



Biosafety Training, MiMol

Practical guidelines

Sept 13th, 2009

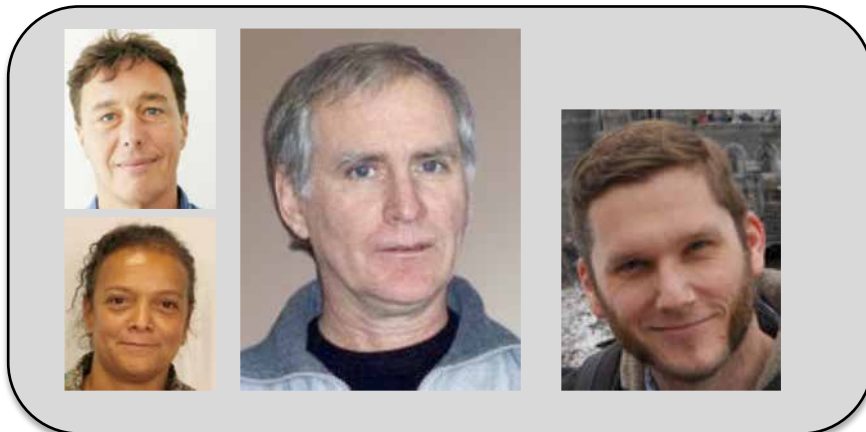
Today's Program

1. Introduction
2. Biosafety risk assessment
3. Biosafety measures
4. Waste treatment
5. Disinfection and Cleaning
6. Spill response
7. Transport of P2 material
8. Accidents

Players involved



Advice/Control



S.T.E.P.S.

BSO

Deputy-BSO



RESPONSIBILITY



Protection of...



Before you start working

- **Do you have an authorization for the work you want to do?**
 - PI has to write a notification (for P2), for P1 global notification (PI still needs to indicate to BSO!)
 - Experimenter has to be indicated on the user list of the dedicated P2 space
- **Do you have the right equipment and the right training to do this work?**
 - Experimenter has undergone a basic biosafety instruction
 - Facilities with the right biosafety level are available
- **Are you physically able to do the work (health conditions)?**
 - pregnancy, immune suppression etc.

Risk assessment: researcher with PI/BSO > Ecogen

- 1st step: which **risk group** does the microbiological agent belong to?

Biosafety levels

- P1 (BSL1): *E. coli* K12, most cancer cell lines
 - Low risk for experimenter and/or environment
- P2 (BSL2): human blood, Salmonella, Pseudomonas, Toxoplasma, Influenza A virus, Staphylococcus aureus, Lentiviral vectors, VSV
 - Moderate risk for experimenter and/or environment
- P3 (BSL3): HBV, HCV, HIV, Dengue virus, EHEC, prions
 - Elevated risk risk for experimenter and/or environment
- P4 (BSL4): Ebola Virus, Nipah virus
 - High risk for experimenter and/or environment



RISK

Laboratory requirements for the specific biosafety levels

BIOSAFETY LEVEL	1	2	3	4
Isolation ^a of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation: - Inward airflow - controlled ventilating system - HEPA-filtered air exhaust				Yes
Double-door entry				Yes
Airlock				Yes
Airlock with shower				Yes
Anteroom				--
Anteroom with shower			^b	--
Effluent treatment (sewage)			^b	Yes
Autoclave: - on site - in laboratory room - double-ended	No	No	Desirable	Yes Yes Yes
Biological safety cabinets	No	Class II	Class II	Class II or Class III
Personnel safety monitoring capability ^d	No	No	Desirable	Yes

For P2:

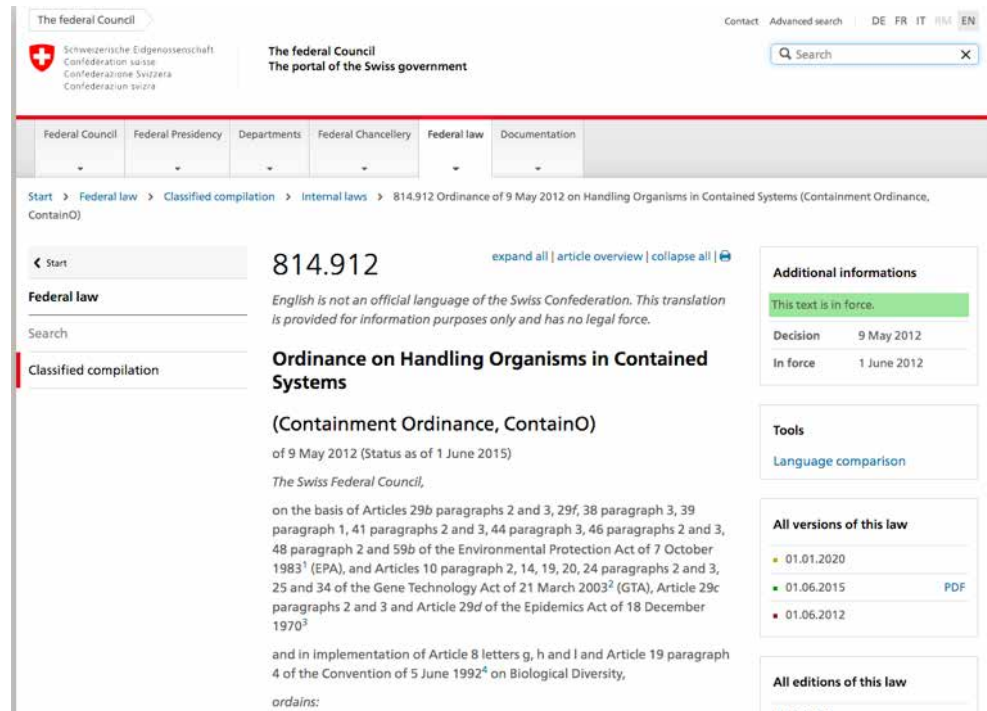
- Access is controlled
- Approved personnel is indicated at the entry door of the P2 lab (as are the notifications)
- Only one door must be used for entry
- Sink for hand washing/disinfection required
- Class II biosafety cabinets
- Sealed floor
- Autoclave on site

^a Environmental and functional isolation from general traffic.

^b Dependent on agent(s) used in the laboratory.

^c For example, window, closed-circuit television, two-way communication.

Classification of organisms



The screenshot shows the official website of the Swiss Federal Council. The header includes the Swiss flag and the text 'The federal Council' in multiple languages. A search bar is located in the top right. The main navigation bar lists various departments, with 'Federal law' selected. The breadcrumb trail indicates the path: Start > Federal law > Classified compilation > Internal laws > 814.912 Ordinance of 9 May 2012 on Handling Organisms in Contained Systems (Containment Ordinance, ContainO).

The main content area displays the title '814.912' and the subtitle 'Ordinance on Handling Organisms in Contained Systems (Containment Ordinance, ContainO)'. It specifies the date 'of 9 May 2012 (Status as of 1 June 2015)' and the authority 'The Swiss Federal Council,'. The text describes the ordinance's basis in various Swiss laws and international conventions. A sidebar on the right provides 'Additional information' (Decision: 9 May 2012, In force: 1 June 2012), 'Tools' (Language comparison), 'All versions of this law' (listing 01.01.2020, 01.06.2015, and 01.06.2012), and 'All editions of this law'.

