallel work-up of a large number of samples, they must be prevented from falling over and kept in a collecting reservoir. They must be securely sealed once the pipetting processes are completed.

Personal protective equipment/protective measures

(23) Personal protective equipment, including suitable protective clothing, is to be provided in accordance with the risk assessment and worn by employees.

The protective clothing comprises at least a lab coat. Protective gloves are to be worn according to the activity, but must always be worn if the hands can come into contact with biological agents, potentially infectious materials, or contaminated objects, surfaces or equipment. If splashes to the face are to be expected, face protection must be used (e.g. safety goggles, mask or face shield).

Protective clothing and other personal protective equipment must be taken off when leaving the protection level area. Personal protective equipment, including protective clothing, must be kept separately from other work clothes and street clothes.

(24) When handling infectious tissue, e.g. during cutting or microscopic examinations, personal protective equipment must be supplemented with disposable aprons. Safety goggles are required when opening cavities; depending on the risk assessment, respiratory protection must be worn if necessary when cutting cysts and lymph nodes, as well as for quick sections.

Note: *Cutting tables with an air-extraction system are recommended in histological laboratories because of the risk posed by formalin.*

5.4 Protection level 3

The protective measures of protection level 3 are intended to **prevent** exposure of employees to biological agents of risk group 3 that can give rise to a serious infectious disease in humans. These measures also serve to protect other persons and are at the same time suitable for protecting the environment.

5.4.1 Activities involving biological agents of risk group 3 that are labelled with (**)

Certain biological agents of risk group 3 that are not normally transmitted by air are marked with two asterisks within the framework of the classification of biological agents. For simplicity's sake, these are referred to in the following as *"biological agents of risk group 3(**)"*. In accordance with Directive 2000/54/EC [3], certain measures of protection level 3 can be dispensed with for these biological agents. It is the responsibility of the Member States to check which measures are to be taken in light of the relevant biological agents' specific properties.

Note: *Contrary to the Directive's stipulation, this section does not define which measures of protection level 3 can be dispensed with, as this approach has shown not to be user-friendly in practice. Rather, the additionally required measures are designated based on the measures of protection level 2. This does not affect the level of protection.*

In addition to the measures of protection level 2 (number 5.3), the requirements described below must be observed for the purpose of protecting employees. They apply to specific activities involving biological agents of risk group 3(**), as well as to non-specific activities involving biological agents of risk group 3(**), if the results of the risk assessment show that the protective measures of protection level 2 are not sufficient. The species-specific protective measures listed in annex 1 must be taken into consideration.

Notes: *The relevant authority must be given at least 30 days' notice of the commencement of these activities for the first time (article 16 (1) number 1 and article 16 (3) of the Biological Agents Ordinance).*
Activities involving certain developmental stages of parasites of risk group 3(**) can be carried out at protection level 2, as they are associated with a lower risk of infection. The corresponding stages, as well as the necessary special protective measures, where applicable, are shown in annex 1.

Structural and technical protective measures

(1) Waste water arising within the protection level area from wash basins and showers must be thermally post-treated. Alternatively, other validated inactivation procedures can be used. Post-treatment can be dispensed with if the results of the risk assessment showed that the arising waste water does not pose a hazard outside of the protection level area. If the laboratory is operating in accordance with the requirements, it can be assumed that the waste water from the hand wash basin is not contaminated with biological agents and does not therefore require post-treatment.

(2) A suitable facility must be present for communication between the laboratory and the outside area. The conditions under which it is possible to work alone must be specified within the framework of the risk assessment.

(3) The safety lighting in the protection level area must be designed so that it is possible to stop work safely in the event of a power failure.

(4) The protection level area must have its own equipment (laboratory equipment).

Note: *In accordance with annex 1, a suitable anteroom is required for activities involving some biological agents of risk group 3(**).*

Organisational protective measures

(5) In addition to the "biohazard symbol", the access door to the protection level area must also be permanently labelled with the notice "Protection Level 3, Restricted to Biological Agents of Risk Group 3(**)", which must be clearly visible from the outside, along with a reference to the access restriction.

(6) The person responsible must restrict access to the protection level area to persons who are necessary for carrying out the activities. An access-control system is required. In justified individual cases, the person responsible may allow access by other persons (e.g. service personnel) under the supervision of personnel with professional expertise.

(7) The protective measures to be observed when removing and decontaminating HEPA filters from a microbiological safety workbench (MSW) are to be specified based on the risk assessment. Corresponding working instructions must be present.

If the risk of infection while changing the filter cannot be ruled out for the maintenance personnel and other persons due to the biological agents used, the relevant transmission routes, and the MSW usage conditions, the filters must be decontaminated while still installed. This can be carried out by *in situ* fumigation with hydrogen peroxide or formaldehyde according to the list of disinfectants and disinfectant procedures [27] tested and recognised by the Robert Koch-Institute (RKI) (see number 5.4.2 paragraphs 25 and 26).

In the event of deviations from the specific procedures listed by the RKI, the effectiveness of the measures taken must be validated.

Notes: *With HEPA filters from MSW in which work involving TSE agents has been carried out, the procedure must be in accordance with ABAS statement [28]. Formaldehyde stabilises the infectivity of TSE agents.*

(8) For specific activities that pose a greater risk, working instructions must be prepared in addition to the operating instructions. Due to the risk of injury and the associated risk of infection, this includes activities involving pointed and cutting instruments, e.g. taking sample materials from animals (see number 5.3 paragraph 16).

Personal protective equipment/protective measures

(9) The protective clothing and personal protective equipment provided for the activities must be put on and, after completing the activity, taken off inside the protection level area. Within the protection level area, an area suitable for putting on and taking off the protective gown must be set up at the entrance. If a vestibule is required in accordance with annex 1, the protective clothing/personal protective equipment shall be put on and taken off here. Suitable collection containers that can be decontaminated must be provided in the area of the entrance or in the vestibule for used protective clothing or personal protective equipment that is to be cleaned.

The protective clothing consists of at least a rear-closing gown with labelling (e.g. of contrasting colour to the protective gowns worn in the other protection level areas), closed shoes, and suitable protective gloves (and with an AQL value $\leq$ 1.5). Subject to the results of the risk assessment, suitable mouth and nose protection (splash and contact protection) and safety goggles (splash protection) might be necessary, depending on the activity.

5.4.2 Activities involving biological agents of risk group 3

The following protective measures must be observed for specific activities involving biological agents of risk group 3 and non-specific activities that have to take place in laboratories of protection level 3.

This section includes <u>all</u> specific protective measures for laboratories of protection level 3 and does not build on numbers 5.2 to 5.4.1.

Notes: *These activities fall under the licensing requirement as set out in article 15 paragraph 1 of the Biological Agents Ordinance. A reliable person with professional expertise must be appointed in accordance with article 10 paragraph 2 of the Biological*

Agents Ordinance (see TRBA 200 "Requirements for professional expertise according to the Biological Agents Ordinance" [11]).

Structural and technical protective measures

(1) Laboratories in which activities of protection level 3 take place must be separated from other areas by an airlock with two interlocking, self-closing doors with a viewing window. The airlock should be of sufficient size according to the use of the protection level area.

Note: *The doors opening in the direction of the escape route should be equipped with a panic function in order to allow the employees to leave the working area in case of danger.*

(2) A disinfectant dispenser that can be operated without hand contact must be present in the airlock to allow disinfection of the hands. A hand wash basin with a hands-free water tap and dispensers for hand detergent and disposable towels must be present.

Notes: *If the laboratory is operating correctly and the organisational safety measures are observed, no contaminated waste water will arise in the airlock.*

Skin-protection and skin-care products must be available outside of the protection level area. The hands must be cared for in accordance with the skin-protection plan.

(3) A constant, controlled negative pressure must be maintained in the protection level area. A pressure gradient must exist between the airlock and the laboratory. The negative pressure present must be easy for the laboratory users to verify (it makes sense if this is also possible from inside) and must be monitored by an alarm with a visual and acoustic signal.

The exhaust air must pass through a high-efficiency particulate absorption filter or a comparable device. The return of contaminated outgoing air into working areas is not permitted. It should be possible to change the filter without releasing biological agents. This must be taken into account when planning the air-conditioning system (see paragraph 26).

Notes: *The ventilation ducts leading to the HEPA filters should be as short as possible.*
See ABAS statement [29] for more information on the use of HEPA filters.

