DRFZ BERLIN
Deutsches Rheuma-Forschungszentrum
Ein Institut der Leibniz-Gemeinschaft

S1 Introduction to work with

Gene modified organisms of risk group 1




Genetic technology

According to Gentech law (GenTG)
& Gentechnik-Sicherheitsverordnung (GenTSV):

Genetic technology is a method for the targeted modification of genetic material.

In vitro combination of nucleic acid molecules

- To a new reproducible molecule (i.e. DNA, RNA)

Introduction of such molecules into a suitable recipient organism

- vectors: plasmids or viruses
- recipient organism: able to transfer such nucleic acid

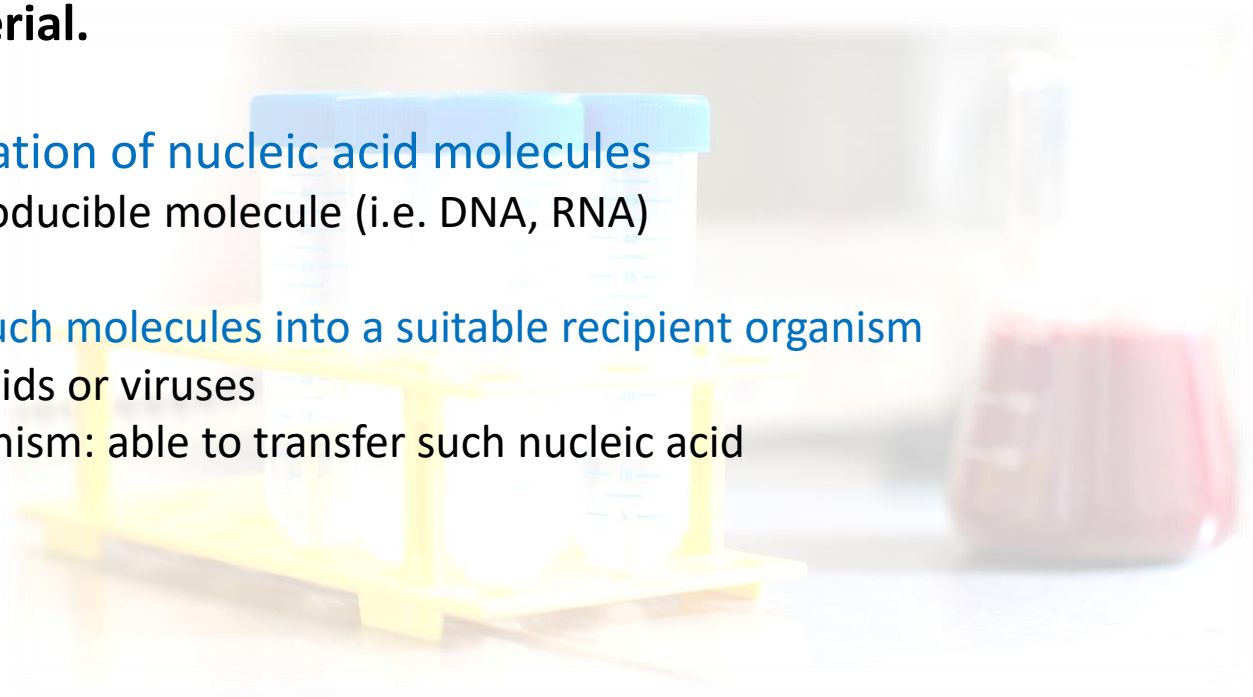

Photo: Jacqueline Hirscher

Safety levels

Safety level 1:
no risk for health and environment

Safety level 2:
low risk for health and environment

Safety level 3: not at DRFZ
moderate risk for health and environment

Safety level 4: not at DRFZ
high risk for health and environment

Photo: Jacqueline Hirscher

At DRFZ we work with:

Gentechnically modified organisms (GMO):

Cells (Anlage 368-00)