<https://www.admin.ch/opc/fr/classified-compilation/20100803/index.html#app3ahref1>

Risk assessment: researcher with PI/BSO > Ecogen

- 1st step: which **risk group** does microbiological agent belong to?
- 2nd step: consider other factors that may affect how you work:
 - **Pathogenicity of agent** and **infectious dose** (animal studies, clinical reports, laboratory acquired infections?)
 - Potential **outcome of exposure**
 - Natural **route of infection**
 - Other routes of infection during **lab manipulation** (needle, airborne, ingestion)
 - **Stability of the agent** in the environment
 - **Concentration of the agent and volume** of material to be used
 - Presence of a **suitable host** (human or animal)
 - Laboratory **activity planned** (sonication, aerosolization, centrifugation, etc.)
 - **Genetic manipulation** that may alter host range or alter sensitivity to known treatments
 - **Countermeasures**: effective prophylaxis or therapeutic interventions

Working with new pathogens?

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/pathogen-safety-data-sheet-template.html

Home - PubMed - ... National Center for... BLAST: Basic Local ... Influenza NCBI iCn3D Overview: w... IRD iHOP t test Most Visited New Tab RV RespVir-Netzwerk Etiketten Print Web of Science etlierversuche-sec.s... BAFU - Biotechnolo... Ecogen

Franglais

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MENU

[Home](#) > [Health](#) > [Health risks and safety](#) > [Biosafety and biosecurity](#) > [Pathogen Safety Data Sheets](#)

Pathogen Safety Data Sheet Template

[Download an editable template to create a Pathogen Safety Data Sheet that complies with Canadian standards.](#)

Section I – Infectious Agent

Name

Name of the pathogen using official taxonomic naming convention (e.g., Genus species, Genus species subsp. subspecies, or Genus spp. for bacteria, parasites, fungi; Species for viruses; Prion Disease Agent for prions).

Agent type: Which of the following classes of agent characterize the pathogen: Bacteria, Fungi, Virus, Parasite, or Prion?

Taxonomy:

Family: e.g., Streptococcaceae

Genus: e.g., Streptococcus

Species: e.g., S. salivarius

Subspecies/Strain/Clonal Isolate: Include if applicable

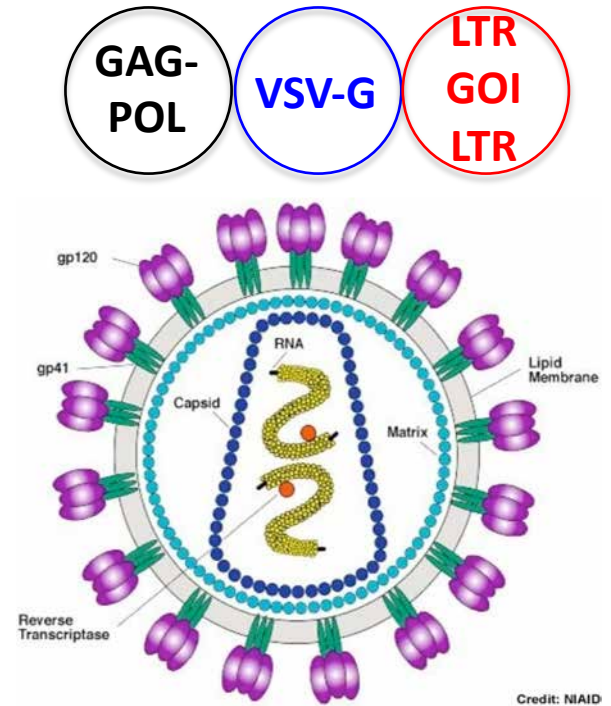
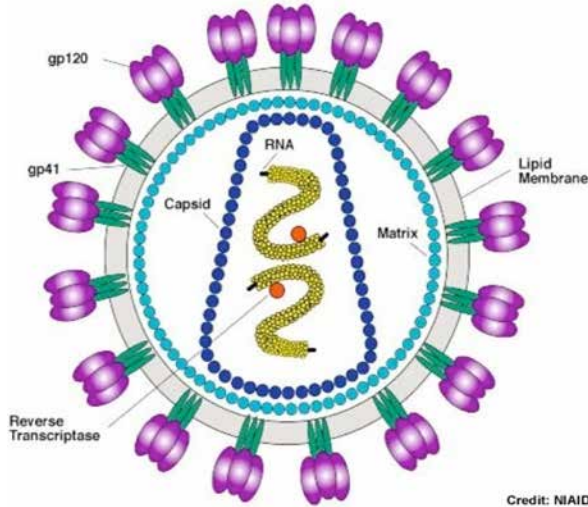
Genetically-Modified Organism or Vector

- Is there a '**novel**' **transgene**?
 - e.g. oncogene, cytokine, toxin, hormone, virulence factor, allergen, antimicrobial resistances?
- Degree of **deletion/attenuation**? e.g. removal of virulence factors, component separation to be replication incompetent.
- **Propagation restricted** to certain cells/hosts?
- Possibility of **activating cellular oncogenes** through integration site if applicable?

**Are there
safer
alternatives ?**



Example 1



HIV:

- No vaccine
- No cure
- Infects predominantly CD4 positive cells
- Integrates into the host genome
- Class:

3

HIV based expression systems:

- Infects all cell types
- No productive infection
- Integrates into the host genome
- Class:

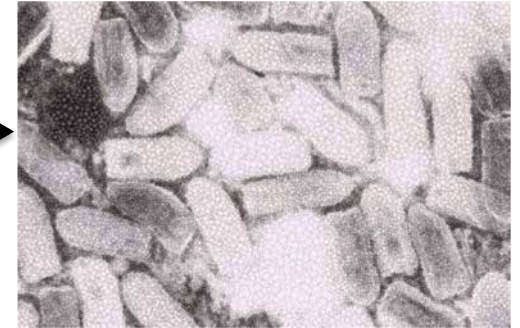
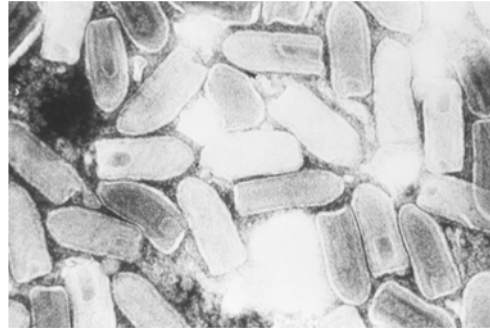
2

Cell lines after lentiviral transduction ?

Class 1

As soon as the lentiviral vectors are removed from the culture, e.g. after passaging the cells or extensive washes, cells can be transferred to P1 level.

