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Technical Rules for Biological Agents	Criteria for the Classification of Biological Agents	TRBA 450
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The Technical Rules for Biological Agents (TRBA) reflect the state of requirements in terms of safety, occupational health, hygiene and work science with respect to activities involving the handling of biological agents. They are drawn up by the

### **Ausschuss für Biologische Arbeitsstoffe (ABAS)**

- Committee for Biological Agents

and adapted by the Committee in accordance with developments. The TRBAs are published by the Federal Ministry of Labour and Social Affairs in the Bundesarbeitsblatt.

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## **1 Area of Application**

This TRBA contains criteria for the classification of biological agents into risk groups according to sections 3 and 4 of the Biological Agents Ordinance.

## **2 Definitions**

A large number of technical terms are needed to give a precise description of the classification criteria and they are explained under 5 at the end of the TRBA in the form of a glossary to provide greater clarity.

## **3 General Remarks**

(1) According to the Biological Agents Ordinance (Biostoffverordnung – BioStoffV) the employer has to carry out a risk assessment for activities with biological agents to ensure the safety and health of the employees. This comprises especially the classification of biological agents, dealing essentially with the effects of a possible exposure to man.

(2) Based upon international conventions and depending on the level of risk of infection, biological agents are classified into four risk groups. The danger of infectious diseases is caused by biological agents of risk groups 2 to 4. Predisposing factors as, for example, genotype, constitution, immunosuppression and diabetes mellitus have to be considered in the process of risk assessment.

(3) The decisive criterion for the classification into risk groups prescribed by the EU regulations is the potential to cause infectious diseases in healthy adults. The concept of the Council Directive 2000/54/EC and of the BioStoffV includes the consideration of sensitising and toxic effects in the risk assessment at the workplace. In the TRBAs 460, 462 and 464 and in the classification lists [1-3] the notes referring to single biological agents concerning toxin production (noted with T) and possible allergic effects (noted with A) provide an aid to orientation.

(4) In general biological agents are classified on a species level. In individual cases it may be necessary in variance to this to classify subspecies, defined varieties (sero- and patho-varieties) or strains. This must be done on the basis of scientific knowledge with reference to the criteria given under No. 4. It is advisable to have a variant classification confirmed by the Committee for Biological Agents.

Subspecies, varieties or individual strains can be classified in a lower risk group than the species if experimental findings or long-standing experience in industrial production can reliably show that their virulence is weaker, or if they have lost known virulence genes.

(5) Opportunistic pathogens within the meaning of this TRBA comprise microorganisms which ordinarily do not cause infectious diseases in healthy humans. Should they nevertheless cause infectious diseases, then their concentration at the site of infection is also decisive in addition to their species-specific characteristics. The state of the host defence mechanisms is more critical in the initiation of an infectious disease caused by opportunist pathogens. Infections of healthy humans caused by opportunistic microorganisms are single events when measured against the number of exposed people. The decision on the classification into risk group 1 or 2 depends on the available data on the individual microorganism. If they are classified into risk group 1, then they must be marked by a proper notation.

(6) With progress in science new microorganisms are routinely discovered and described, and previously unknown pathogenic properties of well-known microorganisms are identified. Organisms classified into a higher risk group may prove to be less hazardous due to an extensive characterisation. In this case the existing lists of classified microorganisms have to be updated, and, if necessary, newly discovered microorganisms or those not listed have to be classified by the employers themselves. In contrast to the evaluation of toxic chemicals the importance of single criteria cannot be determined according to a fixed scheme. On the contrary, a case-by-case evaluation has to be carried out in which single criteria could have individual importance (sections 4.4 to 4.8).

## 4 Criteria for classification

The classification criteria apply to bacteria, viruses, fungi and parasites. Criteria concerning a single group of organisms are printed in italics, the taxon being given.

### 4.1 Systematics

Designation or species designation (including serovar and type, if relevant) and, if necessary, genera, families and orders.

### 4.2 Metabolic properties of pro- and eukaryotes

Point 2 is irrelevant to the classification of viruses and parasites.

The following must be an obligate property.