- *Project leader: Ute Hoffmann*
- In vitro gene modification of cells (e.g. mouse cells) or bacteria (e.g. E. coli K12) of risk group 1 (cloning, transfection) with vectors of risk group 1
- **Please provide Formblatt Z until 15th of February to Ute Hoffmann**
- Analysis of cells from GMO mice
- **Attention:** Gene modification of human cells from blood mostly S2

➢ **All organisms have to be documented in Formblatt Z and send to Ute latest by February 15th yearly**

GMO mice (Anlage 114-97)

- *Project leader: Ute Hoffmann*
- Breeding and experimental usage of knock out, knock in or tg mice
- Please provide „Abschlussbeurteilung“ before starting new breedings, importing or crossing of strains to Theres Manthey

Photo: Jacqueline Hirscher

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S2 Introduction to work with

- Untested human material
- Infectious pathogens
- Gene modified organisms





What does S2 mean?

S2: Safety level 2 according to gene-technic safety regulations

Gentechnik-Sicherheitsverordnung GenTSV

- for work with gene modified organisms of risk group 2

➢ **low risk for health and environment**

S2: Protection level 2 according to biological agents regulations

Biostoffverordnung BioStoffV

- for directed activities with pathogenic micro-organisms of risk group 2
- for non-directed activities with non-tested human material of risk group 3**

➢ **low risk for health and environment**

Photo: Jacqueline Hirscher

S2 work carried out at the DRFZ
Human samples with unknown infectious status

Human samples not tested for infectious micro-organisms

i.e. HAV, HBV, HCV, HIV

risk group 3** (Biostoffverordnung): micro-organisms not transferred by aerosols

Undirected work with these samples

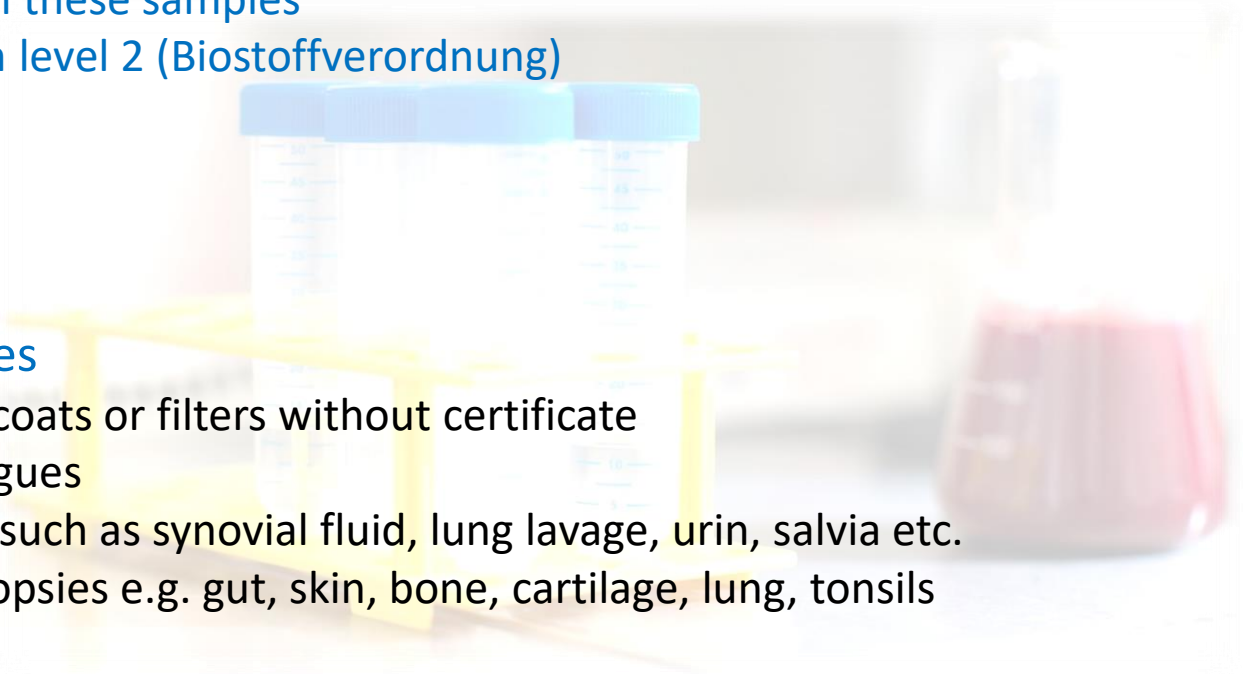
belongs to protection level 2 (Biostoffverordnung)