Example 2



Zaire Ebola Virus

- 50-90% mortality

Vesicular stomatitis virus

- Cow pathogen, usually non-lethal

Vesicular stomatitis virus with Zaire Ebola Virus glycoprotein

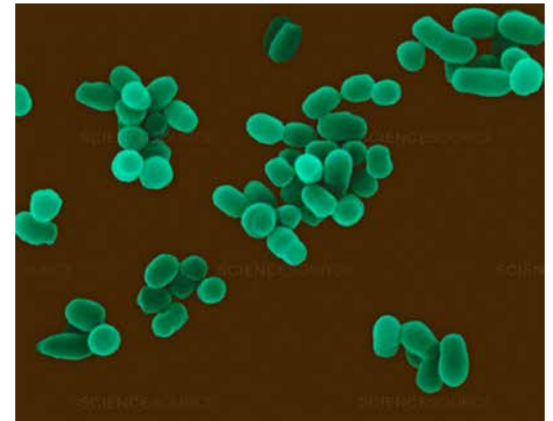
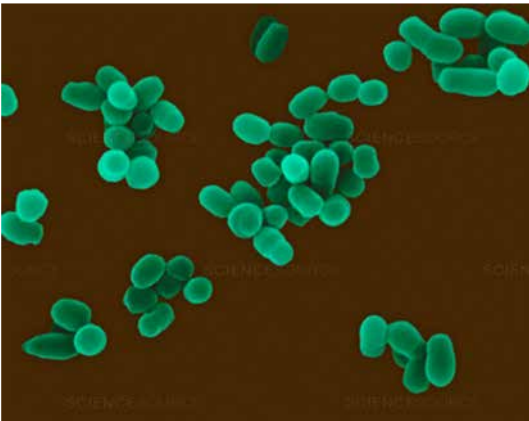
- Replicating virus, infects humans

4

2

2

Example 3



Brucella melitensis
Zoonotic bacterium
Flu like symptoms for
months, rarely lethal

3

Brucella melitensis
Resistant against
spectinomycin and
trimethoprim

?

JULY 01, 2010

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JAVMAnews

ACADEMIA

July 01, 2010



Wisconsin professor suspended for unauthorized research

"William S. Mellon, PhD, associate dean for research policy, said Dr. Splitter's laboratory had produced, without university or government approval, strains of *Brucella* encoded with antibiotic-resistant markers for spectinomycin and trimethoprim. [...] The concern is not a biosecurity issue but a biosafety issue," Dr. Mellon explained. "If someone acquires a laboratory infection, if that happens with a recombinant agent with an antibiotic resistance, you compromise treatment options. For us, that's pretty serious, especially with select agents."

Biosafety measures





Some General Rules



No mouth pipetting ...ever

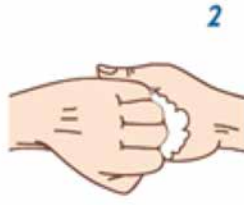


Handwashing

Use liquid soap



Palm to palm



Backs of fingers to
opposing palms with
fingers interlocked



Palm to palm,
fingers interlaced



Rotational rubbing of right
thumb clasped in left palm and
vice versa



Right palm over back of
left hand and left palm
over back of right hand



Rotational rubbing, backwards
and forwards, with clasped
fingers of right hand in left
palm and vice versa

Rinse and dry your hands thoroughly



Total duration 15s:
Should correspond roughly to 1x
♪♪♪Happy Birthday♪♪♪

Washing your hands is an obligatory last step after P2 work

Persistence of pathogens on hands / surfaces

Pathogen	Contamination rate(s) of health care workers' hands (%)	Duration of persistence on hands	Duration of persistence on inanimate surfaces
Acinetobacter spp.	3-15	150 min	3 days-5 mo
C. difficile	14-59	Unknown	vegetative cells: 24 h spores up to 5 mo
E. coli	Unknown	6-90 min	2 h-16 mo
Influenzavirus, parainfluenzavirus	Unknown	10-15 min	12-48 h
HAV	Unknown	Several hours	2 h-60 days
Klebsiella spp.	17	Up to 2 h	2 h-30 mo
MRSA	Up to 16.9	Unknown	4 wk-7 mo
P. vulgaris	Unknown	30 min	1-2 days
Pseudomonas spp.	1.3-25	30-180 min	6 h-16 mo
Rhinovirus	Up to 65	Unknown	2 h-7 days
Rotavirus	19.5-78.6	Up to 260 min	6-60 days
Salmonella spp.	Unknown	3 h	6 h-4.2 yr
S. marcescens	15.4-24	30 min	3 days-2 mo
S. aureus	10.5-78.3	150 min	4 wk-7 mo
"Yeasts," including Candida spp. and T. glabrata	23-81	1 h (79, 564)	1-150 days

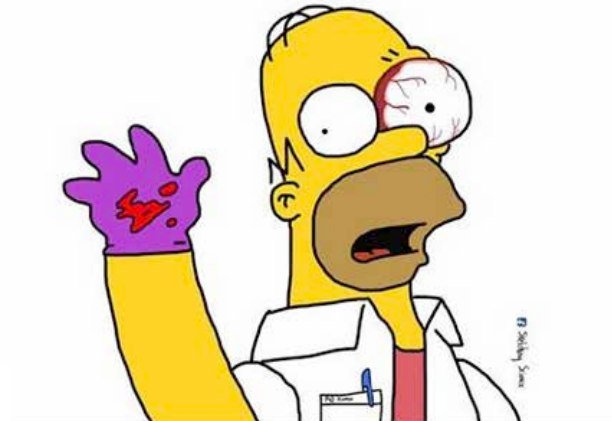
Kampf, G. and Kramer, A. 2004. Clin. Microbiol. Rev., 17 (4), 863-893.