#### 4.2.1 Autotrophic

- phototrophic
- chemotrophic

#### 4.2.2 Heterotrophic

- psychrophilic
- mesophilic
- thermophilic
- alkalophilic/acidophilic

### 4.3 Natural habitat/mode of living

- Occurrence and typical distribution
- Typically apathogenic, opportunistic pathogenic or obligate pathogenic
- Biological barriers concerning host range

#### Viruses:

*For viruses consideration of the host spectrum/vector range only, emphasising the virus reservoirs. What is the natural geographic distribution of the pathogen (virus)?*

#### 4.3.1 Free living (environment)

#### 4.3.2 Saprophytic or parasitic

#### Parasites:

- *with developmental stages in the environment/without developmental stages in the environment*
- *one host cycle/two host cycles/heteroxenous*

- *stages in the environment directly infectious/or only infectious after maturation*
- *with multiplication in the environment/without multiplication in the environment*
- *with vector/without vector*
- *with multiplication in the vector/without multiplication in the vector*

#### **4.3.3 Host range / vectors**

- plant
- animal
- invertebrates
- vertebrates
- mammals
- non-human primates
- human

#### Viruses

*Multiplication and/or dispersal in the vector*

### **4.4 Pathogenicity for humans, virulence**

#### **4.4.1 Factors of pathogenicity/mechanisms of pathogenicity**

- adhesins
- antiphagocytic factors (mucus capsules)
- invasion factors
- toxins (endotoxins or exotoxins)
- immunomodulators

#### Viruses:

*Oncogenic potential or genes coding for proteins which are involved in the regulation of transcription or signal transmission in the host organism, e.g. chemokines, chemokine receptors, cytokine analogues, MHC analogues or genes which influence apoptosis and play a causative role in pathogenicity.*

#### **4.4.2 Pathogenicity/virulence**

- opportunistic strains
- obligate pathogenic strains
- virulent strains
- avirulent strains

If there is lack of clarity concerning the pathogenicity or virulence of a strain, it may be necessary to conduct animal experiments for further determination. The relevant statutory specifications governing animal experiments and the protection of animals must be complied with.

#### **4.4.3 Clinical picture**

- incubation period
- indications and symptoms
- severity and course (chronic, acute)
- complications
- secondary diseases, late sequelae

#### **4.4.4 Infectious dose, index of contagion, infectivity, manifestation**

#### **4.4.5 Persistence, latency**

#### **4.4.6 Possibilities of treatment**

- specific
- unspecific

#### **4.4.7 Possibilities of prophylaxis**

- active immunisation (e.g. live, dead vaccine, toxoid)
- passive immunisation (e.g. immunoglobulin)
- chemoprophylaxis (specific, unspecific, e.g. antibiotics, virustatics)
- exposure prophylaxis

#### **4.4.8 Possibilities of diagnosis**

- clinical diagnosis
- laboratory diagnosis (method, duration, sensitivity, specificity, required material)
- when after infection

#### Parasites:

*Prepatent period, patency*

#### **4.5 Interaction with other microorganisms**

Synergistic infections

#### Viruses:

*Multiple infections*

#### **4.6 Mechanisms and routes of transmission and dispersal**

##### **4.6.1 Mechanisms of transmission and ports of entry**

- airborne/inhalation

- oral (by swallowing)/ingestion
- percutaneous, sexual (through damaged or undamaged skin or mucous membranes)/infection by contact
- bites or stings by vectors (e.g. fox, ticks, insects)
- diaplacental
- perinatal
- galactogen, colostral (lactophor)

#### **4.6.2 Mechanisms and routes of dissemination**

- water
- soil
- air
- plants
- animals

#### **4.6.3 Routes of excretion**

- respiratory air
- secretory products
- excreta

#### **4.6.4 Means of dissemination**

- spores
- conidia
- eggs
- larvae
- metacercariae
- oocysts
- cysts

### **4.7 Epidemiology**

#### **4.7.1 Reservoirs of pathogens, sources of infection, geographic distribution**

- environment (e.g. surface water)
- plants
- animals
- specific carrier (vectors)
- paratenic hosts, transient hosts (e.g. aquatic birds/*vibrio cholerae*), primary hosts of typical anthroozoonoses
- man

#### 4.7.2 Frequency of disease

- incidence
- prevalence
- morbidity
- mortality

#### 4.7.3 Distribution of disease

- sporadic
- epidemic
- endemic
- pandemic

#### 4.7.4 Origin of infection

- endogenous
- exogenous

#### 4.8 Resistance/tenacity

- formation of resistant stages, endospores
- resistance to chemicals, disinfectants
- resistance to chemotherapeutics
- resistance to thermal process
- resistance to radiation including UV
- resistance to dryness