(4) The open handling of biological agents of risk group 3 must be carried out in a microbiological safety workbench (MSW) or in a facility providing comparable personal protection. This also applies to corresponding activities with materials that must be carried out in a laboratory of protection level 3 according to the results of the risk assessment.

(5) An emergency power supply must be installed for safety-related facilities such as ventilation systems, emergency call devices and monitoring equipment.

(6) Emergency lighting must be present. This must be designed to allow work to be stopped safely in the event of a power failure.

(7) All solid and liquid waste from the laboratory must be autoclaved prior to disposal. An autoclave must be present for this purpose outside of the airlock and within the protection level area. The autoclave must be such that contaminated condensate and contaminated exhaust air are not released.

Alternatively, an equivalent, validated inactivation procedure can be used.

Note: *The condensate inside the pressurised gas container is usually sterilised in the process.*

(8) Contaminated process exhaust air must not be emitted into the working area without treatment. It must first be decontaminated by suitable processes (e.g. sterile filtration or thermal exhaust-air treatment). This also applies, for example, to the exhaust air from pumps or bioreactors.

Note: *Please refer to ABAS statement [23] for more information on the treatment of exhaust air.*

(9) Waste water arising in the working area must always be subjected to thermal post-treatment: e.g. by central waste-water sterilisation or another facility for thermal waste-water inactivation in the laboratory (e.g. using an under-sink device). Small quantities of waste water can also be collected in collection containers and then autoclaved. Alternatively, other validated inactivation procedures can be used.

Note: *A wash basin may only be present in the laboratory if the aforementioned post-treatment of waste water is ensured.*

(10) The procedure in the event of maintenance and malfunction must be taken into consideration when planning the safety-related facilities, e.g. the air-conditioning system, the waste-water inactivation system and the autoclave. Care should be taken to ensure easy access – from outside of the protection level area if possible.

(11) The spaces within the protection level area and in the contaminated part of the air-conditioning system, up to and including the first HEPA filter stage, must be sealable for the purpose of fumigation.

(12) Surfaces (work surfaces, wall and floors) must be as seamless and impermeable to water as possible, as well as being easy to clean and resistant to the disinfectants, fumigants and other chemicals used.

Note: *As a general rule, the floor must be designed with a groove to act as a trough. The junctions between permanently installed furniture and the floor or wall must be sealed.*

(13) Surfaces of devices and apparatus that can come into contact with biological agents are to be easy to decontaminate and clean.

(14) Devices must be used that do not release bioaerosols, e.g. centrifuges with aerosol-tight rotors and centrifuge tubes. Devices that are not aerosol-tight can be used in an MSW if necessary and/or, in the case of large devices, in an equivalent physical safety facility. In both cases, it must be ensured that the protective properties of the respective safety facility are not impaired.

(15) Visual links to the exterior must be leakproof and it must not be possible to open them.

(16) If there are multiple laboratories in the protection level area, their doors must also be fitted with a viewing window and must open in the direction of the escape route.

(17) A viewing window or a comparable facility for looking into the working area is required for personal protection.

(18) A suitable facility must be present for communication between the laboratory and the outside area. Especially for persons working alone, an emergency call device that can be activated from the inside, or a similar facility, is required.

(19) The protection level area must have its own equipment.

(20) If the results of the risk assessment show, e.g. for activities involving very easily communicable biological agents of risk group 3, that a hazard to employees and/or the spreading of the biological agents into other areas cannot be reliably prevented, even by taking off the personal protective equipment, a shower must be included in the construction plans for the airlock area (e.g. as a second internal airlock chamber). The shower water must be treated as contaminated waste water.

Organisational protective measures

(21) Activities in laboratories of protection level 3 may only be carried out by reliable employees with professional expertise.

Notes: *The requirements for professional expertise are described in more detail in TRBA 200 [11]. The "reliability of a person" includes general factors such as working reliably and complying with the working instructions or briefings. The definition of further criteria for reliability is ultimately at the employer's discretion.*
A safety check may be advisable for activities involving biological agents that, if misused, may present harmful effects for other persons. In such cases, further information is provided by the relevant department of the Ministry of the Interior.

(22) The access door to the airlock must be permanently labelled with "Protection Level 3", the "biohazard symbol" and a sign prohibiting access by unauthorised persons such that these signs are clearly visible from the outside.

(23) The person responsible must restrict access to the protection level area to persons who are authorised to carry out the activities. An access-control system is required.

In justified individual cases, the person responsible may allow access by other persons (e.g. service personnel) under the supervision of personnel with professional expertise.

(24) Equipment and work surfaces must be disinfected once the activity is completed. Accidental contamination must be eliminated immediately according to the stipulations of the hygiene plan.

(25) Before contaminated devices, facilities or, where applicable, rooms of the protection level area are inspected, repaired or modified, the extent and type of decontamination measures must be defined within the framework of the risk assessment and these measures must be carried out or arranged by the laboratory personnel. The person responsible must issue a written work authorisation. If complete decontamination is not possible, the additional protective measures required must be specified in writing in working instructions for the specific activity. The work must be carried out under supervision.

Note: *The occupational-health prevention measures for the service personnel according to number 6 must be specified within the framework of the risk assessment.*

(26) The way in which HEPA filters are removed and decontaminated must be specified in the risk assessment. These must be removed in such a way that a hazard to the maintenance personnel and other persons can be ruled out.

The following procedures can be used for changing the filter in air-conditioning systems and microbiological safety workbenches (MSW):

1. Bag-in-bag changing system (air-conditioning systems)

The HEPA filters are changed using the bag-in-bag method with subsequent thermal inactivation of the packaged filter in the autoclave (fractionated pre-vacuum method, ideally at 134°C). It must be noted that the secondary packaging (bag-in-bag) is already or becomes vapour-permeable in the first vacuum stage. Alternatively, the HEPA filter can be sent to an approved incineration plant.

2. Fumigation with formaldehyde (MSW and air-conditioning systems)

In order to reduce the biological load, the HEPA filters are fumigated *in situ* with formaldehyde (space disinfection procedure according to the list of disinfectants and disinfectant procedures tested and recognised by the Robert Koch-Institute (RKI) [27] and TRGS 522 [30]). The filters are then decontaminated as described under number 1.

3. Fumigation with hydrogen peroxide (MSW and air-conditioning systems)

The HEPA filters are treated *in situ* with a validated hydrogen-peroxide fumigation procedure. The HEPA filters can then be disposed of as non-infectious waste.

Notes: *Specific specialist procedures for the treatment of HEPA filters in MSW are described in the list of disinfectants and disinfectant procedures tested and recognised by the Robert Koch-Institute [27].*

See ABAS statement [29] for information on changing filters.

See TRGS 522 "Space disinfection with formaldehyde" [30] for information on space fumigation using formaldehyde.

See [31] for information on space fumigation with hydrogen peroxide.

(27) Biological agents of risk group 3 must be stored in the protection level area and protected from unauthorised access. It must be ensured that only authorised persons have access. Biological agents that are pathogenic to humans and that are listed in EU Regulation No. 388/2012 [25] for the control of export of dual-use items must be stored under lock and key.

Measures must be defined that are to be initiated in the event that precautions taken against theft or other misuse have not been effective.

(28) Outside of the protection level area, internal transport of biological agents of risk group 3 or corresponding materials must be conducted in closed, rigid, break-proof and liquid-tight vessels (primary containers) that have been disinfected from the outside and that are permanently inscribed or labelled. It must not be possible for external influences to open these vessels accidentally. The primary containers must be transported in a second break-proof and sealable secondary container labelled with the "biohazard symbol".

Note: *Transport outside of the company premises is subject to the dangerous goods regulations for class 6.2 (usually category A).*

(29) An internal plan must set out the rules specifying which measures are to be taken to prevent dangers that may arise through the release of biological agents in the event that a containment measure fails. In addition to information on the possible specific dangers, the plan must also contain the names of the persons responsible for carrying out the rescue measures.

(30) Working instructions must be present for all activities that are associated with a particular risk of infection.

(31) Needles, syringes and other pointed and sharp instruments and objects are only to be used if absolutely necessary. Safety equipment must be used if technically possible. These must be collected and inactivated after use in puncture-resistant and break-proof disposable containers in accordance with number 4.2.5 paragraph 6 of TRBA 250 [9]. Cannulas must not be reinserted into the cannula cover.

If stabbing and cutting instruments are used in conjunction with animal experiments, annex 2 of TRBA 120 [16] must be taken into consideration.

(32) Pipetting aids must be used.