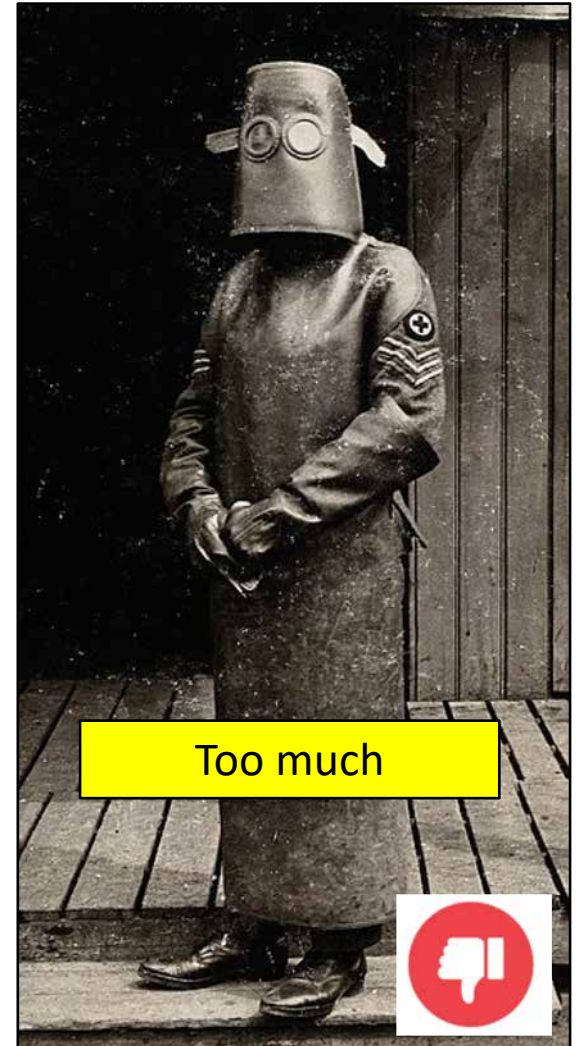
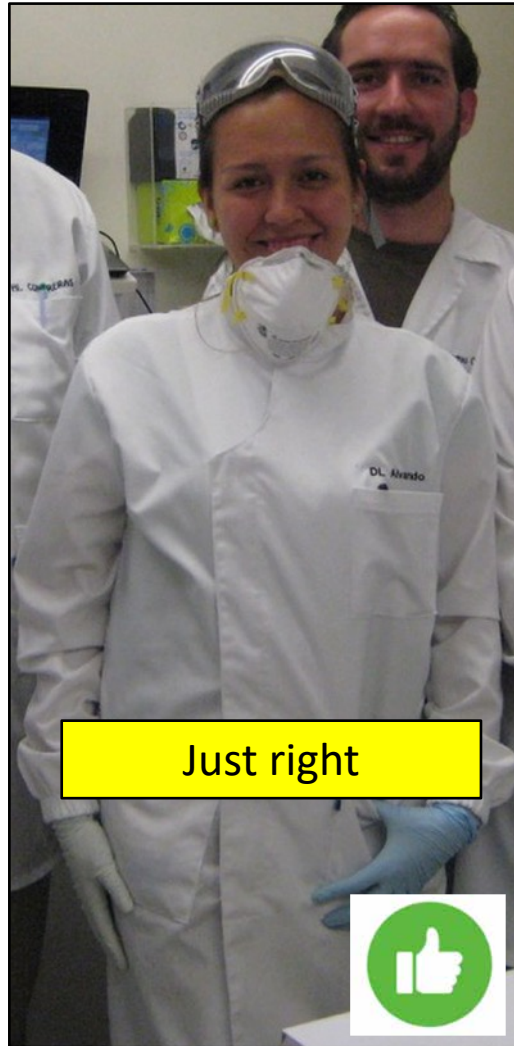
Don't touch with gloves during P2 work



If you wear gloves don't touch your face



PPE (Personal Protective Equipment)



PPE (Personal Protective Equipment)

Gloves (mandatory)

Long lab coat (mandatory also in P1),
ideally hydrophobic

Long pants, closed toe shoes

Goggles/face shield (optional)

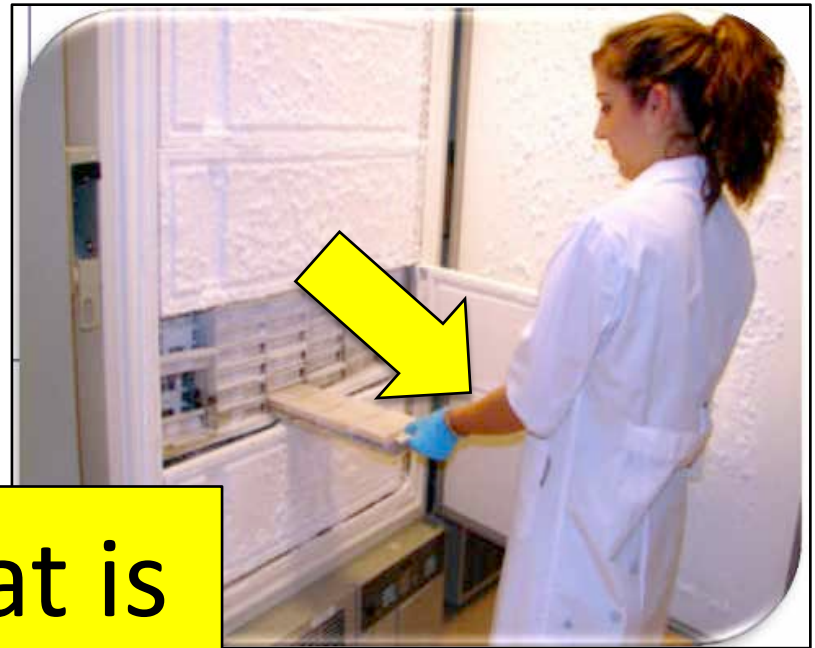
Mask (optional)

PPE has to worn when in the P2

PPE stays in the P2

PPE only makes sense if the **right size**
and the **right material** are used (e.g.
avoid latex gloves) and if they are
used properly.



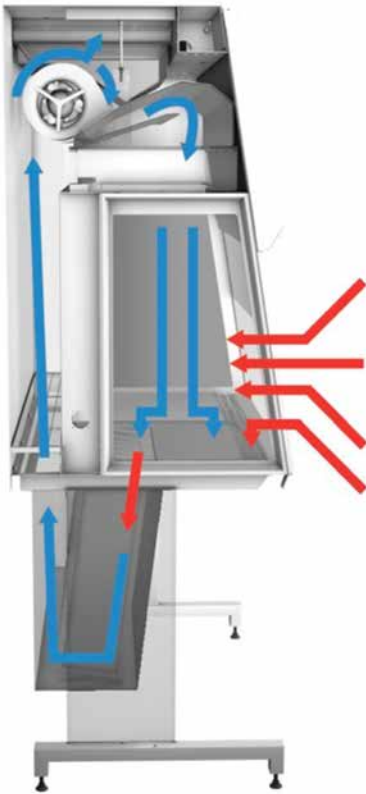


What is
wrong?



