UNIVERSITÄT BERN

BIOSAFETY @ DBMR

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Overview

- Principles of Biosafety – Ordinances
- Personal Protective Equipment, PPE
- «Good lab practice»
- Organism groups / classes of activity / Biosafety Levels
- Waste disposal
- Transport – Spill – Emergency

Information provided on the DBMR webpage Biological safety

- Ordinance on handling organisms in contained systems (Containment Ordinance) of May 9, 2012 (Status as of June 1st, 2014)
- Ordinance on the protection of employees from dangerous microorganisms of August 25, 1999 (Status as of June 1st, 2012)
- Responsibilities of group leaders
- Decision-making aid for the classification of the activities
- Checklist for the notification of level 1 and 2 activities
- Use of level 2 biosafety cabinet
- SOP biological spill kit
- SOP Lentivirus lab D811e
- SOP Hepatitis virus lab D828
- Injury Report

Links:

- Swiss Expert Committee for Biosafety (SECB)
- Federal Office for the Environment (FOEN)

Biosafety vs. Biosecurity

Biosafety = protects people from germs (infecting them)

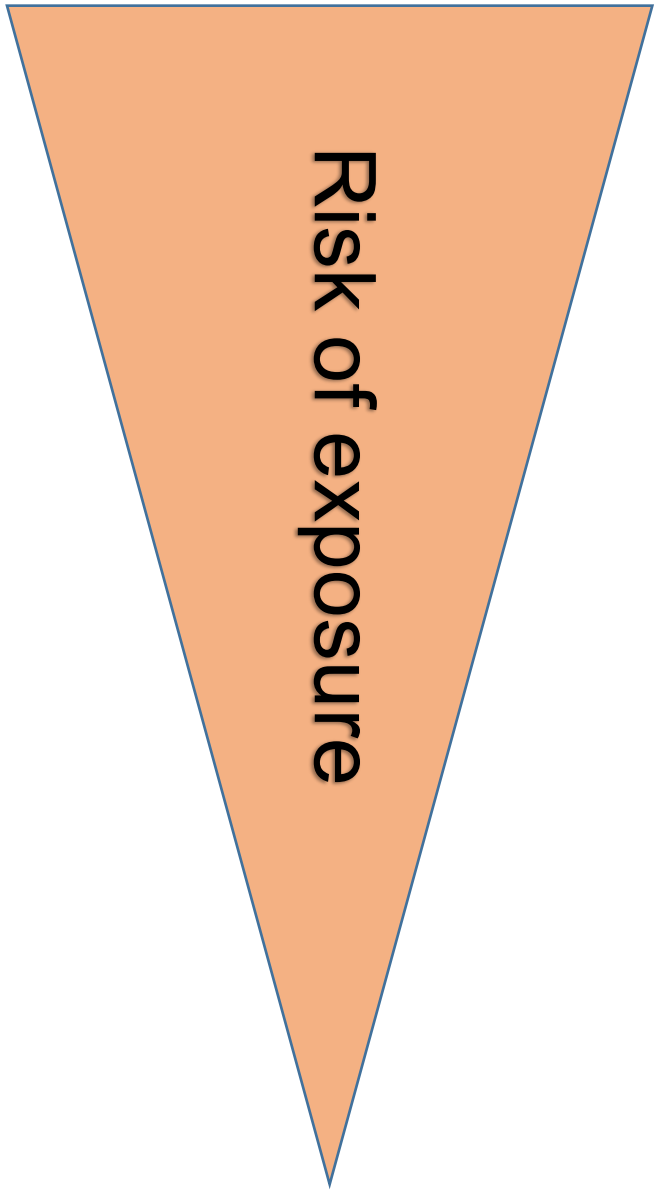
BIOSAFETY is "the **containment principles**, technologies and practices that are implemented to **prevent unintentional exposure to pathogens** and toxins, or their accidental release.“

Biosecurity = protects germs from people (stealing them)

In veterinary and agricultural fields BIOSECURITY means protecting biological resources from foreign or invasive species.

In the lab setting BIOSECURITY means “institutional and personal **security measures** and procedures **designed to prevent** the loss, theft, misuse, diversion or intentional **release of pathogens** (The WHO Laboratory Biosafety Manual)

The Principle



Elimination
(replace)

Substitution
(reduce, refine)

Technical measures
(biosafety cabinets, cages)

Organisational measures
(SOPs, training)

Personal protection
(PPE)

Good laboratory practice

Anything wrong here?