(33) The protection level area must be kept tidy and clean. Only the work equipment that is actually needed may be present on the work surfaces. The protection level area must be disinfected and cleaned regularly according to the stipulations of the hygiene plan.

Note: *Cleaning is to be carried out by personnel with professional expertise, ideally by the employees in the protection level area themselves. In the event of thorough cleaning involving service personnel, this must be carried out under supervision of personnel with professional expertise (see paragraph 23). It must be ensured through corresponding decontamination measures that the service personnel are not exposed to a risk of infection.*

(34) Suitable eye-washing facilities must be kept ready in the laboratory.

Note: *Here, eye-washing bottles in accordance with DIN 12930 are preferable to a permanently installed eyebath for the sake of preventing infection.*

Personal protective equipment/protective measures

(35) The protective clothing and personal protective equipment provided for protection level 3 must be put on and, after completing the activity, taken off inside the airlock. This consists of at least a rear-closing gown with labelling (e.g. of contrasting colour to the protective gowns worn in the other protection level areas), closed shoes, and suitable protective gloves (and with an AQL value $\leq$ 1.5). Subject to the results of the risk assessment, mouth protection (contact protection) or respiratory protection, as well as eye protection (splash protection), may be necessary.

It must be ensured by setting up corresponding areas in the airlock that protective clothing that has been worn is stored separately from other laboratory clothing. Suitable collection containers that can be decontaminated must be provided in the airlock for used protective clothing that is intended for disinfection and cleaning, as well as for used personal protective equipment.

Note: *No jewellery, watches or wedding rings may be worn on the hands and forearms in order to ensure efficient, hygienic disinfection of the hands and that the gloves perform their protective function. Fingernails must be cut short.*

5.5 Protection level 4

The protective measures of protection level 4 serve to protect employees from exposure to biological agents of risk group 4, which put employees and other persons at serious risk of contracting a life-threatening, untreatable infectious disease.
The requirements described below must be observed for the protection of employees and other persons. At the same time, the measures are suitable for protecting the environment.

The following section includes **all** specific protective measures for laboratories of protection level 4 and does not build on numbers 5.2 to 5.4.

Notes: *Activities of protection level 4 fall under the licensing requirement as set out in article 15 paragraph 1 of the Biological Agents Ordinance. A reliable person with professional expertise must be appointed in accordance with article 10 paragraph 2 of the Biological Agents Ordinance (see TRBA 200 "Requirements for professional expertise according to the Biological Agents Ordinance" [11]).*

Structural and technical protective measures

(1) Protection level areas of protection level 4 must be safely structurally separated from other working areas. This can be achieved by constructing a separate building or by structurally sealing off part of a building.

(2) The protection level area comprises one or more laboratories, as well as a four-chamber airlock system as an entrance and exit.

(3) The airlock system must have the following components:

- outer airlock chamber for removing street clothes and putting on undergarments;
- personal shower with space for taking off the undergarments;
- changing room for putting on and taking off the full protective suits;
- inner airlock chamber with the chemical shower for decontamination of the full protective suits.

Note: *For a complex physical structure (multiple laboratories and functional rooms), it may be sensible under certain circumstances to provide two airlock systems. This allows part of*

the area to be deactivated while use of the remaining spaces continues. The escape-route situation is also improved.

(4) A facility must be provided for moving materials or devices in and out through an airlock (e.g. a material airlock that can be fumigated).

(5) The doors of the airlock system must be sufficiently airtight so that there is no possibility of biological agents escaping. They must be interlocking and self-closing so that it is not possible to open them at the same time.

(6) The protection level area must have a controlled, graduated negative pressure that increases from the airlock chambers to the working area in order to prevent air escaping from this area. The respective negative pressure must be easy to verify both from the inside and from the outside – including for the laboratory users – and must be monitored by an alarm system with visual and acoustic signalling.

The supply- and exhaust-air system must be ducted separately from other air-conditioning systems. It must have a non-return and redundant design and an uninterruptible emergency power supply, and must be designed in such a way that no contaminated air can escape even if the air-conditioning system fails.

Supply and exhaust air must each be passed through two HEPA filters (at least H14) connected in series and secured with gas-tight caps. It must be possible to verify that the filters are working correctly while they are installed.

Note: *See ABAS statement [29] for more information on the use of HEPA filters in air-conditioning systems.*

(7) The planning of the air-conditioning system must take into account the concept for space disinfection (e.g. fumigation) and fumigation of the contaminated part of the air-conditioning system (including the filter systems), as well as the safe (low-contamination) changing of filters. The air-conditioning system must be designed so that a filter can be changed without impairing the standard of safety, as the protection level area must otherwise be shut down and disinfected. For larger systems, it is advisable to subdivide the air-conditioning system so that partial operation is possible in the event of a fault or during maintenance work. The supply- and exhaust-air ducts must be gas-tight and suitable for fumigation. The duct routes should be as short as possible and should directly adjoin, or be located within, the protection level area.

Note: *The outdoor climatic conditions must be taken into consideration with regard to the operational requirements of the air-conditioning system (e.g. protection from icing).*

(8) There should preferably be visual links to the exterior, and these should consist of leakproof and break-proof materials. Windows that can be opened are not permitted.

Note: *The visual link should be such that unauthorised persons cannot look in from outside.*

(9) If there are multiple laboratories in the protection level area, their doors must also be fitted with a viewing window and must open in the direction of the escape route.

(10) It must be possible to fumigate the protection level area safely for the purpose of final disinfection. It must not be possible for biological agents to escape at any time.

(11) All points at which supply and disposal lines pass through must be sealed and secured against backflow. Supply lines for gases must be protected with high-efficiency particulate absorption filters; liquid lines must be protected by bacteria-proof filters.

(12) All surfaces must be impermeable to water, easy to clean, and resistant to the disinfectants and fumigants used, as well as to other chemicals. Surfaces must be of a seamless nature, and corners and edges of the room must preferably be rounded for the sake of cleaning/disinfection.

(13) The protection level area must have a sufficiently sized double-ended autoclave whose automatic locking system only allows the door to be opened if the sterilisation cycle has completed without disruptions. The inactivation of contaminated process exhaust air and of the condensed water must be ensured. The autoclave must not be located in the airlock area.

Note: *Please refer to ABAS statement [23] for more information on the treatment of exhaust air from autoclaves.*

(14) The waste water arising in the protection level area must always be subjected to suitable thermal or chemical/thermal post-treatment (central waste-water sterilisation).

(15) As a matter of principle, the procedure in the event of malfunction or maintenance must also be taken into consideration when planning safety-related technical systems, e.g. air-conditioning systems, waste-water treatment systems and autoclaves.

(16) An uninterruptible emergency power supply must be installed for all safety-related facilities, e.g. respiratory air-supply systems for externally ventilated protection suits, ventilation systems and monitoring facilities.

(17) The safety lighting in the protection level area must be designed so that, in the event of a power failure, it is possible to stop work safely and exit via the airlock.

(18) There must be a continuous visual link or camera surveillance link to the working area (laboratories, including functional rooms). There must be an intercom system to the outside or a comparable facility.

(19) It must be ensured through technical measures (e.g. electronic access control) that only authorised persons can enter the protection level area.

(20) Employees must not work alone unless the activities can be controlled safely by one person and there is a continuous means of communication (e.g. via headphones). The conditions under which working alone is possible must be specified within the framework of the risk assessment. Furthermore, in the case of complex facilities, it must be examined within the framework of the risk assessment whether at least two persons must work at the same time for safety reasons.

Note: *Where applicable, it is sensible to wear a personal emergency signalling device when working alone*.

(21) The protection level area must have its own laboratory equipment.

(22) Centrifuges must have aerosol-tight centrifuge inserts or a self-contained, aerosol-tight rotor. As far as possible, centrifuges should be operated in the microbiological safety workbench (MSW). The centrifuge rotors should always be opened in the MSW. It must be ensured that the protective properties of the MSW are not impaired.

(23) As the personal protective equipment described in paragraph 24 must be worn, it is not possible to handle biological agents of risk group 4 openly in a class II MSW.

Note: *The concept of an externally ventilated full protective suit and class II safety workbench represent the current state of the art.*

Organisational and personal protective measures, personal protective equipment

(24) Employees must be protected by an externally ventilated full protective suit during activities in a laboratory of protection level 4, with the respiratory air supply provided by a self-sufficient air supply line. The full protective suit must meet the following criteria:

- mechanical properties: abrasion-resistant, tearproof and airtight;
- chemical properties: resistant to the disinfectant used in the disinfection shower;

- preferably welded-on boots;
- preferably with fastening brackets for gloves.