#### Concerning viruses:

*General resistance outside of a host cell, i.e. remaining capable of multiplication.*

### 5 Glossary explaining the Criteria for Classification

Adhesins	Factors which facilitate the specific adhesion of biological agents to host cells
acidophilic organisms (acidophilic o.)	Organisms which only grow particularly well in an acidic environment (pH ≤ 6)
active immunisation	Intentionally initiated contact of the macroorganism (human, animal) with weakened living or dead pathogens, their cellular structural components or metabolic products (e.g. toxins) with the aim of causing the formation of protective specific immunoglobins (antibodies) (humoral immunity) or the build-up of a protective cellular immunity
alkalophilic organisms (alkalophilic o.)	Organisms which only grow particularly well in an alkaline environment (pH ≥ 8)

antiphagocytic factors	Factors which obstruct the intake of live or dead particles in the interior of phagocytes and thus interfere with a stage of the anti-infective defences
Apathogenic	Does not make sick
autotrophy (autotrophic)	Nutrition exclusively on the basis of inorganic compounds.
chemoprophylaxis (of infectious diseases)	Targeted preventive use of medication to prevent an infectious disease
Diaplacentary	En route through/via the placenta
endemy (endemic)	Permanent presence in a geographically defined area. Medical: Disease which can be verified permanently in a small section of the population.
Endogenous	Arising from an internal cause in the body or originating from one's own body
endogenous viruses	Viruses which are integrated in the genome of the host's germ cells and are passed on via germ line (e.g. some retroviruses)
endogenous infection	Infection due to organisms of the flora naturally present in the host organism whose development normally assumes local influences (e.g. injury) or general influences (immune deficiency)
Endotoxin	1. Any toxin which is only released when cells are dissolved 2. Heat-stable toxin (lipopoly saccharid protein complex) in the outer cell membrane of gram-negative bacteria. It is only released when the bacteria cell is dissolved.
end host	In the case of parasites with change of host in the development process, that host in which the parasite achieves the stage of sexual maturity.
epidemy (epidemic)	Accumulation of transmissible diseases with temporal and geographical limitation
Exogenous	Due to external causes or introduced into the body from outside
exogenous infection	Infection which arises due to the transmission of a pathogen from outside to the host (endogenous viruses can also be transmitted)
exotoxin (=ectotoxin)	Toxin which is given off actively by the producer into the environment.
exposure prophylaxis	Measures taken to prevent the spread of pathogens by eliminating sources of infection, interrupting the transmission routes and restricting the possibility of transmission
heterotrophy (heterotrophic)	Nutrition exclusively on the basis of existing organic compounds
immune modulators	Substances which influence processes in the immune system; mostly used for the purpose of stimulation
infection dose	Number of pathogens which can trigger an infection or which are injected into a test animal
Infectiousness	Degree of the ability of a pathogen to be transmitted from host to host, to adhere to the new host, to reproduce and to establish itself in or on the host's tissue
incubation (incubation period)	Time from the infection (penetration of the pathogen into the body) to the appearance of clinical symptoms
Incidence	Frequency of the fresh occurrence of an illness in a certain population
Colostrum	Relating to the 'foremilk' produced at the end of a pregnancy
contagion index	Ratio of the those manifestly suffering from an infection to the uninfected portion of a part of a population exposed ("contact persons"), normally in relation to 100 such contact persons exposed to infection