«Good laboratory practice» for work with microorganisms

- Close doors and windows
- No smoking, drinking, eating, food storage, application of make-up, eye scratching – never touch face with hands (sniffing)
- **PPE required**
- **No mouth pipetting**
- **Avoid aerosols**
- **Sharps!!**
- **Tidy and organized work space**
- **Change gloves regularly; wash and disinfect hands when finished**
- **Take off gloves when not at bench (phone, door handles)**
- **Regular disinfection of surfaces; is the disinfection solution active?**
- **Hygiene plan**
- **Regular control of microorganism identity**

Containment principles

implemented to prevent the unintentional exposure to biological agent or their accidental release

Protect yourself - use Personal Protective Equipment (PPE) + other measures as outlined in "Ordinance on the protection of employees from dangerous microorganisms"

Activities without a report to the authorities

Protect the environment – prevent damage to the humans, animals and ecosystem by **correct work practices and waste management** as described in "Ordinance on handling organisms in contained systems (Containment Ordinance)"

Activities can be carried out after a report to the authorities +/- permission

Personal Protective Equipment (PPE)

Principle: as much as necessary, as little as possible

The employee is responsible for using protective equipment!

Wear a lab coat and closed shoes, tie back long hair, wear gloves, avoid contact lenses and make-up

Personal Protective Equipment (PPE)

Latex

Nitril

Cryo gloves

Chain gloves

Hand hygiene – why?

Pathogen	Contamination rate(s) of health care workers' hands (%) (references)	Duration of persistenc hands (references)
Acinetobacter spp.	3–15 (132, 335, 519)	≥150 min (33)
B. cereus	37 (569)	Unknown
C. difficile	14–59 (362, 491)	Unknown
E. coli	Unknown	6–90 min (33, 151)
"Gram-negative bacteria"	21–86.1 (4, 7, 166, 187, 271, 302, 378)	Unknown
Influenzavirus, parainfluenzavirus	Unknown	10–15 min (25, 46)
HAV	Unknown	Several hours (354, 3
HCV	8–23.8 (11)	Unknown
Klebsiella spp.	17 (81)	Up to 2 h (33, 81, 151,
MRSA	Up to 16.9 (378, 412, 542)	Unknown
P. vulgaris	Unknown	≥30 min (33)
Pseudomonas spp.	1.3–25 (53, 119, 144, 420, 607)	30–180 min (33, 11
Rhinovirus	Up to 65 (191, 457)	Unknown
Rotavirus	19.5–78.6 (490)	Up to 260 min (22
Salmonella spp.	Unknown	≤3 h (427)
S. marcescens	15.4–24 (90, 492)	≥30 min (33)
S. aureus	10.5–78.3 (90, 101, 179, 359, 378, 412, 546)	≥150 min (33)
VRE	Up to 41 (202)	Up to 60 min (402
"Yeasts," including *Candida* spp. and *Torulopsis glabrata*	23–81 (90, 112, 221, 378, 541)	1 h (79, 564

Kampf, G & Kramer, A (2004) *Epidemiologic background of hand hygiene and evaluat* Clin. Microbiol. Rev., 17(4), 863-893

Hand hygiene – how and when?