(25) Two pairs of gloves of category III (and with an AQL value ≤1.5) must be worn to protect the hands, with at least the outer glove attached in a sealed manner to the cuff of the protection suit (e.g. clamping bracket).

(26) This is the procedure for entering and exiting via the airlock:

Entering via the airlock: All clothing, watches and jewellery must be taken off in the first airlock chamber and light undergarments put on for the full protective suits. Disposable gloves are put on. The protective suit is put on in the changing room and the laboratory is entered through the inner airlock chamber without activating the disinfection shower. After the inner airlock chamber is vacated, this is subjected to a short shower cycle with decontaminant and a short water phase.

Exiting via the airlock: After work is completed a shower cycle is carried out in the disinfection shower to decontaminate the full protective suit. After a rinse with water, the suit is taken off in the changing room and remains there. The undergarments are taken off in the personal shower and a hygiene shower is taken if necessary.

Note: *In the disinfection shower, it must be ensured that the shower wets the entire surface of the protective suit. The shower must be of sufficient duration to ensure the complete disinfection of the protective suit. The subsequent rinse with water must remove the disinfectant completely in order to prevent contact between the corresponding chemical components. The disinfection procedure must be validated.*

(27) Activities in the protection level area may only be carried out by reliable employees with professional expertise.

Notes: *The requirements for professional expertise are described in more detail in TRBA 200 [11]. The "reliability of a person" includes general factors such as working reliably and complying with the working instructions or briefings. The definition of further criteria for reliability is ultimately at the employer's discretion.*

A safety check may be advisable for activities involving biological agents that, if misused, may present harmful effects for other persons. In such cases, further information is provided by the relevant department of the Ministry of the Interior.

(28) The time of the laboratory user's entry and exit via the airlock is to be documented directly, and the activities carried out must be recorded promptly.

(29) The protection level area must be permanently labelled with "Protection Level 4", the "biohazard symbol", and a sign prohibiting access by unauthorised persons so that these signs are clearly visible from the outside.

(30) Working areas must be kept tidy and clean. Only the work equipment that is actually needed may be present on the work surfaces. Equipment and work surfaces must be disinfected according to the hygiene plan once the activity is completed. Any accidental contamination must be eliminated immediately in the proper fashion.

(31) All solid and liquid waste must be collected safely and inactivated using the double-ended autoclave. Waste water must be disposed of via the central waste-water sterilisation system.

(32) Biological agents of risk group 4 must be stored safely and under lock and key. Only authorised persons are to have access. The stock level and whereabouts of biological agents of risk group 4 must be documented.

(33) The internal transport of biological agents of risk group 4 or materials that contain these agents must be carried out in closed, rigid, break-proof and liquid-tight vessels (primary containers) that have been disinfected from the outside and that can be permanently labelled or

inscribed. It must not be possible for external influences to open these vessels accidentally. The primary containers must be transported in a second break-proof, firmly sealed secondary container that has been disinfected from the outside and labelled with the “biohazard symbol”. It must be ensured that biological agents of risk group 4 cannot be spread to the outside while exiting the protection level area via the airlock.

Note: *When inactivated materials are taken out via the airlock for further processing in another laboratory, one may, for example, use a secondary container with perforations that permits disinfection of the surface of the primary container in the immersion bath or through fumigation.*

(34) Working instructions in accordance with article 14 paragraph 4 of the Biological Agents Ordinance must be present for all activities that take place in the protection level area. This relates in particular to:

- users entering and exiting via the airlock;
- the putting on and taking off of protective clothing, as well as the corresponding steps of disinfection;
- the bringing in of materials via the airlock (e.g. specimens, animals if necessary);
- the disposal of liquid and solid waste;
- cleaning and disinfection measures according to the hygiene plan, as well as the procedure in the event of any accidental contamination;
- the procedure in the event of accidents;
- repair and maintenance.

(35) The conduct in the event of breakdowns, accidents and emergencies, as well as the corresponding obligations of information, reporting and notification, must be regulated in an internal plan in accordance with article 13 paragraphs 3 and 4 of the Biological Agents Ordinance.

This plan must also include regulations for the prevention of dangers that may arise through the release of highly pathogenic biological agents in the event of a containment measure failing. The plan must contain:

- information on specific dangers;
- names of the persons responsible for carrying out the rescue measures;
- information on the extent of safety exercises and the regular performance of these exercises.

It must be coordinated with the relevant internal and external rescue and safety workers and must be designed so that the safety workers are able to define their rescue and safety measures.

Warning systems and means of communication whose correct operation is guaranteed must be established for immediately warning employees and alerting the rescue and safety services.

(36) The employees in the protection level area must be briefed before commencing the activity, after prolonged breaks in the activity, and when the workflows and working procedures are changed. To ensure smooth operation, the briefings must each include practical exercises/training sessions on safety-related activities, workflows, and working procedures. The appointed person in accordance with article 10 paragraph 4 of the Biological Agents Ordinance must be involved. The carrying out of the briefing must be documented.

(37) In principle, stabbing or cutting instruments may not be used. If, however, this is necessary in conjunction with animal experiments or when processing pathologically relevant ma-

terials and no other suitable procedure is available, the extent to which safety equipment can be used must be checked. Cannulas must not be reinserted into the cannula cover. Used instruments must be disposed of safely in appropriate puncture-resistant waste containers.

Note: *For more information on animal experiments, see annex 2 to TRBA 120 [16].*

(38) If work with laboratory animals is carried out in laboratories of protection level 4, number 4.5 of TRBA 120 “Keeping of laboratory animals” [16] must also be observed.

6 Preventive occupational medicine

(1) Preventive occupational medicine includes conducting a general occupational-health consultation and implementing preventive occupational healthcare in accordance with the Ordinance on Occupational Medical Prevention (ArbMedVV) [32].

(2) Depending on the specific hazards determined, occupational-health questions are to be incorporated and assessed in the risk assessment. Occupational-health expertise is to be drawn upon in particular for:

1. Activities involving risks of infection for which compulsory or optional occupational healthcare is to be arranged or offered (see section 6.2).

and

2. Activities

- that present a risk of exposure to biological agents with effects that are sensitising, toxic, or otherwise harmful to health;
- that necessitate hygiene measures and/or special disinfection measures;
- that necessitate special first-aid measures and post-exposure prophylaxis;
- for which personal protective equipment must be worn (e.g. protective gloves, respiratory protection);
- in which exposures of the skin can occur that necessitate skin-protection measures.

As a first priority, this process must involve the appointed medical officer, who has specific knowledge of the hazards at the various workplaces.

6.1 General occupational health consultation

(1) The employees must be given a general occupational-health consultation within the framework of the briefing in accordance with number 5.1 (7). This is to be carried out with the participation of the doctor appointed to provide preventive occupational healthcare in accordance with number 6.3. The doctor is deemed to have participated if, for example, they have trained the people giving the briefing or have collaborated in the preparation of suitable teaching materials for preventive occupational medicine.

(2) The topic areas on which the employees must be informed and advised are to be specified subject to the results of the risk assessment. They relate to, among others:

1. Possible activity-specific health hazards as a result of the biological agents that are used or are present.

   Here, particular account must be taken of:

a. the typical transmission routes or uptake pathways or those associated with the activity;

b. the possible disease patterns and symptoms;

c. medical factors that may lead to an increase in the risk, such as:

- diminished immune defence (e.g. due to an immunosuppressant treatment or a disease such as diabetes mellitus);
- the presence of chronic obstructive respiratory diseases in conjunction with activities involving potentially sensitising biological agents;
- impaired barrier function of the skin;
- another individual disposition; or
- pregnancy and breastfeeding; as well as

d. the possibilities for prophylactic vaccination.

2. The code of conduct that must be observed, e.g. with regard to hygiene requirements, skin protection and skin care, and their consistent implementation.

3. The medical aspects of necessity, suitability and use of personal protective equipment (e.g. protective gloves, protective clothing, respiratory protection), including handling, maximum wearing times, replacement cycle and possible physical and psychological stresses.

4. The first-aid and post-exposure prophylaxis measures, as well as the procedure in the event of cuts and stabs.

5. The necessary occupational healthcare check-ups (compulsory and optional examinations), the scope and benefit of such examinations, and possible vaccinations.

6. The offer of an occupational healthcare check-up when a disease occurs if a causal connection with the activity is suspected.