Containment

- Class II bio safety cabinet
- Dedicated P2 laboratory with restricted access



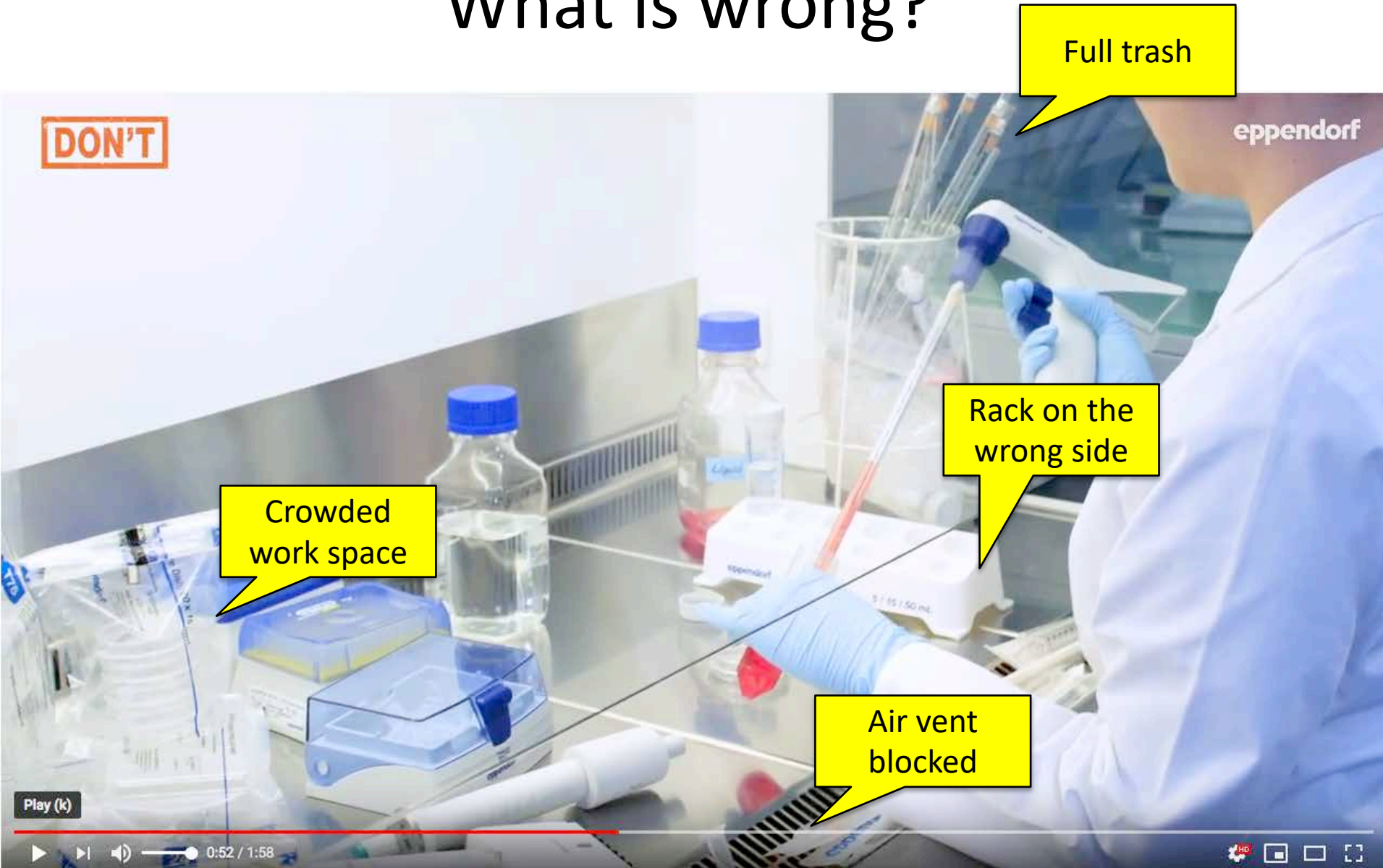
Requires frequent cleaning



Not a fume hood



What is wrong?



Organize your work bench



CLEAN

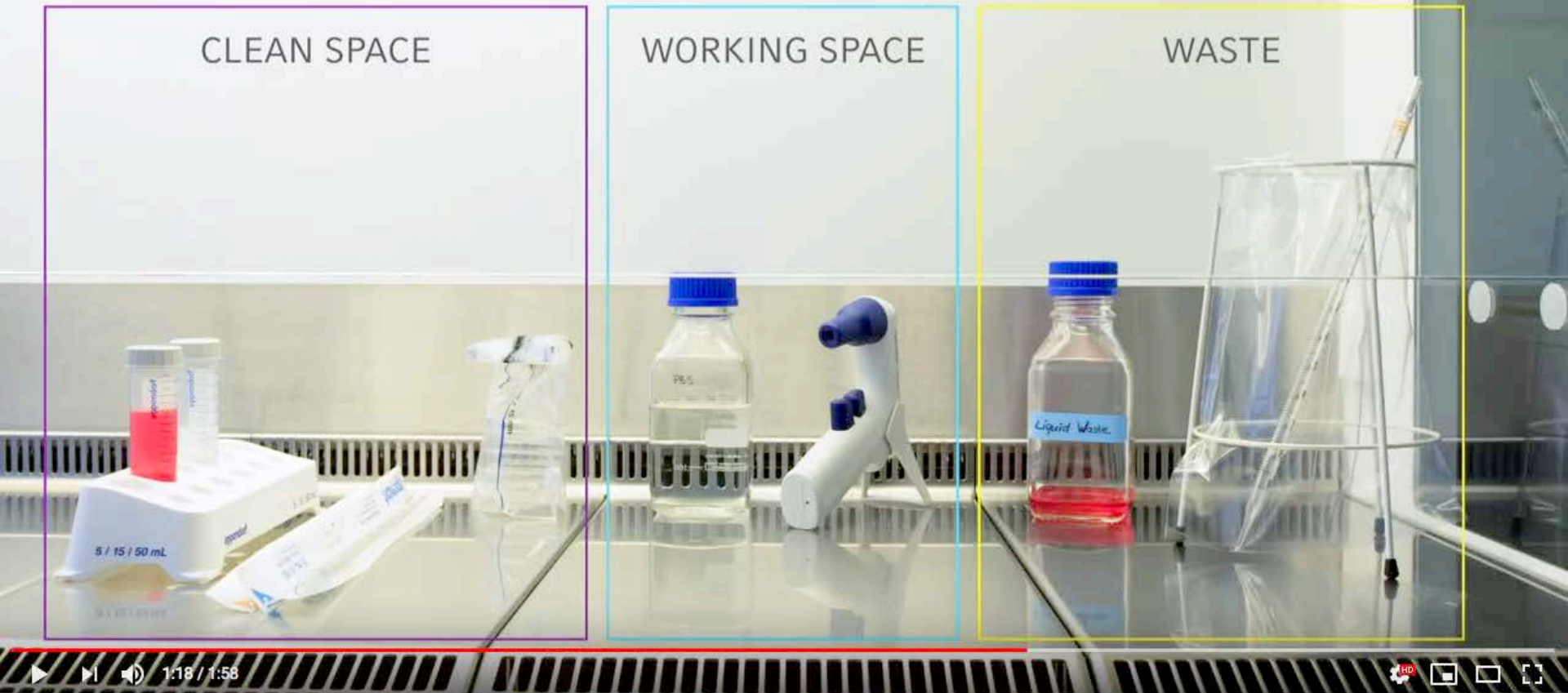
eppendorf

DIRTY

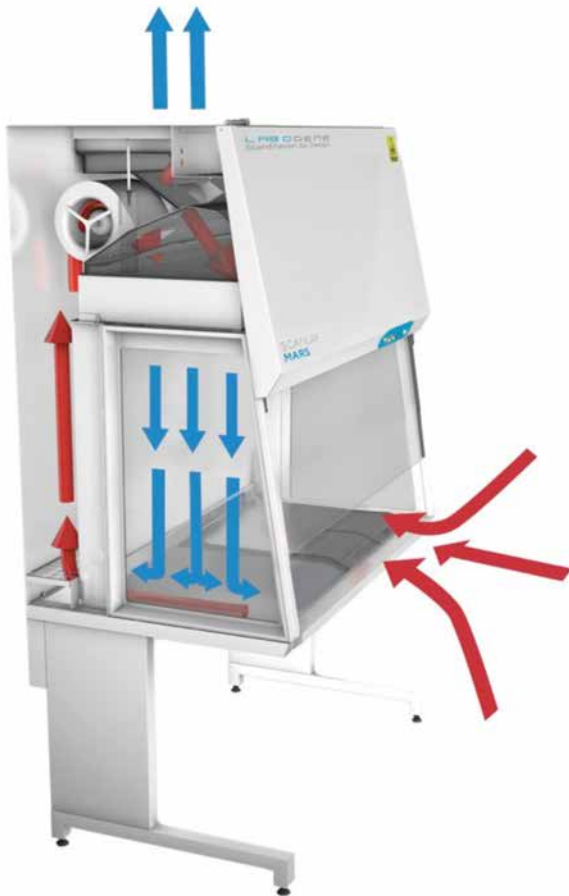
CLEAN SPACE

WORKING SPACE

WASTE



Avoid Aerosols



Waste treatment

Suboptimal situation



FILIÈRES DES DÉCHETS À RISQUES BIOLOGIQUES

Déchets solides P2/P3



FILIÈRE ROUGE

Les déchets solides de culture et les objets contaminés par des organismes P2/P3 doivent être collectés dans des sacs pour autoclaves de couleur rouge. Ces déchets doivent être inactivés par autoclavage.