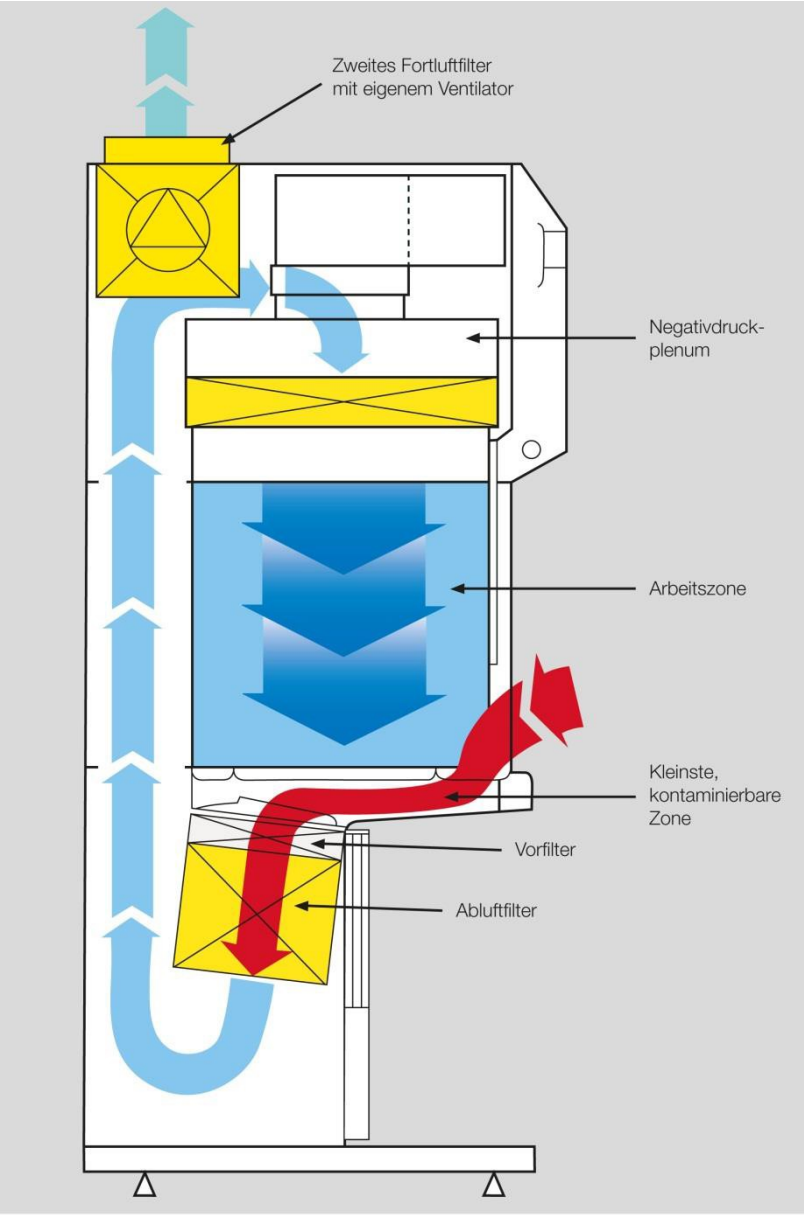
HOW:

- Lather min 15 sec
- Wash all surfaces (around/under nails)
- Dry hands with clean paper towel

WHEN:

- After removing gloves or other PPE
- Before leaving the working area (this includes a dash to the computer!)
- Immediately after any exposure

Biosafety cabinet with laminar flow



HEPA = high-efficiency particulate absorber

Biological Safety Cabinets (BSCs) are designed to provide:

1. Personnel protection
   - air is drawn around the operator into the front grille of the cabinet

2. Environmental protection
   - Exhaust air is HEPA-filtered (particle-free)

3. Product protection
   - The downward laminar flow of HEPA-filtered air provides product protection, i.e. sterile environment for cell culture

BSCs require segular service and testing (by SKAN; organized via Inselspital)

Cryostat / frozen sections: special precautions required

- Handle all specimens as if they were infectious at all times.
- Always wear double gloves, eye glasses, mask and lab coat whenever using the cryostat. A N95/FFP3 mask is recommended to protect from aerosols.
- Never reach into the cryostat for any purpose without wearing gloves.
- Do not create any aerosol within the cryostat. Never use aerosol freezing spray to cool tissue.
- The cryostat must be defrosted and decontaminated with a tuberculocidal disinfectant at an interval appropriate for the institution; this must be weekly for instruments used daily.

  Never use bleach in the cryostat since it will corrode the metal components.

814.912 Containment Ordinance and classification of activities

Ordinance on Handling Organisms in Contained Systems (Einschliessungsverordnung, ESV)