6.2 Occupational healthcare check-up

Occupational healthcare check-ups allow early detection of work-related health problems, as well as determination of whether an increased health hazard is present when carrying out a specific activity. An occupational healthcare check-up can be limited to a verbal consultation if physical or clinical examinations are not necessary for the consultation.

Occupational healthcare check-ups include compulsory, optional and requested examinations.

6.2.1 Compulsory examination

(1) Compulsory examinations, within the scope of application of this technical rule, are to be arranged with regard to the risk of infection according to part 2 (1) of the annex to the Ordinance on Occupational Medical Prevention for specific and non-specific activities involving the biological agents stated in the table therein. The examination's completion is a prerequisite for working with the biological agents named in column 1. The areas listed in column 2 with the exposure conditions of column 3 are decisive for non-specific activities.

(2) For activities involving biological agents labelled as preventable by vaccination *) in the table in part 2 (1) of the annex to the Ordinance on Occupational Medical Prevention, a vaccination with corresponding medical advice must be offered within the framework of the compulsory examination. The employee does not have to accept the offer of vaccination, as the vaccination is not compulsory.

(3) If it is determined within the framework of the first examination that the employee has sufficient immunity to the biological agent (the reason for examination), follow-up examinations can be dispensed with as long as the immunity lasts.

(4) Subject to the risk assessment, further reasons for compulsory examinations can additionally arise (in accordance with the annex to the Ordinance on Occupational Medical Prevention) in the event of, for example:

- activities involving certain hazardous substances where a limit value is exceeded;
- health-relevant exposure to dust from laboratory animals in animal rooms and systems;
- working in wet conditions for four hours or more per day on a regular basis (e.g. wearing liquid-tight gloves);
- activities that necessitate the wearing of respiratory protection apparatus of group 2 (e.g. FFP3 particle-filtering half mask) or group 3.

Note: *For more information on activities involving exposure to dusts from laboratory animals, see TRBA 120 number 5.3 (4) [16].*

6.2.2 Optional examination

(1) Reasons for optional examinations pursuant to part 2 (2) of the annex to the Ordinance on Occupational Medical Prevention are generally present in specific activities involving biological agents of risk group 3 or non-specific activities of protection level 3, provided these are not included in part 2 (1) of the mentioned annex.

This also applies to specific activities involving biological agents of risk group 2 and non-specific activities of protection level 2 in laboratories.

If the effects of the biological agents are preventable by vaccination, the examination includes the offer of vaccination following medical advice.

(2) Reasons for optional examinations also exist in relation to events when:

1. A serious infection or disease is to be expected as a consequence of exposure to a biological agent and post-exposure prophylaxis measures are possible (e.g. after stabs or cuts by instruments contaminated with blood).
2. A health problem (infection, disease, sensitisation or poisoning) has occurred for which a causal connection with the activity is possible (see article 5 (2) of the Ordinance on Occupational Medical Prevention). If there are indications that other employees performing comparable activities may also be in danger, these employees must also be offered examinations.
3. An activity is completed for which compulsory examinations were required (follow-up examination). This does not apply to activities involving biological agents whose effects are preventable by vaccination if sufficient immunity remains at this point in time.

(3) Subject to the risk assessment, further reasons for optional examinations can additionally arise in accordance with the annex to the Ordinance on Occupational Medical Prevention; for example:

- activities involving certain hazardous substances or mixtures thereof (e.g. n-hexane, n-heptane, 2-butanone, 2-hexanone, methanol, ethanol, 2-methoxyethanol, benzene,

toluene, xylene, styrene, dichloromethane, 1,1,1-trichloroethane, trichloroethene, tetrachloroethene);

- activities involving carcinogenic or mutagenic substances or preparations of category 1 or 2 for the purposes of the Hazardous Substances Ordinance;
- working in wet conditions for more than two hours per day on a regular basis (e.g. wearing liquid-tight gloves);
- activities that necessitate the wearing of respiratory protection apparatus of group 1 (e.g. FFP2 particle-filtering half mask);
- activities using display screen equipment.

6.2.3 Requested examination

In accordance with article 11 of the Occupational Safety and Health Act, the employer must allow the employees to undergo occupational healthcare check-ups if health damage connected to the activity cannot be ruled out. Within the scope of application of these technical rules, this may be the case, for example, when there has been exposure to bioaerosols with sensitising and toxic properties or when work is carried out in wet conditions for less than two hours a day.

Annex 1

Species-specific protective measures for biological agents of risk group 3(**)

Bacteria

Species	Disease	Possible transmission routes to humans[1]	Inactivation procedure	Special instructions/ other
Escherichia coli (EHEC*)*	Diarrhoea, haemorrhagic colitis, haemolytic-uraemic syndrome (HUS)	Contact infection; oral through pathogen-containing food, especially raw beef, unpasteurised milk and contaminated raw vegetables (e.g. through faecal manuring)	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Zoonotic
Mycobacterium microti	Vole (wild rodent) tuberculosis	Aerogenic within animal populations and – directly or indirectly – from animal to human (?); (oral? percutaneous – injuries, bites?)	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. (Where applicable, observe manufacturer's instructions on special application times.) Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Zoonotic
Mycobacterium ulcerans	Buruli ulcer	Percutaneous – injuries, including stabs and cuts	See *Mycobacterium microti*	Anthroponotic
Rickettsia akari	Rickettsialpox	Parenteral, e.g. through stabs and cuts; vectorial [bites from mites (*Allodermanyssus sanguineus*); both larvae and sexually mature mites suck blood, and the pathogens are transmitted transovarially to the next mite generation]	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Zoonotic, 2
Rickettsia canadensis	Human diseases not definitely proven!	Parenteral, e.g. through stabs and cuts; vectorial [bites from ticks *(Dermacentor andersoni, Haemaphysalis leporispalustris)*]	See *Rickettsia akari*	Zoonotic?, 2
Rickettsia heilongjiangensis	Far Eastern spotted fever	Parenteral, e.g. through stabs and cuts; vectorial [bites from ticks (*Dermacentor silvarum*, *Haemaphysalis concinnae*)]	See *Rickettsia akari*	Zoonotic, 2
Rickettsia montanensis	Human diseases not proven!	Vectorial [bites from ticks (*Dermacentor variabilis* and *D. andersoni*)]?	See *Rickettsia akari*	Zoonotic?, 2

Species	Disease	Possible transmission routes to humans[1]	Inactivation procedure	Special instruc-tions/ other
Salmonella Typhi	Typhus abdominalis	Contact infection; faecal-oral; food, drinking water	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Anthroponotic, 3
Shigella dysenteriae, Type 1	Dysentery	Contact infection; faecal-oral; food; drinking water	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Anthroponotic

Parasites

Species	Disease	Activities under the conditions of protection level		Possible transmission routes to humans[1]	Inactivation procedure	Special instructions/ other
		3(**)	2			
Echinococcus granulosus	Cystic echinococcosis, dog tapeworm infestation)	Proglottids, eggs	Metacestodes (= cystic stages)	Enteral; aerogenic via contaminated hair or dandruff from the final host (canids) with subsequent swallowing not ruled out; faeces	Hand hygiene by wearing protective gloves, plus thorough hand washing after taking off gloves; surface disinfection using phenolic preparations with sufficiently long application time (typically 4 h); treatment of instruments (inactivation of all life stages) and waste inactivation, e.g. by heating to 70°C core temperature for 15 minutes or prior deep-freezing at -80°C; standard chemical disinfectants for disinfection of hands, skin, surfaces and instruments are normally ineffective!	Zoonotic, 4, 5, 6, 7, 8, 9
Echinococcus multilocularis	Alveolar echinococcosis, fox tapeworm infestation	Proglottids, eggs	Metacestodes (= cystic stages)	Enteral; aerogenic via contaminated hair or dandruff from the final host (fox, dog, cat, wolf, among others) with subsequent swallowing not ruled out; faeces	See *Echinococcus granulosus*	Zoonotic, 4, 5, 6, 7, 8, 9
Echinococcus oligarthrus	Polycystic echinococcosis, Central or South American echinococcosis	Proglottids, eggs	Metacestodes (= cystic stages)	Enteral; aerogenic via contaminated hair or dandruff from the final host with subsequent swallowing not ruled out; faeces	See *Echinococcus granulosus*	Zoonotic, 4, 5, 6, 7, 8, 9
Echinococcus vogeli	Polycystic echinococcosis, Central or South American echinococcosis	Proglottids, eggs	Metacestodes (= cystic stages)	Enteral; aerogenic via contaminated hair or dandruff from the final host with subsequent swallowing not ruled out; faeces	See *Echinococcus granulosus*	Zoonotic, 4, 5, 6, 7, 8, 9
Leishmania braziliensis	Mucocutaneous leishmaniasis, Espundia or Uta	Metacyclic stages in the vector		Vectorial [bite from the vector mosquito (*Phlebotomus*, *Lutzomyia* species)]	Kill vectors and heat to 60°C or transfer to 70% alcohol	Zoonotic, 2, 10
			Promastigote stages in the culture, amastigote stages in the vertebrate host	Parenteral through stabs or open wounds	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A; treatment of instruments and waste inactivation through thermal disinfection (heat to 60°C core temperature for at least 15 min)	10
Leishmania donovani	Visceral leishmaniasis, Kala azar	Metacyclic stages in the vector		Vectorial [bite from the vector mosquito (*Phlebotomus*, *Lutzomyia* species)]	See *Leishmania braziliensis*	Zoonotic, 2, 10
			Promastigote stages in the culture, amastigote stages in the vertebrate host	Parenteral through stabs or open wounds	See *Leishmania braziliensis*	10