Les sacs rouges sont autoclavés par les services des différents départements. Le patch indicateur fait apparaître le mot «autoclavé».

FILIÈRE JAUNE

Les sacs rouges autoclavés sont ensuite placés dans des sacs jaunes.

Les sacs jaunes, fermés, sont déposés par le personnel des laboratoires dans les containers de la filière jaune.

Déchets solides de culture P1, OGM et autres déchets de laboratoire potentiellement contaminés.



FILIÈRE JAUNE

Les déchets solides de culture P1 et autres OGM, les déchets de laboratoires sujets à contaminations chimiques ou biologiques ainsi que les prélèvements d'organismes potentiellement infectieux doivent être collectés dans des sacs jaunes.

Les sacs jaunes, fermés, sont déposés par le personnel des laboratoires dans les containers de la filière jaune.

Déchets coupants/piquants



FILIÈRE COUPANTS PIQUANTS

Les déchets coupants piquants doivent être inactivés par immersion dans de l'eau de javel immédiatement après leur utilisation. Ces déchets doivent être placés dans des boîtes jaunes «sharps».

Les boîtes jaunes, fermées définitivement, sont déposées par le personnel des laboratoires dans les containers de la filière jaune.



CONTAINERS DE LA FILIÈRE JAUNE

Local: 1^{er} sous-sol/
bâtiment D
N° de porte:
DS1.2048.a

L'ensemble de ce matériel de conditionnement (sacs rouges, jaunes et boîtes «sharps») est à votre libre disposition dans le local de la filière jaune 1^{er} s/sol bâtiment D

Ne pas trop remplir les sacs et ne jamais mettre de seringues ni d'objets piquants/tranchants dans les sacs

P1 and P2 waste stays in the respective laboratories until inactivated or deposited in the dedicated bins of the filiere jaune.

No black bags (trash) in the P2.

Liquid waste needs to be inactivated on site, by appropriate chemical or physical measures.

Make sure that your method of inactivation works for the organism you try to inactivate.

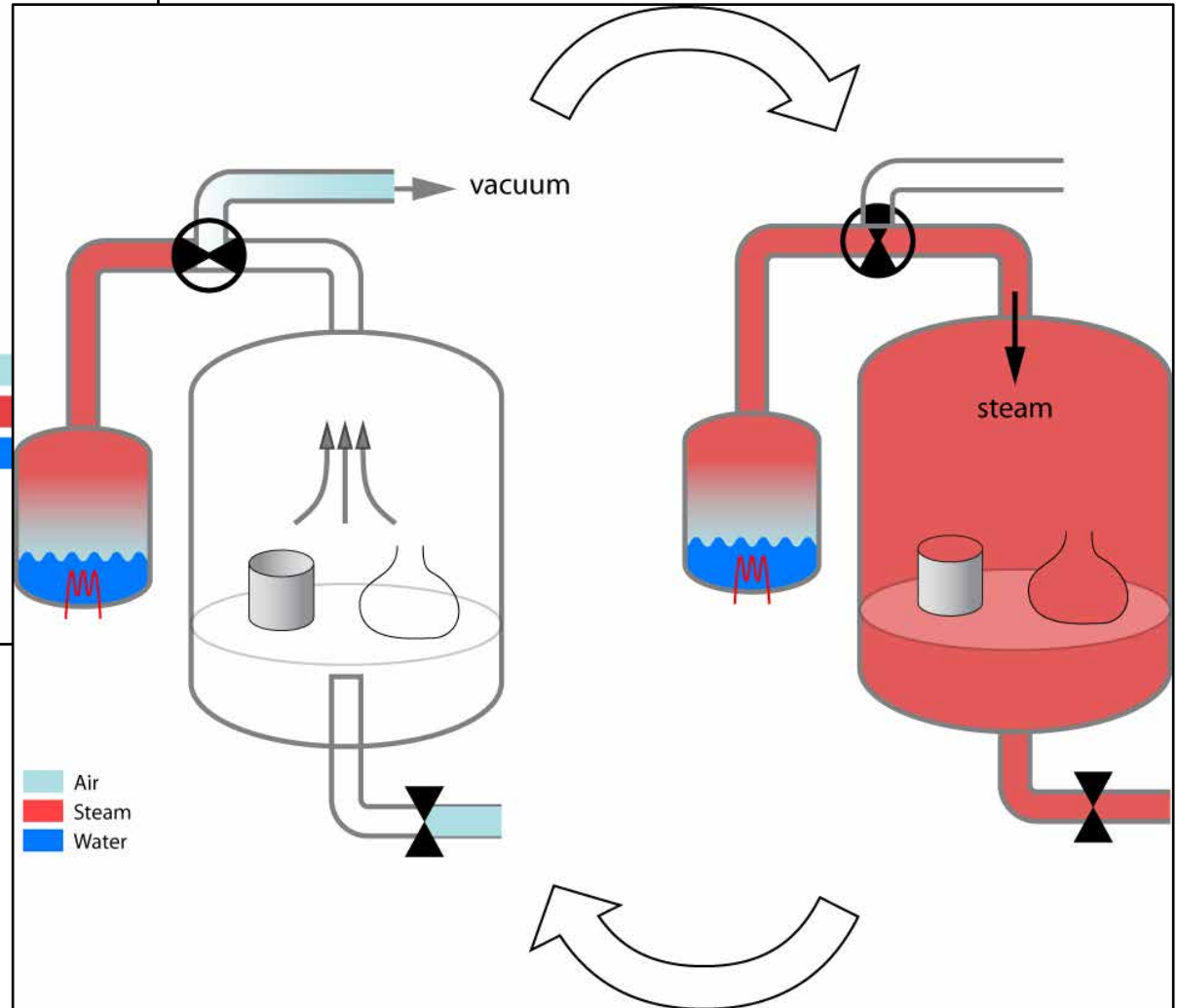
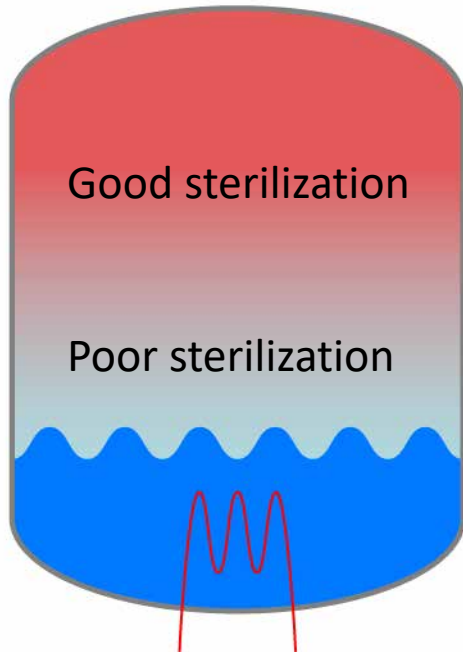
Autoclave