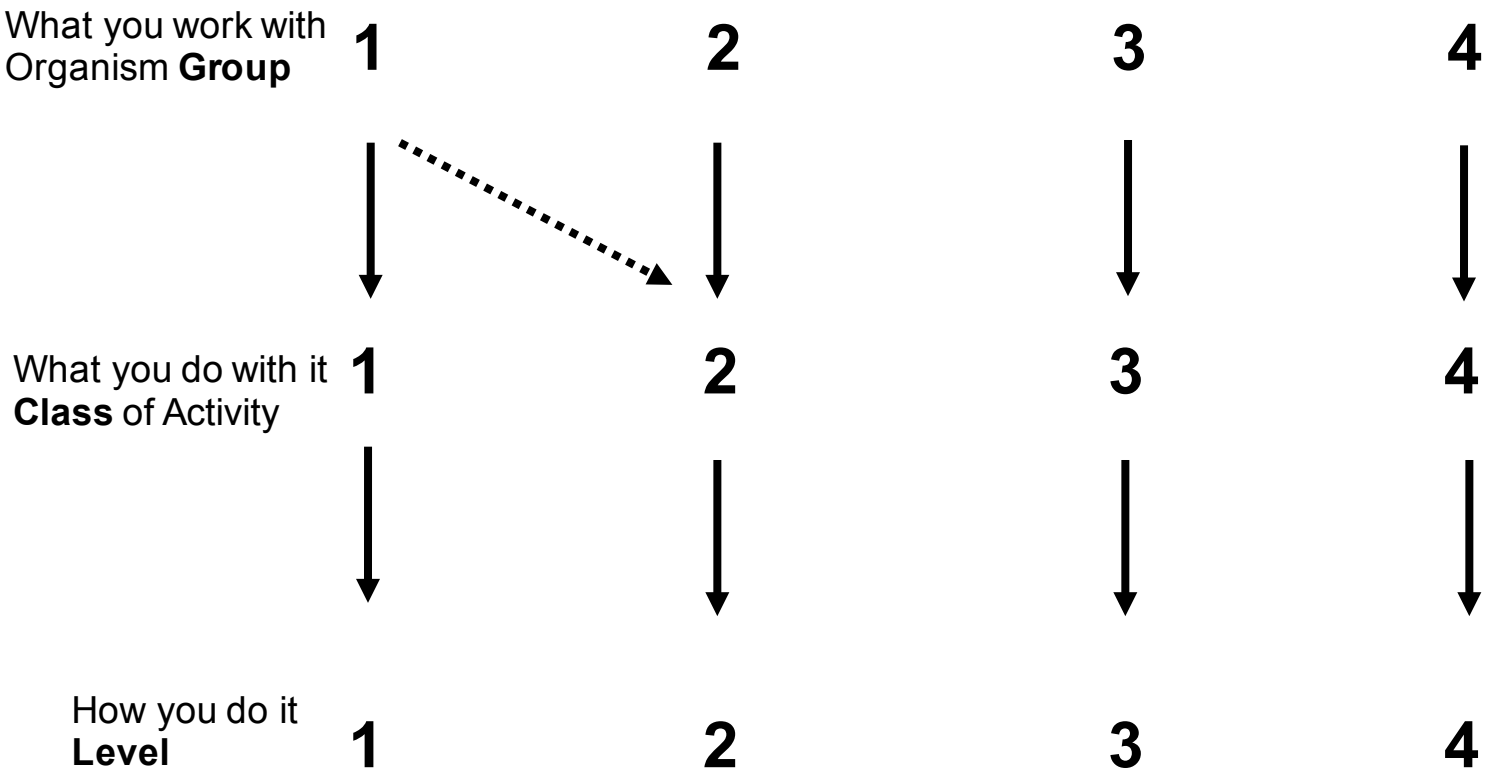
(Containment Ordinance, ContainO) of 9 May 2012 (Status as of 1 June 2015) *The Swiss Federal Council*

Purpose: to protect human beings, animals and the environment from hazards or harm caused by handling organisms, their metabolic products and wastes in contained systems.

Subject matter and scope of application of ContainO

- This Ordinance regulates the **handling of genetically modified, pathogenic or alien organisms** in contained systems.
- The **transport** of organisms intended for handling in contained systems is governed by Articles 4, 15 and 25 only.
- Handling organisms **in the environment** is governed by the Release Ordinance of 10 September 2008[1].
- The **protection of people** and the environment against serious damage resulting from major accidents involving microorganisms is regulated by the Major Accidents Ordinance of 27 February 1991[2].[3]
- The **protection of employees** when handling microorganisms is governed by the Ordinance of 25 August 1999[4] on the Protection of Employees from Dangerous Microorganisms.

Biosafety level definition



Group 1: organisms whose occurrence presents **no risk** or a negligible risk;

Group 2: organisms whose occurrence presents a **low** risk;

Group 3: organisms whose occurrence presents a **moderate** risk;

Group 4: organisms whose occurrence presents a **high** risk.

Class 1: activities with no risk or a negligible risk to humans, environment and biodiversity (CH-specific!)