Project leader

Ute Hoffmann

Examples of samples

- Blood from buffy coats or filters without certificate
- Blood from colleagues
- Other body fluids such as synovial fluid, lung lavage, urin, salvia etc.
- Solid tissues as biopsies e.g. gut, skin, bone, cartilage, lung, tonsils
- Stool samples



S2 work carried out at the DRFZ
Human samples with unknown infectious status

Risks

Absorption

- by inhalation of aerosols
- via the skin especially in case of injuries or chronically pre-damaged skin
- via eye or mucosa in case of splashing

Infection

- with hepatitis A (HAV), B (HBV) and C (HCV) virus
- with human immunodeficiency virus (HIV)

Allergenic and toxic potential

All samples are potentially infectious !

TRBA 100 (Technische Regeln für biologische Arbeitsstoffe)

S2 work carried out at the DRFZ
Infectious pathogens

Micro-organisms as virus or bacteria

- can cause human disease
- might be hazardous to employees and environment
- belong to risk group 2 (low risk, Biostoffverordnung)

Pathogens currently used at DRFZ

- Pseudomonas aeruginosa (PI: Chiara Romagnani)
- S2 intestinal commensals including spore formers (PI: Ute Hoffmann)
- Helicobacter hepaticus,
  Citrobacter rodentium,
  LCMV (lymphocytic choriomengitis virus),
  Influenza A Virus (A/Puerto Rico/8/1934(H1N1)),
  Vacciniavirus
  Vesikuläres Stomatitis Virus
  (PI: Ahmed Hegazy and Max Löhning)

S2 work carried out at the DRFZ
Infectious pathogens

Risks

- Absorption
  - by inhalation of aerosols (including spores)
  - via the skin especially in the case of injuries or chronically pre-damaged skin
  - via eye or mucosa in case of splashing

- Infection

- Allergenic or toxic potential

Facultative pathogenic for humans and vertebrates but usually no transmission between the two host groups

Please contact the project leader before you start with your work for detailed introduction! This is mandatory!

S2 work carried out at the DRFZ

Gene modified organisms (GVO):

Gene technical experiments:

Transfer of *nucleic acids* (i.e. plasmids, vectors) from *donor* to *recipient* with low risk for health and environment (same as infectious pathogens)

Belong to risk group 2 (Gentechnikgesetz)

