

# > Microbiological safety cabinets (MSC)

*Guideline for the use of microbiological safety cabinets when handling human-pathogenic microorganisms*



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*Guideline for the use of microbiological safety cabinets  
when handling human-pathogenic microorganisms*

*2<sup>nd</sup> updated edition, December 2014; first published in 2008*

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**Publisher**

Federal Office for the Environment (FOEN)  
The FOEN is an office of the Federal Department of Environment, Transport, Energy and Communications (DETEC).  
Federal Office of Public Health (FOPH)  
The FOPH is an office of the Federal Department of Home Affairs (FDHA)

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**Suggested form of citation**

Biebinger S., Burkart D., Gschwind M., Hutter M., Roulin S., Wiesendanger B. 2014: Microbiological safety cabinets (MSC). Guideline for the use of MSC when handling human-pathogenic microorganisms. 2<sup>nd</sup> updated edition, December 2014; first published in 2008. Federal Office for the Environment, Bern. The environment in practice no. UV-0816-E: 21 pp.

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**Link to PDF file**

[www.bafu.admin.ch/uv-0816-e](http://www.bafu.admin.ch/uv-0816-e)  
(no printed version available)

This publication is also available in German, French and Italian.

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## > Abstracts

This implementation guide presents measures to protect against infectious aerosols. The main focus is on the use of microbiological safety cabinets (MSC). It is intended to show the most important criteria and considerations for using an MSC when handling human-pathogenic organisms. But it also summarises further measures to prevent aerosols.

In der vorliegenden Vollzugshilfe werden Massnahmen zum Schutz vor infektiösen Aerosolen vorgestellt. Der Fokus liegt insbesondere auf der Verwendung und dem Einsatz der mikrobiologischen Sicherheitswerkbank (MSW). Es soll ein Hilfsmittel sein in dem die wichtigsten Kriterien und Überlegungen für die Verwendung einer MSW beim Umgang mit humanpathogenen Organismen aufgezeigt werden. In summarischer Form wird darin aber auch auf weitere Massnahmen zur Aerosolvermeidung hingewiesen.

La présente directive expose les mesures à prendre pour se protéger contre les aérosols infectieux. Elle met l'accent sur l'utilisation d'un poste de sécurité microbiologique (PSM). Elle constitue un outil d'aide contenant les principaux critères et réflexions relatifs à l'utilisation d'un PSM lors de la manipulation de microorganismes pathogènes pour l'homme. D'autres mesures destinées à lutter contre les aérosols y sont également présentées sous forme condensée.

Il presente aiuto all'esecuzione presenta misure atte ad evitare la formazione di aerosol infettivi ed è incentrato sull'utilizzo e sull'impiego del banco di lavoro di sicurezza microbiologica (BSM). Questo testo è inteso come uno strumento ausiliario volto a illustrare i criteri e le riflessioni più importanti ai fini dell'utilizzo di un BSM nell'ambito della manipolazione di microrganismi patogeni per l'uomo. Tuttavia, riassume anche altre misure volte a prevenire la diffusione di aerosol.

**Keywords:**

Containment Ordinance (CO), biosafety, microbiological safety cabinet, laboratories

**Stichwörter:**

Einschliessungsverordnung (ESV), Biosicherheit, mikrobiologische Sicherheitswerkbank, Laboratorien

**Mots-clés:**

ordonnance sur l'utilisation confinée (OUC), sécurité biologique, poste de sécurité microbiologique, laboratoires

**Parole chiave:**

ordinanza sull'impiego confinato (OIConf), sicurezza biologica, banco di lavoro di sicurezza microbiologica, laboratori



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## > Foreword

The Containment Ordinance (CO) stipulates the use of a microbiological safety cabinet (MSC) in laboratories at safety level 2 and higher. However, it also permits the omission of an MSC in individual cases, i.e. where this does not compromise safety.

The present implementation guide explains when a laboratory needs such an MSC for the handling of human-pathogenic organisms, and when it can be omitted. It is aimed primarily at institutions of education and research, and also at the authorities responsible for enforcing the Containment Ordinance. The details given are intended to bring clarity and uniformity of practice in implementing the Containment Ordinance.