Class 2: activities with a low risk (disease possible, but no spread and effective prevention measures)

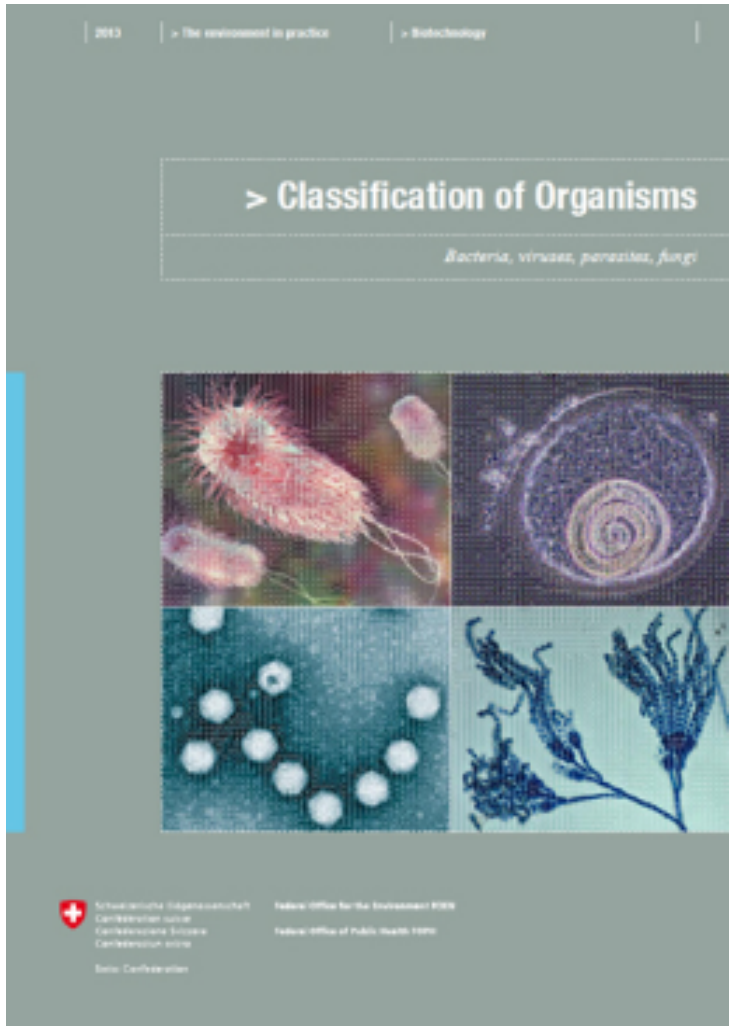
Class 3: activities with a moderate risk (viruses, bacteria, fungi, no easy spread but treatment available)

Class 4: activities with a high risk (only viruses, contagious on contact, no prevention or treatment)

Biosafety Level (BSL) = measures, depend on Class of activity

Inactivation reduces risk, propagation enhances risk, mutations increasing pathogenicity enhance risk

Coordination Centre for Biotechnology, Federal Office for the Environment (FOEN)



Federal Office for the Environment /Topic Biotechnology/ Publications and studies / Classification of Organisms

Year	2013
Number	UV-1114-E
Publisher	Federal Office for the Environment FOEN, Federal Office of Public Health FOPH
Series	The environment in practice

Class 1 activity: Global Notification for DBMR when using GMO (all groups and activities listed; new groups MUST be added)

Class 2: Individual Notifications for each project (primary cultures, viral expression systems)

Waste disposal

According to ContainO, activities with genetically modified or pathogenic organisms require special measures:

- Inactivation of microorganisms in contaminated material and waste, and on contaminated equipment:
  Class 1: Safe disposal, Class 2: In the building
- Minimise or prevent the escape of organisms during internal transport between various work areas
- The following may be disposed of as hazardous waste:
  a. contaminated material, animal carcasses and diagnostic samples
  b. solid cultures

All waste from Class 1 activities, containing GMO, undergo **inactivation** or disposal as **hazardous waste**

BSL waste

- *Biohazard waste*: ALL materials used for cell and/or bacteria cultures (contact with cells) AND recombinant nucleic acids in all forms, natural and synthetic (e.g., DNA, RNA, shRNA, etc.)

Special waste management: Biohazard waste

LIQUID

Autoclave or chemically inactivate waste prior disposal.

See **MSDS** of the disinfectant for proper disposal

White

Bleach ≥ 0.25 % = harmful to aquatic life with long lasting effect.
DO NOT autoclave bleach!