Species	Disease	Activities under the conditions of protection level		Possible transmission routes to humans[1]	Inactivation procedure	Special instructions/ Other
		3(**)	2			
Plasmodium falciparum	Malaria tropica	Sporozoites in the vector		Vectorial [bite from the vector mosquito (*Anopheles* species)]	Kill vectors and heat to 60°C or transfer to 70% alcohol	Anthroponotic, 2, 10
			Asexual stages in the culture or in the vertebrate host	Parenteral through stabs or open wounds	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A (RKI list) [27]; treatment of instruments and waste inactivation through thermal disinfection (heat to 60°C core temperature for at least 15 min)	10
Plasmodium knowlesi	Malaria	Sporozoites in the vector		Vectorial [bite from the vector mosquito (*Anopheles* species)]	See *Plasmodium falciparum*	Zoonotic, 2, 10
			Asexual stages in the culture or in the vertebrate host	Parenteral through stabs or open wounds	See *Plasmodium falciparum*	10
Taenia solium	Bladder worm infestation, cysticercosis	Proglottids, eggs		Oral-enteral	Hand hygiene by wearing protective gloves, plus thorough hand washing after taking off gloves; surface disinfection by phenolic preparations with sufficiently long application time (typically 4 h); treatment of instruments (inactivation of all life stages) and waste inactivation through thermal disinfection (heat to 70°C core temperature for 15 min); standard chemical disinfectants for disinfection of hands, skin, surfaces and instruments are normally ineffective!	Zoonotic, 4, 6, 7, 8
			Larvae (cysticerci)	None	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A (RKI list) [27]; treatment of instruments and waste inactivation through thermal disinfection (heat to 60°C core temperature for at least 15 min)	10
Trypanosoma brucei gambiense, T. b. rhodesiense	African sleeping sickness	Metacyclic stages in the vector's faeces		Vectorial [bites from the vector fly (*Glossina* species)]	Kill vectors and heat to 60 °C or transfer to 70% alcohol	Zoonotic, 2, 10
			Trypomastigote forms in the culture, trypomastigote forms in the vertebrate host	Parenteral through stabs or open wounds	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range A (RKI list) [27]; treatment of instruments and waste inactivation through thermal disinfection (heat to 60°C core temperature for at least 15 min)	10

Viruses

Species	Enve-lope	Disease	Possible transmission routes to humans[1,11]	Inactivation procedure	Special instruc-tions/ other
Flaviviridae					
Hepatitis C virus (HCV)	Yes	Hepatitis C	Parenteral (through e.g. stabs or cuts); vertical; sexual; blood transfusion	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range AB (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. (Where applicable, observe manufacturer's instructions on special application times.) Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Anthroponotic, 12
Louping ill virus (LIV)	Yes	Louping ill	Parenteral (through e.g. stabs or cuts); vectorial [bites from ticks (*Ixodes ricinus*)]	See Hepatitis C virus	Zoonotic, 2
Neudörfl virus (NEUV)	Yes	Central European tick-borne encephalitis	Parenteral (through e.g. stabs or cuts); vectorial [bites from ticks (*Ixodes* species]; enteral	See Hepatitis C virus	Zoonotic, 2, 3
Wesselsbron virus (WESSV)	Yes	Flu-like disease	Parenteral (through e.g. stabs or cuts); vectorial (bites from the vector mosquitoes, e.g. *Aedes* species)	See Hepatitis C virus	Zoonotic, 2
Central European tick-borne encephalitis virus (TBEV-Eu)	Yes	Tick-borne encephalitis (TBE)	Parenteral (through e.g. stabs or cuts); vectorial [bites from ticks (*Ixodes* species]; enteral	See Hepatitis C virus	Zoonotic, 2, 3
Hepeviridae					
Hepatitis E virus (HEV)	No	Hepatitis E	Oral-enteral (faecal-oral)	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range AB (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. (Where applicable, observe manufacturer's instructions on special application times.) Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Anthroponotic

Species	Enve-lope	Disease	Possible transmission routes to humans[1,11]	Inactivation procedure	Special instruc-tions/ other
Hepadnaviridae					
Hepatitis B virus (HBV)	Yes	Hepatitis B	Parenteral (through e.g. stabs or cuts); bite (experiments on primates); vertical; sexual; blood transfusion	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range AB (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. (Where applicable, observe manufacturer's instructions on special application time.) Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Anthroponotic, 3, 12
Retroviridae					
Human T-cell leukaemia virus 1[14] (HTLV-1)	Yes	T-cell leukaemia, tropical spastic paraparesis	Parenteral (through e.g. stabs or cuts); vertical; sexual (sperm); blood transfusion	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range AB (RKI list) [27] or an equivalent agent. Corresponding lists, e.g. from professional associations, can assist in the selection process [33], [35]. (Where applicable, observe manufacturer's instructions on special application times.) Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instrument disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Anthroponotic, 12
Human T-cell leukaemia virus 2[14] (HTLV-2)	Yes	T-cell lymphoma?, neurological diseases?	Parenteral (through e.g. stabs or cuts); vertical; sexual (sperm)?; blood transfusion	See Human T-cell leukaemia virus 1	Anthroponotic, 12
Human immunodeficiency virus 1[14] (HIV-1)	Yes	Acquired immunodeficiency syndrome (AIDS)	Parenteral (through e.g. stabs or cuts); sexual; vertical; blood transfusion	See Human T-cell leukaemia virus 1	Anthroponotic, 12
Human immunodeficiency virus 2[14] (HIV-2)	Yes	AIDS with long survival time	Parenteral (through e.g. stabs or cuts); sexual; vertical; blood transfusion	See Human T-cell leukaemia virus 1	Anthroponotic, 12

Species	Enve-lope	Disease	Possible transmission routes to humans[1,11]	Inactivation procedure	Special instruc-tions/ other
Rhabdoviridae					
Australian bat lyssavirus (ABLV)	Yes	Rabies	Parenteral (through e.g. stabs, cuts or bites) or via mucous membranes (e.g. eyes) or wounds; aerosols	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range AB (RKI list) [27] or an equivalent agent according to the VAH or DVG lists [33, 35] (where applicable, observe manufacturer's instructions on special application times); Treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instru-ment disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Zoonotic
Duvenhage virus (DUVV)	Yes	Rabies	Parenteral (through e.g. stabs, cuts or bites) or via mucous membranes (e.g. eyes) or wounds; aerosols	See Australian bat lyssavirus	Zoonotic, 3
European bat lyssavirus type 1 (EBLV-1)	Yes	Rabies	Parenteral (through e.g. stabs, cuts or bites) or via mucous membranes (e.g. eyes) or wounds; aerosols	See Australian bat lyssavirus	Zoonotic, 3
European bat lyssavirus type 2 (EBLV-2)	Yes	Rabies	Parenteral (through e.g. stabs, cuts or bites) or via mucous membranes (e.g. eyes) or wounds; aerosols	See Australian bat lyssavirus	Zoonotic, 3
Rabies virus (RABV)	Yes	Rabies	Parenteral (through e.g. stabs, cuts or bites) or via mucous membranes (e.g. eyes) or wounds; aerosols	See Australian bat lyssavirus	Zoonotic, 3
Togaviridae					
Chikungunya virus (CHIKV)	Yes	Chikungunya fever	Parenteral (through e.g. stabs or cuts); vectorial [bite from vector mosquitoes (*Aedes* species)]	Disinfection of hands, skin and surfaces with a chemical disinfectant with the efficacy range AB (RKI list) [27] or an equivalent agent according to the VAH or DVG lists [33, 35] (where applicable, observe manufacturer's instructions on special application times); treatment of instruments primarily through, where necessary, disinfectant pre-cleaning and subsequent thermal disinfection (or sterilisation); instru-ment disinfection by the immersion method is possible but subordinate (for details see KRINKO recommendation for preparation of medical products [34]); inactivation of waste primarily through thermal disinfection	Zoonotic, 2
Everglades virus (EVEV)	Yes	Fever, headaches and pains in the limbs, neurological symptoms	Parenteral (bite, through e.g. stabs or cuts); vectorial [bite from vector mosquitoes (*Aedes* species)]	See Chikungunya virus	Zoonotic, 2
Mucambo virus (MUCV)	Yes	Mild feverish disease	Parenteral (through e.g. stabs or cuts); vectorial	See Chikungunya virus	Zoonotic, 2