The implementation guide was prepared by the Working Group on safety cabinets of the Intercantonal Group ERFA BIO, and completed under the aegis of the FOPH. We would like to take this opportunity to thank all members of the Working Group and the FOPH for their commitment in elaborating this implementation guide.

Gérard Poffet  
Vice Director  
Federal Office for the Environment (FOEN)

# 1 > Introduction

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## 1.1 Content, purpose and significance

When working with human-pathogenic organisms in laboratories, laboratory staff should be protected from infections, and the environment from contamination. In accordance with the Ordinance on the Contained Use of Organisms (Containment Ordinance, CO, SR 814.912) of 9 May 2012 and the Ordinance of 25 August 1999 on Occupational Safety in Biotechnology (OOSB; SR 832.321), a microbiological safety cabinet (MSC) is therefore required for particular activities, and measures must be taken to prevent the production of aerosols.

The purpose of this Guideline is to put into concrete terms the statutory provisions for measures, in particular the use of MSC, to protect against infectious aerosols. The document indicates the most important criteria for and considerations in the use of an MSC in the handling of human-pathogenic organisms.

*This document **focuses** on the use of MSC.*

*It also summarises additional measures for preventing aerosols. Other aspects relevant to MSC, such as technical information, operation and maintenance, are mentioned because of their importance, but they are not the subject of this Guideline. Instead we refer to the appropriate specialist literature.*

The target audiences of this document are institutions of education and research, other bodies that handle human-pathogenic microorganisms in contained systems, and the authorities responsible for enforcing the CO and the OOSB.

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## 1.2 **Scope**

The present Guideline deals exclusively with the handling of human-pathogenic organisms, covering the protection both of the environment and the general population (CO), and of employees (OOSB).

The stipulations apply to the handling of human-pathogenic microorganisms in Group 2 (low-risk microorganisms) and Group 3 (moderate-risk microorganisms). The handling of Group 4 microorganisms is not covered by these stipulations.

The use of an MSC may also be required to handle environmentally damaging, or animal- or plant-pathogenic, organisms. Some of the principles set out in this document also apply to these organisms. But since there may be other relevant considerations concerning risk in these situations, they are not covered in this document. The FOEN can be consulted on issues associated with these organisms.

## 2 > Use of the microbiological safety cabinet

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### 2.1 Protective effect of a microbiological safety cabinet

*Microbiological safety cabinets (MSC) are internationally classified into 3 classes. Since this document focuses primarily on the use of MSC, we do not give the different technical details (for definition and detailed description of the various types or synonymous designations: see Glossary and Pamphlet B011 of BG-Chemie, 9/2004, in German).*

*In the present document, the designation “microbiological safety cabinet (MSC)” is used exclusively to mean a Class II cabinet, which is the most commonly used type.*

Assuming that “good microbiological practice” is consistently applied, a hygiene plan is adhered to in a disciplined fashion, and a suitable training programme is in place, using an MSC can achieve the following:

- > Protection of workers from direct exposure to infectious aerosols
- > Protection of immediate laboratory environment from contamination by released aerosols (protection of the population and the environment)
- > Protection of the product or the materials used from contamination by micro-organisms in the environment.

The MSC is also a physical barrier and provides a degree of protection from splashes, which is particularly important when handling pathogens that cause infection via the mucous membranes. There are however other measures for preventing or protecting against splashes. It should be noted that a Class II MSC is not completely sealed and – depending on the model being used – provides no protection, or only slight protection, from radioactive substances or toxic chemicals.

### Risks from cross-contamination and accidental inoculation

When the MSC is used, hands, clothing or laboratory equipment may be contaminated by aerosols, spills or direct contact with spilled or contaminated biological material. The risk of such contamination, which could result in the transfer of microorganisms via injuries or mucous membranes (mouth, eyes, nose), is reduced to a certain extent by working inside the MSC. However, cross-contamination, i.e. transfer of microorganisms by such contamination, can be prevented only if additional safety measures are also adhered to (e.g. avoid hand-to-face contact; avoid touching surfaces and objects outside the MSC such as door handles, telephones and writing implements with contaminated gloves or dirty hands).