SOLID

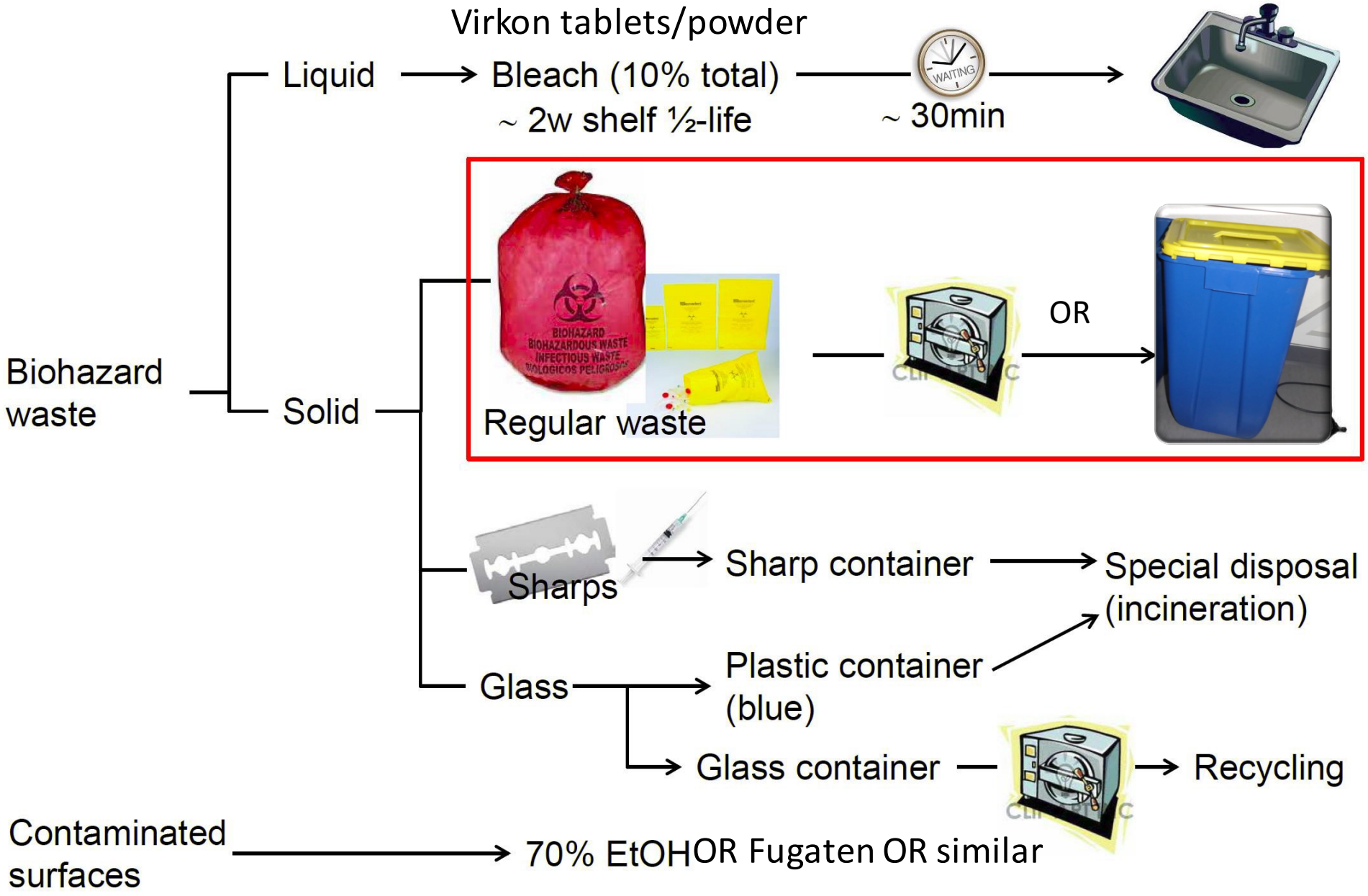
UN3291

1. Close lid tightly
2. Desinfect lid

Sharps, needles

Puncture-proof sharp container

Practical waste guide for BSL1



Behälter welche nicht richtig verschlossen sind, dürfen nicht mehr vom Transportdienst abtransportiert werden!

richtig

falsch

Bei Fragen wenden Sie sich bitte an Ernst Hurni, Abteilungsleiter Transport, AG Entsorgung Tel. 2 28 91