Species	Enve-lope	Disease	Possible transmission routes to humans[1,11]	Inactivation procedure	Special instruc-tions/ other
Tonate virus (TONV)	Yes	Fever, headaches, among others	Parenteral (through e.g. stabs or cuts); vectorial [bite from vector mosquitoes (*Culex* species)]	See Chikungunya virus	Zoonotic, 2
Other viruses					
As yet unidentified hepatitis viruses		?	?	?	
Unconventional agents that are associated with transmissible spongiform encephalopathies (TSE)					
Pathogens of Creutzfeldt–Jakob disease		Creutzfeldt–Jakob disease	Parenteral and enteral (e.g. contaminated instruments or injection of contaminated therapeutic agents)	Disinfection or sterilisation procedure for prions [15, 36]	Anthropo-notic
Pathogens of variant Creutzfeldt–Jakob disease		New variant Creutzfeldt–Jakob disease	Parenteral, enteral	Disinfection or sterilisation procedure for prions [15, 36]	Anthropo-notic
Pathogens of Gerstmann–Sträussler–Scheinker syndrome		Gerstmann–Sträussler–Scheinker syndrome	Parenteral and enteral? (e.g. contaminated instruments or injection of contaminated therapeutic agents)	Disinfection or sterilisation procedure for prions [15, 36]	Anthropo-notic
Pathogens of the disease "Kuru"		Kuru ("muscle tremors")	Parenteral, enteral	Disinfection or sterilisation procedure for prions [15, 36]	Anthroponotic
Pathogens of fatal familial insomnia		Fatal familial insomnia	Parenteral, enteral	Disinfection or sterilisation procedure for prions [15, 36]	Anthropo-notic
Pathogens of bovine spongiform encephalopathy		Bovine spongiform encephalopathy	Parenteral, enteral	Disinfection or sterilisation procedure for prions [15, 36]	Zoonotic?, 13
Other related animal TSE			Parenteral, enteral	Disinfection or sterilisation procedure for prions [15, 36]	Zoonotic?, 13

Explanatory remarks, specific instructions

The numbers in the tables for bacteria, parasites and viruses refer to explanatory remarks and special measures that must be taken for activities involving biological agents of risk group 3(**). Where no information is given, no definitive statement can be provided due to lack of knowledge.

1 Transmission routes

Transmission: Transport of an infectious agent from a source of infection (e.g. infected material, pathogen-containing culture, infected animal, infected human) to the human.
The transmission route for typical laboratory activities is placed first. The natural transmission route is shown after this.

Oral-enteral: Transmission through the gastrointestinal tract as a point of entrance for the pathogen (e.g. faecal-oral).

Parenteral: Transmission bypassing the gastrointestinal tract (e.g. through intramuscular/intravascular injection, blood transfusion, organ transplantation, cuts and stabs, living vectors).

Sexual: Pathogens transmitted through sexual contact.

Vectorial: Transmission by living vectors, e.g. through bloodsucking/bites by certain species of lice, mosquitoes, bugs, mites or ticks. Natural carriers are stated for the vectors.

Vertical: Transmission via the germline, placenta or breast milk, or through infection of the birth canal.

2 When infected vectors (arthropods) are being handled, these animals must be prevented from escaping. All activities involving vector transmission must be carried out in a laboratory that can contain arthropods securely. Access must be gained via an airlock that can contain arthropods securely.

3 Activities involving these pathogens should be carried out by employees that have sufficient immunity and that have received appropriate training.

4 The laboratory must have its own equipment that is used exclusively in the laboratory.

5 Protective clothing, personal protective equipment and work equipment must be autoclaved after work is completed and before cleaning. The autoclave must be located inside the working area.

6 If activities cannot be carried out in a microbiological safety workbench, e.g. the dissection of infected large animals, the laboratory must be accessed via an airlock that is designed as a separate room with a black/white area (i.e. contaminated/uncontaminated).

7 Eye and mouth protection can be dispensed with when working in the microbiological safety workbench. All activities that must be carried out outside of the safety workbench must be performed with splash protection (protective shield or visor), mouth protection, eye protection and respiratory protection (FFP 2 respirator).

8 All work must be carried out in sterilisable basins that are heat-sterilised after work is completed, as it is not possible to decontaminate the workplace.

9 In dissections, any dust formation must be avoided; keep the fur of final hosts wet (e.g. fox, dog, cat); ideally immerse in water containing detergent prior to dissection. Filtration of exhaust air can then be dispensed with.

10 Inactivation of contaminated waste water is not necessary, as the infectious stages die off quickly in the waste water (*Leishmania*, *Trypanosoma*, *Plasmodium*, isolated larvae of *Taenia solium*). Because of their clear macroscopic visibility and their localisation in the musculature, larvae of *Taenia solium* do not normally reach the waste water.

11 The individual transmission route cannot be clarified for some viral infections.

12 Virus can be transmitted to certain primates.

13 There is no proof of an infection of humans with pathogens of other animal TSE. Nevertheless, protective measures are recommended for activities in the laboratory, such as when handling biological agents of risk group 3(**). One exception is laboratory work with an identified pathogen of scrapie, for which protection level 2 is sufficient.

14 Virus originates from the virus of certain non-human primates. In principle, oral transmission is also possible (e.g. through virus-containing breast milk, seminal fluid, liquids with sufficient virus titre).

? No reliable information is available.

Annex 2

Literature:

[1] Verordnung über Sicherheit und Gesundheitsschutz bei Tätigkeiten mit biologischen Arbeitsstoffen (Biostoffverordnung – BioStoffV) [Ordinance on safety and health protection at work involving biological agents (Biological Agents Ordinance)] of 15th July 2013 (BGBl I No. 40 p. 2514–2528)
http://www.gesetze-im-internet.de/bundesrecht/biostoffv_2013/gesamt.pdf

[2] TRGS 526 “Laboratorien” [Laboratories]
www.baua.de/TRGS

[3] Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work
http://eur-lex.europa.eu/LexUriServ/

[4] TRBA 460 “Einstufung von Pilzen in Risikogruppen” [Classification of fungi into risk groups]
www.baua.de/TRBA

[5] TRBA 462 “Einstufung von Viren in Risikogruppen” [Classification of viruses into risk groups]
www.baua.de/TRBA

[6] TRBA 464 “Einstufung von Parasiten in Risikogruppen” [Classification of parasites into risk groups]
www.baua.de/TRBA

[7] TRBA 466 “Einstufung von Prokaryonten (Bacteria und Archaea) in Risikogruppen” [Classification of prokaryotes (bacteria and archaea) into risk groups]
www.baua.de/TRBA

[8] TRBA 468 “Liste der Zelllinien und Tätigkeiten mit Zellkulturen” [List of cell-lines and activities involving cell cultures]
www.baua.de/TRBA

[9] TRBA 250 “Biologische Arbeitsstoffe im Gesundheitswesen und in der Wohlfahrtspflege” [Biological agents in health care and welfare facilities]
www.baua.de/TRBA

[10] TRBA 400 “Handlungsanleitung zur Gefährdungsbeurteilung und für die Unterrichtung der Beschäftigten bei Tätigkeiten mit biologischen Arbeitsstoffen” [Guideline for risk assessment and for the instruction of employees in relation to activities with biological agents]
www.baua.de/TRBA

[11] TRBA 200 “Anforderungen an die Fachkunde nach Biostoffverordnung” [Requirements for professional expertise according to the Biological Agents Ordinance]