The MSC does not protect against infections caused by accidental inoculation (puncture wounds/cuts). As far as possible, therefore, handling sharp or pointed objects should be avoided, and unbreakable consumables or disposable material should be used.

## 2.2 Relevant criteria for the risk of infection

The decision on whether human-pathogenic microorganisms may be handled outside a safety cabinet (Safety measure 21 in Annex 4, Table, point 2.1, CO) depends on the risk of infection present in specific activities involving microorganisms. This is often difficult to assess under laboratory conditions. To evaluate the risk of infection under laboratory conditions it is necessary to perform a risk assessment that takes into account various criteria. In addition to pathogenicity or virulence, the most important of these are the possible routes by which the microorganism used may be transmitted and the potential of any working step to create aerosols.

### Routes of transmission

Various routes of transmission are known for pathogenic microorganisms (airborne, oral, contact, accidental inoculation), which under natural conditions can lead to colonisation of the body and thus to illness.

**Natural routes of transmission**

The probability of transmitting pathogenic organisms may be higher under laboratory conditions than under natural ones. The reason is that high concentrations of pathogenic microorganisms are often used, and many work steps could produce aerosols.

**Possible routes of transmission in the laboratory**

### Potential of specific activities for creating aerosol or droplets

Aerosols are often produced when handling microorganisms (liquid cultures, agar plates etc.). A small quantity of aerosol can occur even when preparing a gram stain or inoculating a plate with a platinum loop.

According to the activity, the amount of aerosols or droplets produced may be greater (e.g. shaking liquid cultures) or smaller (e.g. inoculating with platinum loop). See also "Further literature".

Examples of laboratory activities with potential for creating aerosols:

- > Work steps involving risk of spills
- > Work with liquid cultures (inoculating cultures; decanting)
- > Shaking, vortexing, stirring
- > Pipetting (especially emptying pipettes)
- > Opening bottles and culture flasks
- > Centrifuging
- > Ultrasound disruption
- > Homogenising infectious tissue
- > Handling freeze-dried microorganisms, lyophilisation
- > Inoculating secondary cultures with inoculation loops (in particular, flaming of metal loops)
- > Handling needles and syringes
- > Washing consumables
- > Scraping off
- > Sorting cells by Fluorescence Activated Cell Sorter (FACS) (older open-style types)

Further criteria for assessing the infection risk include:

- > Volume and concentration of the microorganism used
- > Infective dose of the microorganism used
- > Current prophylaxis of laboratory staff and general population
- > Resistance profiles of the organisms used (possible treatment)
- > Knowledge of laboratory infections and their causes<sup>1</sup>

<sup>1</sup> Particularly relevant here are laboratory infections where the cause is unknown and which have occurred as a result of aerosol transmission. However, it can be assumed that laboratory infections and their causes are often not known or published. This means that even if a particular microorganism is not known to cause a laboratory infection, this cannot be the only criterion for omitting safety measures.

### 2.3 Use of the safety cabinet

In accordance with the CO and OOSB, appropriate measures should be taken to prevent aerosols during activities at safety level 2 or higher (CO, Annex 4, and OOSB, Annex 3, Table, safety measure 22).

Using an MSC is one way to protect against aerosols; MSC are standard equipment for laboratories at safety level 2 or higher, and are stipulated by both Ordinances for activities from safety level 2 upwards (CO, Annex 4, and OOSB, Annex 3, Table, safety measure 21).

Equipping a laboratory with an MSC may be omitted at safety level 2 in substantiated cases, if the Federal Office responsible issues an authorisation to do so within the field of the CO. The applicant must however provide justification for not equipping the laboratory with an MSC, or proof that substitute measures are adequate to guarantee the protection of humans, and in particular employees, and the environment. At safety level 3 an MSC must always be present.

The issue of equipment should be distinguished from the issue of which work steps really do have to be performed in the MSC. The decision on which particular work steps should be carried out within the safety cabinet must be used depends on the risk assessment for each individual case. This must take into account the protective effect of the MSC (see 2.1) and the criteria mentioned above (see 2.2). According to the hazard present, priority may be given to reducing the immediate risk of infection to laboratory personnel, or to avoiding the contamination of the laboratory environment by released aerosols.