Transport of biological samples

Classification

- Exceptions
- Exempt animal/human specimen (with very low risk of infectious material)
- Class 6.2
  - ✓ UN 3373 Biological substance, category B
  - ✓ UN 2814 Biological substance, category A - Infectious substance, affecting humans
  - ✓ UN 2900 Biological substance, category A - Infectious substance, affecting animals
- Class 9
  - ✓ UN 3245 Genetically modified microorganisms / organisms
  - ✓ UN 1845 Dry ice

Exceptions are substances:

- Unlikely to cause disease, non-pathogenic
- All pathogens have been neutralized or inactivated
- Environmental substances (food, water, soil)
- Biological substances for transfusion and transplantation
- **Nucleic acids** (including plasmids)

Transport of biological samples: basic principles

The main goals

- Safe arrival of material in good condition for further work
- Avoiding exposure of people to biological agents and their release into the environment

The requirements

- Correct packaging (three layer principle)
- Correct labelling (UN number, description: biological substance category B)
- Documentation (for customs, etc.)

Transport packaging for UN 3373 substances

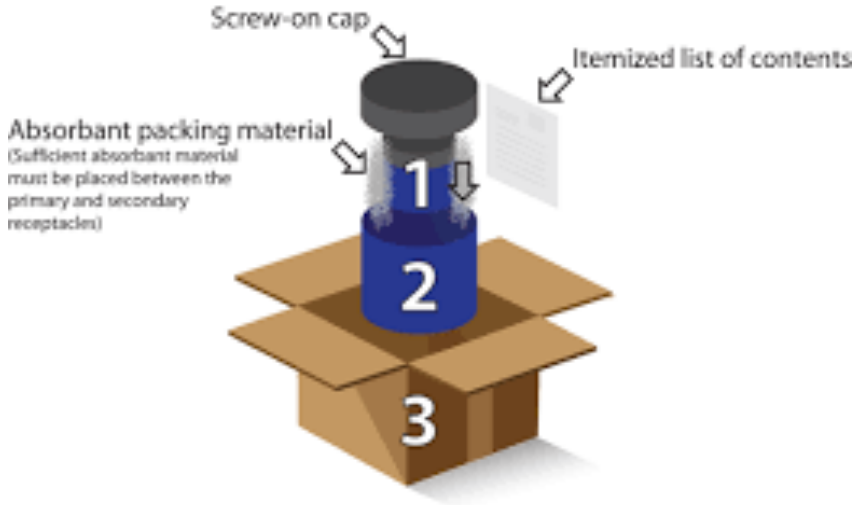
Any packaging for biological substances must include **three components**:

•A **primary receptacle**: the tube, vial or other container typically made of glass or rigid plastic (including the stopper, cap or other closure elements) that is in direct contact with the specimen.

•A **secondary packaging** (including cushioning and other materials) that fully encapsulates the primary receptacle. For liquids: include absorbent material in sufficient quantity to absorb all contents of the primary receptacle)

•An **outer packaging** for shipping or transit.

Outer or secondary contained MUST be rigid



1. Primary receptacle (leakproof, 95kPa)
2. Secondary receptacle (leakproof)
3. Outer container (w/list of itemized contents)

Transport of biological samples: daily work, challenges and solutions

Problem: DBMR has many sites, necessitating transfer of biohazardous material between labs

Solution 1: “Grab & Go”

WRONG!

Solution 2: “three layer principle” and spill containment

CORRECT

Biohazard Spill (BSL2 Labs)

DO NOT ENTER!

BIOLOGICAL SPILL
DECONTAMINATION IN PROGRESS

Put on PPE outside the contaminated area, in the following order:

- FFP3 mask (no surgical mask!)
- Goggles
- Light gloves (nitrile)
- Protective clothing
- PVC cleaning gloves
- Booties

Proceed to decontamination

- Dissolve 1 sachet perform classic concentrate OXY into the flask with distilled water
- Cover spill with enough absorbent material
- Pour disinfectant solution onto the absorbent, wait at least 10 minutes
- Collect absorbent with scraper and dustpan
- Clean area with towels soaked with disinfectant solution
- Discard all waste in biological waste container

Remove PPE in the following order:

- PVC cleaning gloves
- Goggles
- FFP3 mask
- Booties
- Protective clothing
- Light gloves
- Discard PPE in biological waste container