[12] TRBA 450 “Einstufungskriterien für biologische Arbeitsstoffe” [Criteria for the classification of biological agents]
www.baua.de/TRBA

[13] TRBA/TRGS 406 “Sensibilisierende Stoffe für die Atemwege” [Sensitising substances for the respiratory tracts]
www.baua.de/TRBA

[14] TRBA 130 “Arbeitsschutzmaßnahmen in akuten biologischen Gefahrenlagen” [Occupational safety measures in acute biohazard situations]
www.baua.de/TRBA

[15] ABAS Resolution 603 “Schutzmaßnahmen bei Tätigkeiten mit Transmissibler Spongiformer Enzephalopathie (TSE) assoziierter Agenzien in TSE-Laboratorien” [Protective measures for activities involving transmissible spongiform encephalopathy (TSE) associated agents in TSE laboratories]
www.baua.de/TRBA

[16] TRBA 120 “Versuchstierhaltung” [Keeping of laboratory animals]
www.baua.de/TRBA

[17] TRBA 220 “Sicherheit und Gesundheit bei Tätigkeiten mit biologischen Arbeitsstoffen in abwassertechnischen Anlagen” [Safety and health for activities involving biological agents in sewage plants]
www.baua.de/TRBA

[18] BGI 629 “Laboratorien – Ausstattung und organisatorische Maßnahmen” [Laboratories – equipment and organisational measures] from the “Berufsgenossenschaft Rohstoffe und chemische Industrie” [Professional association of raw materials and chemical industry], Jedermann-Verlag, Heidelberg, ISBN 978-3-86825-002-2

[19] TRBA 500 “Grundlegende Maßnahmen bei Tätigkeiten mit biologischen Arbeitsstoffen” [Basic measures to be taken for activities involving biological agents]
www.baua.de/TRBA

[20] BGI/GUV-I 853 “Betriebsanweisungen nach Biostoffverordnung” [Operating instructions as specified in the Biological Agents Ordinance] from the German Social Accident Insurance (DGUV)

[21] Gesetz über die Durchführung von Maßnahmen des Arbeitsschutzes zur Verbesserung der Sicherheit und des Gesundheitsschutzes der Beschäftigten bei der Arbeit − Arbeitsschutzgesetz (ArbSchG) [Act on the implementation of occupational safety and health measures to improve the safety and health of employees at work – Occupational Safety and Health Act] of 7.8.1996 (BGBl. I p. 1246), last amended on 2.2.2009 (BGBl. I p. 160)
http://www.gesetze-im-internet.de/bundesrecht/arbschg/gesamt.pdf

[22] BGI 863 “Sicheres Arbeiten an mikrobiologischen Sicherheitswerkbänken” [Working safely at microbiological safety workbenches] from the Berufsgenossenschaft Rohstoffe und chemische Industrie [Professional association of raw materials and chemical industry], Jedermann-Verlag, Heidelberg

[23] Stellungnahme zur Wahl der Autoklavenabluftbehandlung bei Neuanlagen, Nachrüstungen oder Ergänzung [Statement on the selection of autoclave exhaust-air treatment in new systems, retrofits or additions].
http://www.baua.de/de/Themen-von-A-Z/Biologische-Arbeitsstoffe/ABAS/aus-dem-ABAS/Autoklaven.html

[24] ASR A 1.3 “Sicherheits- und Gesundheitsschutzkennzeichnung” [Safety and Health Signs]
http://www.baua.de/de/Themen-von-A-Z/Arbeitsstaetten/ASR/ASR-A1-3.html

[25] Regulation (EU) No 388/2012 of the European Parliament and of the Council of 19 April 2012 amending Council Regulation (EC) No 428/2009 of 5 May 2009 setting up a Community regime for the control of exports, transfer, brokering and transit of dual-use items
http://eur-lex.europa.eu/LexUriServ/

[26] TRGS 401 “Gefährdung durch Hautkontakt: Ermittlung – Beurteilung − Maßnahmen” [Risks resulting from skin contact – identification, assessment, measures]
www.baua.de/TRGS

[27] List of disinfectants and disinfectant procedures tested and recognised by the Robert Koch-Institute, as at 31.05.2007 (15th edition)
Bundesgesundheitsblatt−Gesundheitsforschung−Gesundheitsschutz, 50: 1335-1356 (2007)
http://www.rki.de/DE/Content/Infekt/Krankenhaushygiene/Desinfektionsmittel/Desinfektionsmittelliste/Desinfektionsmittelliste_node.html

[28] Stellungnahme zum Wechsel, Inaktivierung und Entsorgung von Filtern aus mikrobiologischen Sicherheitswerkbänken in TSE-Laboratorien [Statement on the replacement, inactivation and disposal of filters from microbiological safety workbenches in TSE laboratories].
http://www.baua.de/de/Themen-von-A-Z/Biologische-Arbeitsstoffe/ABAS/aus-dem-ABAS/Stellungsnahmen-Labortechnik.html

[29] Stellungnahme zum Einsatz von HEPA-Filtern in raumlufttechnischen Anlagen in Schutz-/Sicherheitsstufe 3 und 4 - Laboratorien und Tierhaltungsbereichen [Statement on the use of HEPA filters in air-conditioning systems in protection/safety level 3 and 4 – laboratories and animal enclosures].
http://www.baua.de/de/Themen-von-A-Z/Biologische-Arbeitsstoffe/ABAS/aus-dem-ABAS/Stellungsnahmen-Labortechnik.html

[30] TRGS 522 “Raumdesinfektion mit Formaldehyd” [Disinfection of rooms using formaldehyde]
www.baua.de/TRGS

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Hyg. Med. 35: 204-208.

[32] Verordnung zur arbeitsmedizinischen Vorsorge (ArbMedVV) [Ordinance on Occupational Medical Prevention] of 18.12.2008, (BGBl. I p. 2768), last amended on 26.11.2010. (BGBl. I p. 1643)
http://www.gesetze-im-internet.de/bundesrecht/arbmedvv/gesamt.pdf

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mph-Verlag, Wiesbaden (2011), ISBN 978-3-88681-106-9

[34] Empfehlung der Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO) "Anforderungen an die Hygiene bei der Aufbereitung von Medizinprodukten" [Recommendation of the Commission for Hospital Hygiene and Infectious Disease Prevention (KRINKO) "Hygiene requirements for the preparation of medical products"]
http://www.rki.de/DE/Content/Infekt/Krankenhaushygiene/Kommission/kommission_node.html

[35] Desinfektionsmittel-Liste der DVG (Deutsche Veterinärmedizinische Gesellschaft) [Disinfectant list of the German Veterinary Medical Society (DVG)]
http://www.dvg.net/index.php?id=1449

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Inaktivierung und Entfernung von Prionen bei der Aufbereitung von Medizinprodukten [Inactivation and removal of prions in producing medical products]
Bundesgesundheitsblatt 2004, 47: 36-40.

Further **links** on the topic of biological safety:

BAuA Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
http://www.baua.de

ABAS Ausschuss für Biologische Arbeitsstoffe (Committee for Biological Agents)
http://www.baua.de/abas

RKI Robert Koch-Institut
http://www.rki.de

ZKBS Zentrale Kommission für Biologische Sicherheit
http://www.bvl.bund.de/cln_027/nn_491824/DE/06__Gentechnik/093__ZKBS/zkbs__node.html__nnn=true

BVL Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
http://www.bvl.bund.de

BfR Bundesinstitut für Risikobewertung
http://www.bfr.bund.de/de/start.html

EBSA European Biosafety Association
http://ebsa.org/portal/

ABSA American Biosafety Association
http://www.absa.org

NIH (National Institutes of Health)
http://www.nih.gov

ATTC (American Type Culture Collection)
http://www.lgcpromochem-atcc.com

Leibniz-Institut DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
http://www.dsmz.de

BG RCI (Berufsgenossenschaft Rohstoffe und Chemische Industrie)
http://www.bgrci.de
(Link to sample operating instructions from the BG RCI:
http://www.bgrci.de/praevention/fachwissen/laboratorien/arbeitshilfen/inhalt-arbeitshilfen/musterbetriebsanweisungen-fuer-laboratorien/)

VAH (Verbund für Angewandte Hygiene)
http://www.vah-online.de

DVV (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e. V.)
http://www.dvv-ev.de

DVG (Deutsche Veterinärmedizinische Gesellschaft)
http://www.dvg.net

KRINKO (Kommission für Krankenhaushygiene und Infektionsprävention)
http://www.rki.de/DE/Content/Kommissionen/KRINKO/