contagiousness (contagious)	Ability to infect, infectious: the pathogens of an infected organism are secreted via various routes and can be transmitted to others – directly or indirectly
Latency	In the case of bacteria, viruses, parasites: temporarily concealed state of an infectious disease  In the case of bacteriophages: phase from infection until emergence of the first infectious stages
mesophilic organisms	Organisms which grow particularly well at temperatures of about 20-40°C
Morbidity	Frequency of illness: number of ill persons in a population in relation to 100,000 individuals per year
Mortality	Number of deaths in a population in relation to 100,000 individuals per year
obligatorily pathogenic	obligatory: exclusive, essential, unconditional  pathogen: triggering, causing, inducing an illness
opportunistically pathogenic	Only causes illness when the host organism's defence capacity is impaired by local factors (e.g. wounds) or general factors (e.g. immunosuppression)
(per)oral transmission	Transmission by swallowing, with the digestive tract as the entry port for the pathogen (e.g. faecal-oral)
pandemy (pandemic)	Accumulation of transmissible diseases with temporal, but no geographical limitation
parasite (parasitical)	Living entity which resides temporarily or permanently <b>on</b> the body of other organisms (hosts) (ectoparasite) or in the body of other organisms (endoparasite) and feeds at their expense
paratenic host	Host in which a parasitic stage of an animal penetrates, but does not develop further with persistent infectiousness
parenteral transmission	Transmission bypassing the gastro-intestinal tract (e.g. by i.m./i.v. injection, blood transfusion, organ transplant, cuts and pricks, sting or bite of living vectors)
passive immunisation	Administration of specific antibodies of human or animal origin
patency (1)  prepatent period(2)	(1) Period in which parasites or their development stages can be detected in blood, faeces, urine or the skin of the host  (2) Period from the infection of a host with parasites to their verification in blood, faeces, urine or the skin
Perinatal	Period around the birth
persistence persistant	In terms of the persistent viability of pathogens without multiplication
phototrophic organisms	Organisms which use light as an energy source
Prevalence	Frequency of a certain feature or a certain disease at a certain time
psychrophilic organisms	Organisms which only grow well at temperatures of $\leq 20$ °C
saprophyte saprophytic	Organism which feeds off dead organic substances
Sporadic	isolated (in occurrence); scattered
synergistic effect	The total effect of a number of events or pathogens is greater than the sum of their individual effects
thermophilic organisms	Organisms which only grow particularly well at temperatures of $\geq 40$ °C
Transmission	Transport of an infection pathogen from an infection source (e.g. infected material, pathogen-bearing culture, infected animal, infected human) to a human or other hosts
Vector	1. Carrier (transmitter) (living or non-living) of infection pathogens. 2. (GenTG): A biological carrier which introduces nucleic acid elements into a

	new cell
intermediate host	In the case of multi-host parasites the host in which the parasite reproduces asexually or undergoes a metamorphosis in the larval stages

## 6 Indications for the Evaluation of Specialist Literature on the Pathogenicity of Microorganisms

### 6.1 Preliminary remark

The objective is the classification of microorganisms in risk groups according to the state of scientific knowledge. The following points must be noted, but they are no criteria for exclusion of a citation.

### 6.2 Literature sources

- Primary literature/scientific literature  
*(evaluation of the frequency of reliable information, peer review, listed in ISI)*
- Single case description/specialist article/Review/publication of a specialist committee  
*(review with quotations of the original works which are accessible, relevant experience of the specialist committee)*
- Expertise of the author/author-collective  
*(publications of authors, relevant experience of the institutes)*
- Age of the publication in relation to more recent publications

### 6.3 Description of the of the infectious process

- Identification of the pathogen  
*(suitable methods for identification, use of reference strains)*
- Exposed group of people
  - Workers  
*(description of the working conditions, type of exposure, risk assessment, details of protective measures)*
  - The general public  
*(natural exposure via the environment, direct/indirect contact, zoonosis)*
- Patient  
*(healthy, compromised defence system / previous history of ill health, detailed description of the finding)*
- Proposals/indications for a classification  
*(national/international classification, reasons for the classifications)*

## References

- [1] Directive 2000/54/EC of the European Parliament and the Council on the protection of workers from risks related to exposure to biological agents at work. Official Journal of the European Communities  
No. L 262/21 dated 17.10.2000
  
- [2] Merkblätter "Sichere Biotechnologie - Eingruppierung biologischer Agenzien" der Berufsgenossenschaft der chemischen Industrie;  
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Parasiten (BGI 632, previously ZH 1/345)  
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Jedermann-Verlag Dr. Otto Pfeffer oHG; Postfach 10 31 40; 69021 Heidelberg
  
- [3] Notice of the Federal Ministry of Health: "Liste risikobewerteter Spender- und Empfängerorganismen für gentechnische Arbeiten".  
Bundesgesundheitsbl. 3/01, P. 246, also under the term Gentechnik on the Internet at [www.rki.de](http://www.rki.de)