Steam/Autoclaving

- **Fun fact:** Steam has 3,600 times >> heat energy than dry air at same temp
- Common steam-sterilizing temps are:
 - 121°C (standard, 15mins with 100 kPa pressure)
 - 132°C (inactivates prions)
- Steam is lighter than air, thus efficient sterilization requires:
 - an external steam generator
 - vacuum system

Air removal by pulsed pre-vacuum



Vessels and bags have to be open to allow the replacement of air by steam

Indicators to control sterilization procedures

Chem-indicators (immediate information)



Before



After

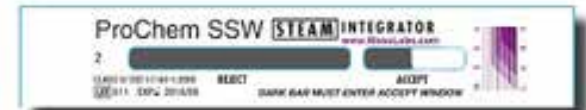
Unprocessed



Processed-Fail



Processed-Accept



Bio-indicators (result ~24h later)



Dry-heat sterilization

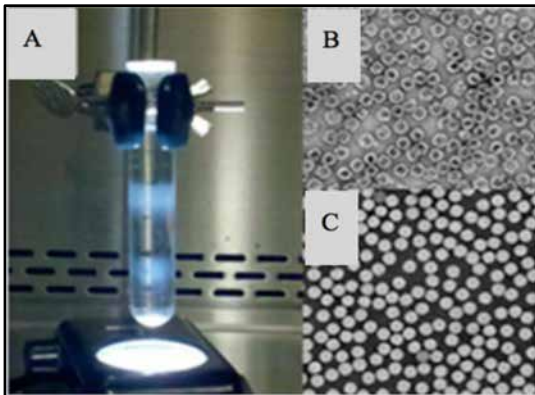
- To reach the same grade of sterilization as with 121°C steam, the temperature in dry heat sterilizers needs to be increased to 160-170°C for periods of 2 to 4 hours.
- Many plastic and rubber components will not survive this procedure and may impose a risk of fire
- Organic matter is burned



Sharps



No Recapping! ...ever



Disinfection and Cleaning



Choice of method depends very much on your experimental settings!

Cleaning of P2 labs

- Has to be performed by trained and certified personal
- Floors should be cleaned once a week with an appropriate disinfectant
- Work areas should be cleaned with an appropriate disinfectant after finishing an experiment

Some Disinfectants used in MiMol

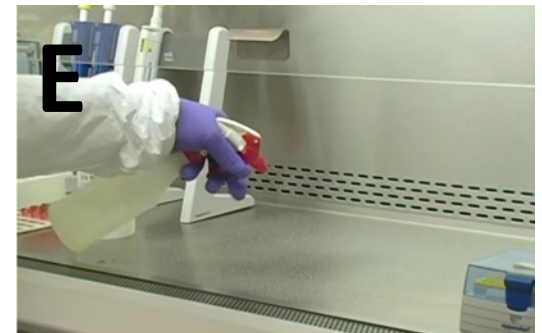
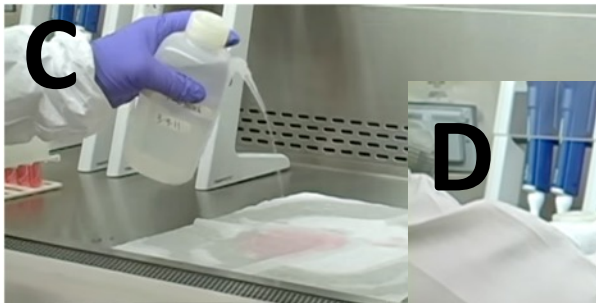
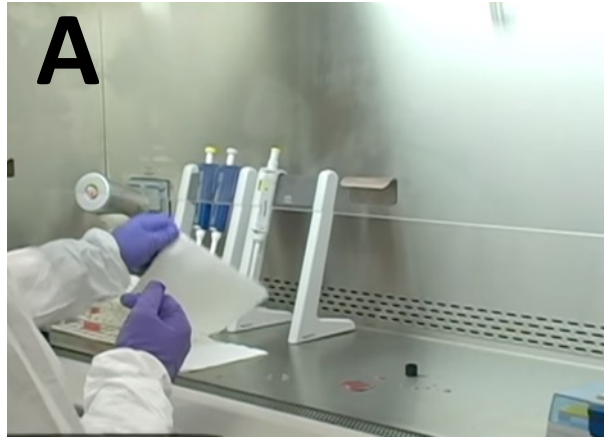
Disinfectant	Compound	Active concentration	Application
Ethanol	ethanol	70 %	surface
Bleach (Javelle)	Sodium hypochlorite	10 %	liquid waste
Bleach (Javelle)	Sodium hypochlorite	2 %	surface
VIRKON-S	Pentapotassium bis(peroxymonosulphate) bis(sulphate)70693-62-840-55% Sodium C10-13-alkylbenzenesulfonate68411-30-310-12% Malic acid6915-15-77-10% Sulphamic acid5329-14-64-6% Sodium toluenesulphonate12068-03-01-5% Dipotassium peroxodisulphate7727-21-1<3% Dipentene138-86-3<0.25%	0.5%	Surface, equipment, liquid waste (final concentration 0.5%)
Mikrozyd	Ethanol, 1-propanol	100%	surface (steel)
H ₂ O ₂ (liquid /gas)	peroxide	35%	surface/gas accessible areas
Biosanitizer	H ₂ O ₂	2%	Floor, surfaces

Make sure the disinfectant is killing your bug of interest!