The following principles apply to the microorganisms used:

#### **Group 3 microorganisms**

*When activities that may produce **aerosols** are performed with Group 3 human-pathogenic microorganisms, **an MSC must routinely be used.***

**Reason:** *The specific properties of these microorganisms (e.g. low or unknown infective dose, high pathogenicity, lack of treatment/prophylaxis) mean they represent an increased risk. Because of this increased risk, the release of aerosols in the laboratory environment and the resulting contamination (and carry-over) should be avoided.*

## Group 2 microorganisms

When activities that may produce **aerosols** are performed with Group 2 human-pathogenic microorganisms, **an MSC is necessary** for:

- > *Microorganisms that are airborne transmissible under natural conditions (see definition in the Glossary), and where according to the current state of scientific knowledge, infection of employees by aerosols produced cannot be ruled out.*  
Example: *Neisseria meningitidis*
- > *Microorganisms that are not airborne transmissible under natural conditions but could cause infection under laboratory conditions through aerosols or droplets coming into direct contact with the facial mucous membranes (eyes/nose/mouth) or on non-intact skin (wounds).*  
*These include e.g. microorganisms that are normally transmitted through contact, inoculation or orally, but where transmission and subsequent infection under laboratory conditions are possible. Examples: Vaccinia virus, human Herpes viruses (e.g. Epstein-Barr Virus)*
- > *Microorganisms for which the risk of infection through aerosols cannot be ruled out entirely.*  
Examples: *pathogenic microorganisms for which the route of transmission and infective dose are still largely unknown.*

*Use of an MSC can be omitted if activities using **Group 2 microorganisms** are being carried out, where transmission through aerosols or droplets with subsequent infection of staff can **largely be ruled out** even under laboratory conditions. Risk assessment should be performed on a case-by-case basis. If there is any remaining uncertainty, the MSC may not be omitted.*

*Examples: most Group 2 enteropathogenic, opportunistic bacteria (e.g. *Campylobacter* and *Salmonella enterica* except Serovar Typhi)*

**Reason:** *The CO and OOSB stipulate that aerosols must be minimised at safety level 2. This applies particularly if the staff are at immediate risk of infection.*

If a risk assessment has shown that use of an MSC is not appropriate, or if working in the MSC is not technically possible or sensible, suitable substitute measures must be taken in accordance with the risk assessment (see 2.5 and Chapter 4: Further measures to protect against aerosols or droplets).

The **flow chart** at the end of this document may help the risk assessment and consideration of whether to use an MSC (Chapter 5: Flow chart for risk assessment).

## 2.4 Special cases

### Medical microbiological diagnostics

The analysis of clinical samples in medical microbiological diagnostics is a special case, in that it is generally not known whether or what pathogenic microorganisms the samples contain. The extent of the potential infection risk is thus difficult to assess and depends on the nature and origin of the samples, as well as on the microorganisms to be investigated.

Basically, therefore, a risk assessment should also be carried out for the analysis of clinical samples in medical diagnostics, to ascertain whether an MSC is needed for particular work steps or not. This assessment should involve the same considerations as for research activities, i.e. the possibility of aerosol formation in the individual steps of the activity, the transmissibility of the organisms, and the risk of infection of the staff.

Detailed instructions for using an MSC in diagnostics, which take into account the special requirements of handling clinical samples, are given with examples in the Guideline Safety measures in human medical microbiology diagnostic laboratories<sup>2</sup>.

### Analysis of environmental samples suspected to contain Group 3 microorganisms (suspected bioterrorism samples)

Like medical microbiological diagnostics, the assessment of infection risk in the analysis of suspected for example bioterrorism environmental samples depends on the nature and origin of the samples, and the suspicion in question. For example, in the case of weaponised anthrax powder it must be assumed that, because of the associated infection risk of aerogenic transmission, handling powder samples is much more hazardous than manipulating cultures of *Bacillus anthracis* or handling of clinical samples.

Primary analysis and any further investigation of suspected bioterrorism environmental samples must be performed in accordance with level 3 safety measures and in an MSC. At present, these dangerous analyses may be performed only in laboratories suitably equipped for this (Regional laboratories).<sup>3</sup>

<sup>2</sup> FOEN (2008): [www.environment-switzerland.ch/uv-0815-e](http://www.environment-switzerland.ch/uv-0815-e)

<sup>3</sup> BAG (2006), Diagnostik im B-Bereich – Stand und Perspektiven des Regionallabornetzwerkes. BAG-Bulletin 33/06.