Class 2 activities and BSL2 Labs

Classification and inclusion criteria

- Anything involving Group 2 organisms (primary cells, immortalized cell lines)
- Viral vector expression systems (Lenti, retro, HSV, SFV)
- Expression of sequences with hazard potential (oncogenes, cytokines, si/sh/miRNAs)

BSL2 lab requirements

- Restricted access, biohazard sign, instruction of personnel
- Class 2 MSC, mandatory PPE, labcoats NEVER leave the lab
- Rigorous recontamination, inactivation of waste, spill kit, transport measures
- Registration of activities
- Involvement of BSO – risk assessment, training

Safety measures	Safety level	
	1	**2**
Building		
Restricted access to the work area	-	+ Bioharzard sign, key, login for access
Animal rooms separated by lockable doors	Only in installations with vertebrates	Only in installations with vertebrates
Facilities for personal decontamination in the work area	-	+ Hand wash basin, Fugaten or 70% EtOH
Biohazard warning sign	-	+
Rooms with easily cleanable floors	+	+
Equipment		
Surfaces resistant to water, acids, alkalis, solvents, disinfectants and decontaminants	+ Work bench	+ Work bench
Microbiological safety cabinet	-	+ BSL2 cabinet
Measures against aerosol formation and dissemination	-	+ Minimise aerosols via centrifuge lids, tubes, no vortexing
Autoclave	Available	In the same building
For the animal species cages, that are easily decontaminated	Washable	Decontaminable

BSL1 vs. BSL2 Labs

Work Organisation	**1**	**2**
Suitable clothing for the work area	laboratory activities: laboratory clothing	For laboratory activities: laboratory clothing
Personal safety equipment Personal safety measures must be adapted to the activity and the organisms used.	+	+ More / better PPE PPE DOES NOT leave the BSL2 lab
Regular disinfection of the workplaces	-	+
Inactivation of microorganisms in contaminated material and waste, and on contaminated equipment	Safe disposal, All GMO into UN 3291	In the building: a. contaminated material, b. solid cultures Autoclave on-site or UN 3291
Minimise or prevent the escape of organisms during internal transport between various work areas	Minimise	Minimise

If both Class 1 and Class 2 activities are carried out in the same lab:
BSL2 rules apply to the whole lab !!!

In emergency

What to do if exposure to biohazardous material has occurred

- Follow SOP for cleaning / decontamination; use knowledge and common sense to perform risk assessment
- Report the incident to safe@dbmr.unibe.ch CC silvia.roesselet@dbmr.unibe.ch
  - Research Group, name of Group Leader
  - Name of the affected person, DOB
  - Date, time and place of the incident
  - Type of biological material involved
  - Describe the incident (with or without injury, first help measures)

Call:

Personalärztliches Dienst Inselspital (PAD)

'22038 Open daily: 8:00-16:30

Out of working hours: Medizinischer Notfall (Dienstoberarzt Medizin) '181-7520

Hepatitis B Vaccination

Process of the Hepatitis B Vaccination

To: **all employees working at the DBMR with an employment at the University of Bern**.

1. Questionnaire regarding the Hepatitis B-vaccination and entry questionnaire: fill out 2 forms and send them together with your vaccination record to the personal medical service / “Personalärztlichen Dienst (PAD)” of the Inselspital.
2. With the documents the registration of the patient will be organized by the PAD
3. The DBMR/University employees don’t have to register themselves at the registration desk
4. The vaccination record will be examined by the doctor
5. The doctor will prescribe the necessary Hepatitis B vaccination dose/serology and prescribes it on the questionnaire document
6. With this procedure the PAD gets the exact information what they have to use for the Hepatitis B vaccination before the employee arrives for the vaccination
7. Employees are called directly by the PAD to make an appointment

Forms available from PIs of BSO

Hepatitis B Vaccination - What PAD needs to know

Hepatitis B-Vaccination	**Note date of vaccination**
1. Vaccination	**Date:**
2. Vaccination	**Date:**
3. Vaccination	**Date:**
Further vaccine doses	**Date:**

Vaccine Antibody Titers (Anti-HBs) Please send copy of laboratory report

Date: **Result:**

Forms available from PIs or BSO

Generally:

- Be aware of hazards
- Increase your knowledge to allow for sober evaluation of risks and benefits
- Read SOPs and instructions
- Consult authorities if in doubt

  (local BSO, FOEN)
- For PPE – as little as possible,

  as much as necessary
- Use common sense !

Thank you for attending, please distribute the information and apply it!