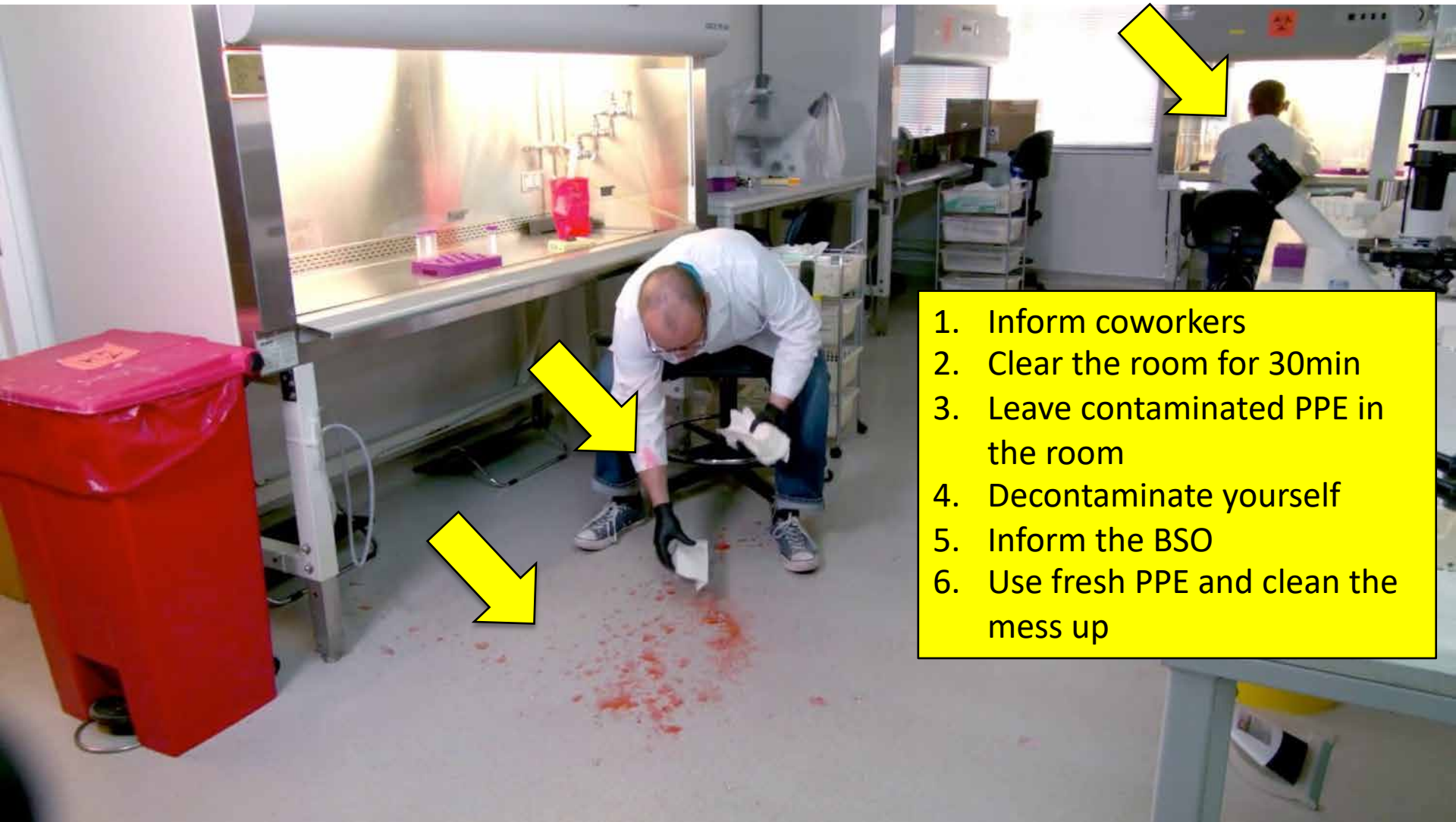
Spill response



Small contained spills



Large spills – What would you do?



1. Inform coworkers
2. Clear the room for 30min
3. Leave contaminated PPE in the room
4. Decontaminate yourself
5. Inform the BSO
6. Use fresh PPE and clean the mess up

Large Spills

Biological Spill Cleanup



In P2 labs we are required to have either a spill response kit or at least 5 rolls of absorbent material and appropriate detergent to respond to spills.



**Do not spray
ethanol into a spill.**



Spill in a centrifuge:

- Let aerosols settle for 30min
- If possible open in BSC
- Clean with detergent soaked towels from top to bottom
- Wipe with ethanol

Transport of infectious material



Triple packaging principle and proper labeling

For questions

Kevin.Reichen

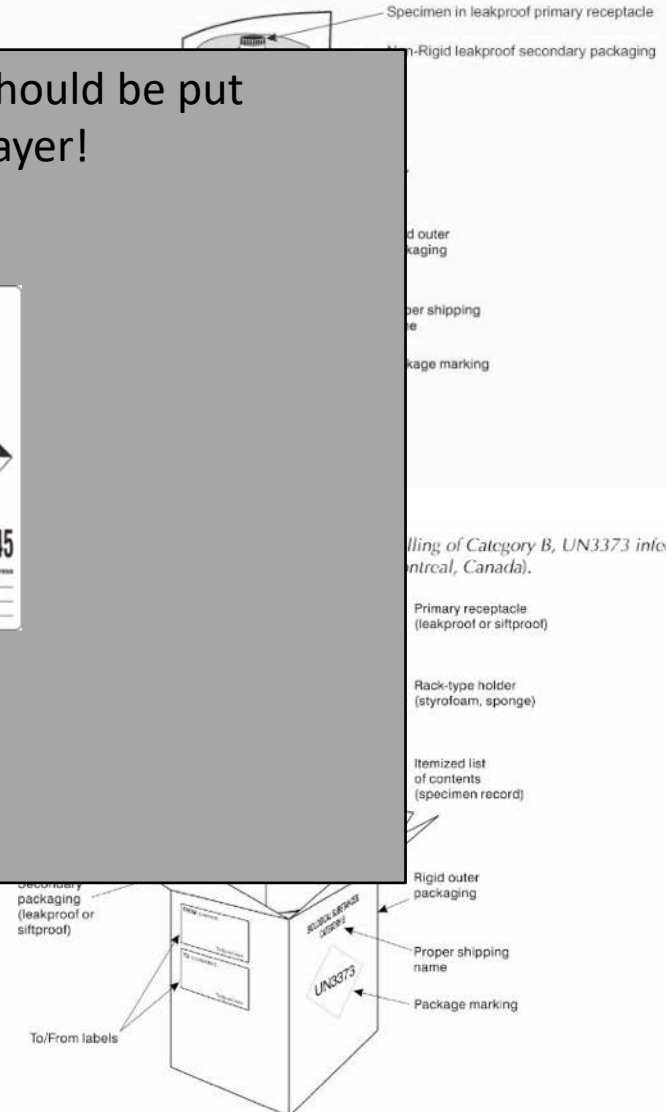
Cooling packs, dry ice or wet ice should be put outside of the second packaging layer!



**BIOLOGICAL SUBSTANCE
CATEGORY B**

23CHF

Fig. 5. Example of the triple packaging system for the packing and labelling of Category B, UN3373 infectious substances with non-rigid leakproof secondary packaging (Figure kindly provided by IATA, Montreal, Canada).



Accidents (happen)



Report to PI and BSO

Joseph.Curran@unige.ch

Ext: 95799

Mirco.Schmolke@unige.ch

Ext: 94360

Accident Benin

- Urgences HUG

- Samaritains - Pharmacie
N° interne: **555

SECURITE INTERNE- CMU

Agent de jour / nuit
N° interne: **555

LOGE DES HUISSIERS - CMU

N° interne: 95900
022 37 95900

SECURITE - UNIVERSITE

N° interne: 1222
022 379 1222



Effraction
Vandalisme
Indésirables



Feu
Explosion / Gaz
Alarme
chimique
Radioprotection
Biosécurité



Accident
Malaise

suva





Please do not forget to sign the attendance sheet!