2.5

### Work where use of an MSC is not possible or reasonable

Certain work steps cannot be carried out in an MSC for technical reasons, even though they may produce aerosols, and therefore, on the basis of the criteria given above, should be performed in the MSC. To respect the principle of proportionality, these work steps may – in exceptional cases and based on a risk assessment – be carried out outside the MSC, provided suitable substitute measures are taken (Chapter 4: Further measures to protect against aerosols or droplets).

The same criteria that apply to handling pathogenic microorganisms also apply to experimental infections and when handling infected animals. For certain animal procedures (e.g. handling large animals, inoculating animals with syringes), handling in an MSC may be problematic or impossible.

**Handling infected animals**

For procedures such as changing cage litter, or animal dissection, there are special workbenches that permit greater freedom of movement. This equipment should not however replace an MSC entirely. The device specification must be adhered to in each case. If there is a possibility of infection because of aerosol formation when handling animals, additional measures should therefore be taken. At higher levels of risk, isolation units should be used.

The handling of certain parasites and their vectors is a further special case. Handling insect vectors in an MSC can be particularly problematic (laminar flow; MSC provides additional hiding places for insects), so that here the risk assessment should be performed on a case-by-case basis and if necessary, alternative measures should be taken.

In some cases, equipment used for infectious organisms, and from which the release of aerosols is possible, may be too large to be placed inside an MSC. Examples include large centrifuges or Fluorescence Activated Cell Sorters (FACS). Suitable technical measures can usually be applied to the equipment to prevent the escape of infectious aerosols. (Chapter 4: Further measures to protect against aerosols or droplets).

**Use of large laboratory equipment that produces aerosols**

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## 3 > Safe handling of the microbiological safety cabinet

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Care should be taken to ensure that the MSC is tested before first use or after substantial modifications (e.g. change of location). When positioning the device, ergonomic principles should be observed. The correct placement of the MSC within the laboratory plays an important role. Any disturbance in airflow in the MSC can reduce its efficacy. The devices should therefore be sited as far as possible from door openings, windows or air conditioning systems.

The MSC will function properly only if the necessary rules are adhered to in its use. One important condition is the provision of operating instructions and the training of laboratory staff. Furthermore, there should be clear instructions for cleaning, disinfection, maintenance, servicing (including filter changes and regular function monitoring) and repairs, as well as procedures for malfunctions (see BG Chemie Pamphlet B011, 9/2004). Servicing and maintenance of the MSC must be carried out according to the manufacturer's instructions; the devices must reflect the current state of technology.

In operating the MSC, care should be taken not to cover the air vents, to avoid quick movements within the MSC, and to keep only a minimum amount of material inside the MSC.

The use of open flames in the MSC is contested as they could cause a disturbance of airflow in the MSC and possible heat damage to the HEPA filter (alternatives are given in Chapter 4). They should therefore be avoided if possible. Likewise, equipment (e.g. centrifuges) inside the MSC could lead to air turbulence that diminishes the function of the MSC. Placing such devices inside the MSC should therefore be avoided if possible.

## 4 > Further measures to protect against aerosols or droplets

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In addition to using an MSC, a range of further measures can be taken by laboratory staff to protect themselves against released aerosols or droplets. These measures should be employed whenever necessary. They are particularly important for laboratory tasks that cannot be performed inside the MSC, e.g. when using large equipment that does not fit inside the apparatus.

To prevent the escape of aerosols during centrifugation, tightly sealed, unbreakable receptacles should be used. Equipment should be used that has special aerosol protection covers (with airtight seal) for the rotor (for table centrifuges) or the buckets (for larger centrifuges).

**Centrifuges with aerosol protection**

Aerosols can be produced in centrifugation not only through unsealed tubes, but also from contamination on the outside of the tubes. In both cases, using aerosol protection covers is an effective way to prevent aerosols being released into the room. After centrifugation, the tubes in the closed rotor (for table centrifuges) or the adaptors (for larger centrifuges) should be transferred to the MSC and opened there. This applies particularly to cases of imbalance, or if leakage (a defective tube) is suspected.

In addition to wearing protective clothing and gloves, which are part of good microbiological practice, laboratory staff may also need to wear filter masks (e.g. FFP2 or FFP3) and/or protective goggles or shields.

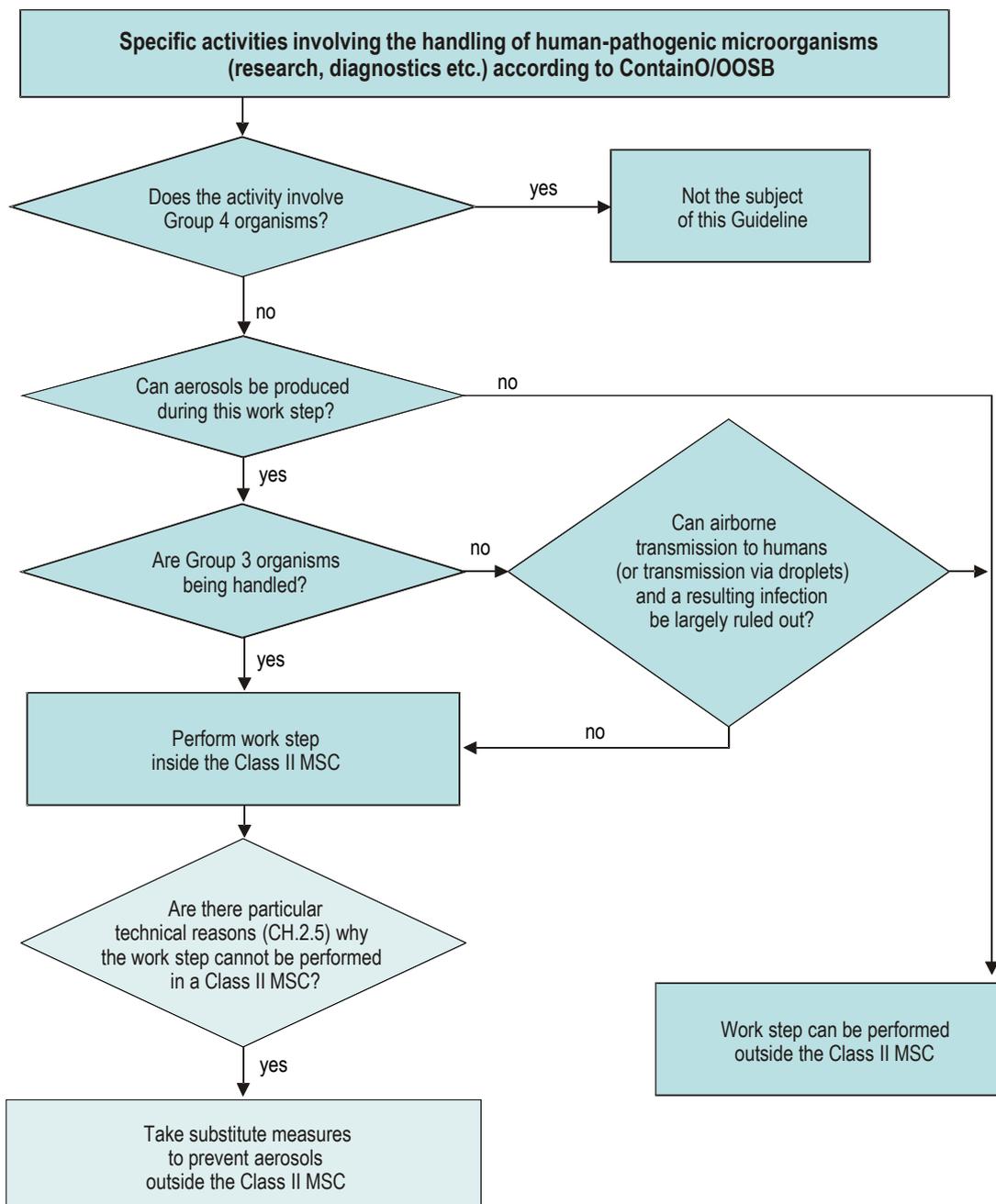
**Personal safety measures**

- > Ultrasound: no use of open receptacles (immersion probe)
- > Use of disposable inoculation loops instead of flaming platinum loops
- > Use of loop microincinerators instead of flaming platinum loops
- > Use of aerosol-protected pipettes; if possible do not completely eject liquid from the pipette
- > Use of tightly sealed sample and culture flasks
- > Avoiding spills when decanting or pouring liquid cultures
- > Sealing agar plates with tape (e.g. spore-forming bacteria or fungi)
- > Regular decontamination, especially of the MSC and the centrifuges
- > Regular testing and changing of exhaust air filters.

**Examples of further measures**

## 5 > Flow chart

Flow chart for risk assessment



## > Annex

### Glossary

#### Airborne transmission

Transmission of pathogens through the air, particularly by inhaling infectious aerosols or dust. This is the main route of transmission for infectious diseases of the airways, but under laboratory conditions it can also lead to the transmission of other diseases.

#### Aerosols

Airborne liquid droplets and solid particles that consist of biological materials and/or their metabolites or that can be attached to them. Because of their small size (typically 0.1–10 µm) they float in the air and can be inhaled. During normal laboratory activities, aerosols are formed from liquids through the influence of mechanical forces (vibration, pressure, emptying pipettes etc.). They can also be formed as a result of splashes or spills of liquids. The smaller the droplets or droplet cores that arise through evaporation, the more easily they are transported by the air and can enter the lung alveoli.

#### CO

Ordinance of 25 August 1999 on the Contained Use of Organisms

#### FOEN

Federal Office for the Environment

#### FOPH

Federal Office of Public Health

#### FVO

Federal Veterinary Office

#### HEPA filter

High efficiency particulate air filter

#### Infection

Penetration of microorganisms (e.g. bacteria, viruses, fungi, parasites) into a macroorganism (e.g. human), where they remain and reproduce (colonisation); in a broader sense, the infectious disease.

#### MSC

Microbiological safety cabinet: device with ventilator and air filtration to protect the user and the environment from infectious aerosols.

Three classes of MSC are distinguished:

(For details see BG Chemie Pamphlet B011, 9/2004, in German)

A **Class I MSC** is a workbench with airflow directed inwards, used exclusively to protect people (not products).

A **Class II MSC** (which is the focus of this document) has an aperture through which the user can perform working procedures inside the cabinet. It is so constructed that an air barrier is created at the

aperture through the inwards-flowing air and a vertical airflow in the work area. Filtration of the exhaust air ensures that aerosols cannot escape. According to the structure, four subtypes of Class II are distinguished.

A **Class III MSC** is a tightly sealed cabinet with internal underpressure, which is operated using gas-tight, sealed rubber gloves.

It should be noted that in the English-speaking literature and in laboratory practice, a variety of terms are used for MSCs.<sup>4</sup>

#### OOSB

Ordinance of 25 August 1999 on Occupational Safety in Biotechnology

#### SUVA

Swiss Accident Insurance Fund

#### Transmission

In a scientific sense, the transport of microorganisms and their colonisation of the body, which can lead to an infection or a disease.

#### Transmission through carry-over

Transmission of pathogens via contaminated laboratory equipment clothing or hands (typically contaminated by spills, splashes or aerosols). Pathogens can then be transmitted (carry-over by the hands, contact contamination) through direct contact with contaminated equipment or materials (including hand-to-mouth contact), through existing wounds or via the mucous membranes (mouth, eyes, nose).

#### Virulence

The infective strength of a pathogen, which determines the level of its pathogenicity. The characteristic ability to penetrate healthy tissue, to reproduce there, and to damage or partially destroy the host organism through toxicity.

<sup>4</sup> In the English-speaking literature and in laboratory practice, various terms are used for an MSC (e.g. laminar flow, safety work station, hood). These terms are used very loosely and sometimes also describe other equipment similar to MSC but fulfilling different functions (e.g. fume cupboard). The terms "microbiological safety cabinet (MSC)" and "biological safety cabinet (BSC)", are however unambiguous, and are used in English for Class II MSC.

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