Project leader

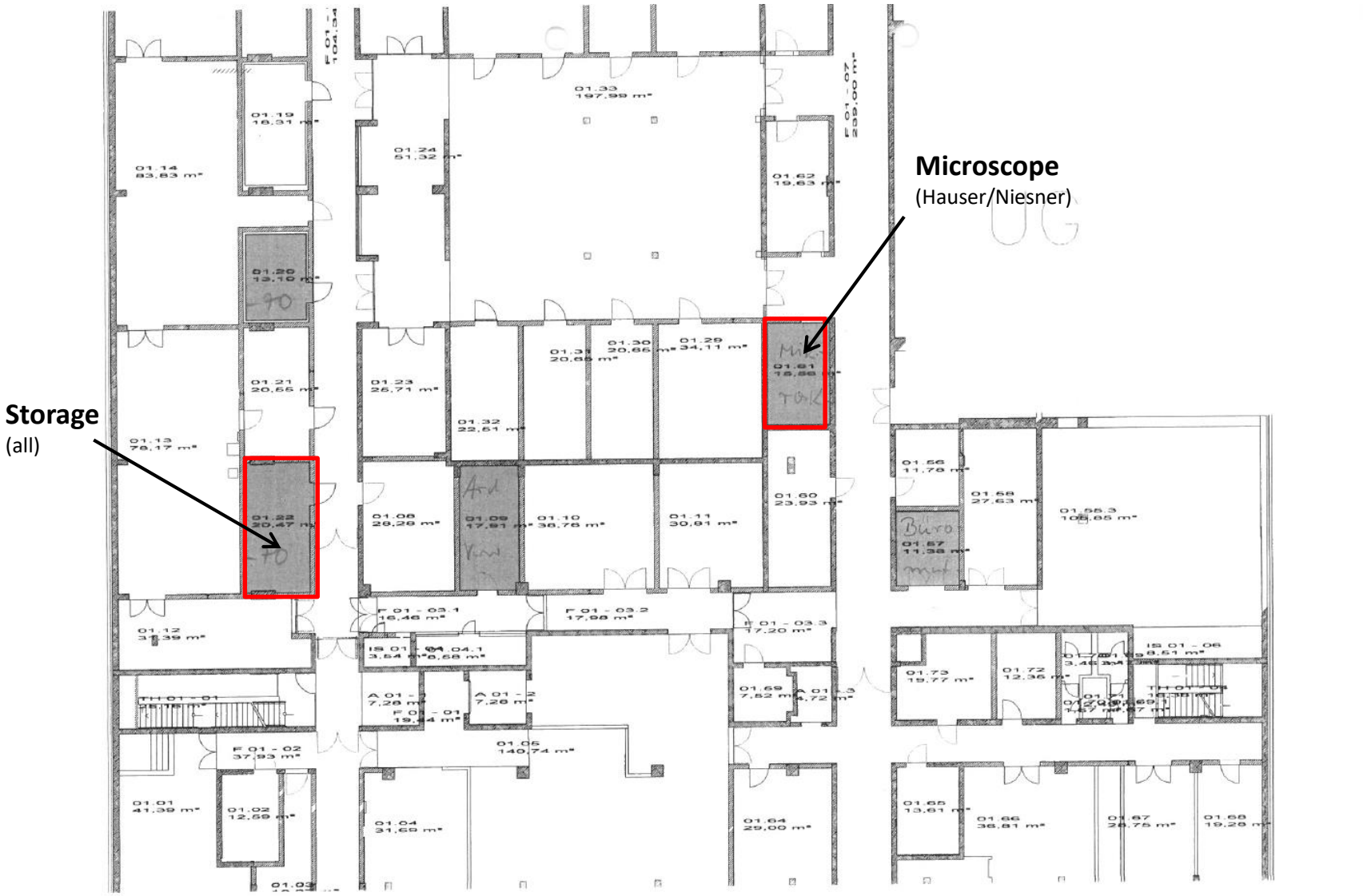
Ute Hoffmann

Current S2 projects at DRFZ

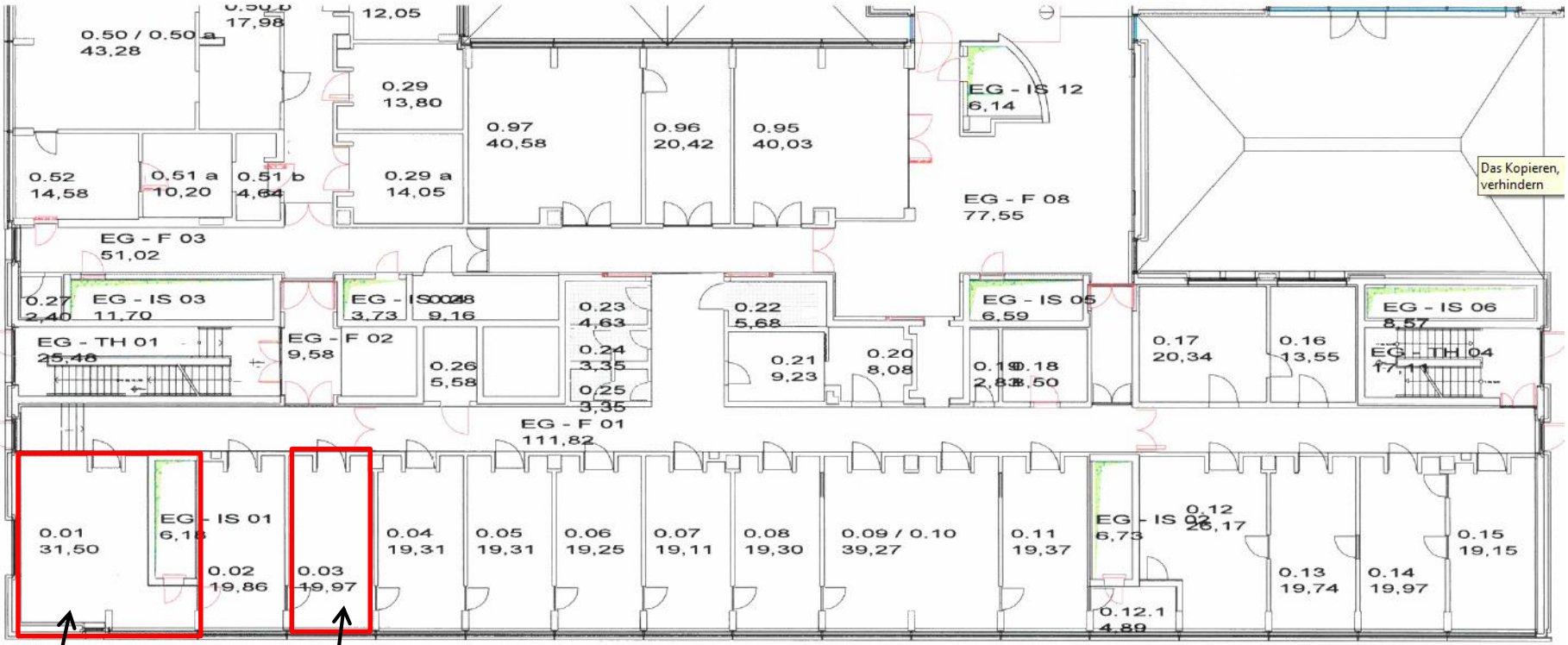
- Interaction of HCMV-infected cells with NK cells using recombinant replication competent HCMV particles (Vector: TB40-BAC4) *AG Romagnani*
- Switch of genes of circadian rhythm in T cells by CRISPR-Cas 9 using recombinant replication deficient pseudotyped lentiviral virus particles (transfer vector: lentiviral pL-CRISPR.EFS.GFP) *AG Buttgereit*
- Knock out of genes in primary human T cells by CRISPR-Cas9 (vector: pSpCas9 (BB)-2A-GFP (PX458) *AG Polansky*
- Infection of cartilage cells with lentiviral plasmids (vector: pLenti-Xi-Blast-rLAMP1-eGFP) *AG Buttgereit*
- CRISPR/Cas9-based manipulation of candidate genes in hemotopoietic cells *AG Dörner*
- Retroviral transduction of intestinal cells (pSIRV-NF-kB-eGFP) *AG Chang*
- Lentiviral transduction of NK cells *AG Romagnani*
- Gamma retroviral T cell receptor transduction into primary human T cells to investigate the specificity of the T cell receptor *AG Hiepe*
- CRISPR/Cas9-based manipulation of different genes involved in signalling pathways in peripheral blood mononuclear cells (PBMZ) and specific immune cell populations of patients with autoimmune diseases and healthy individuals *AG Mashreghi*

Safety level 2 rooms at the DRFZ

Basement



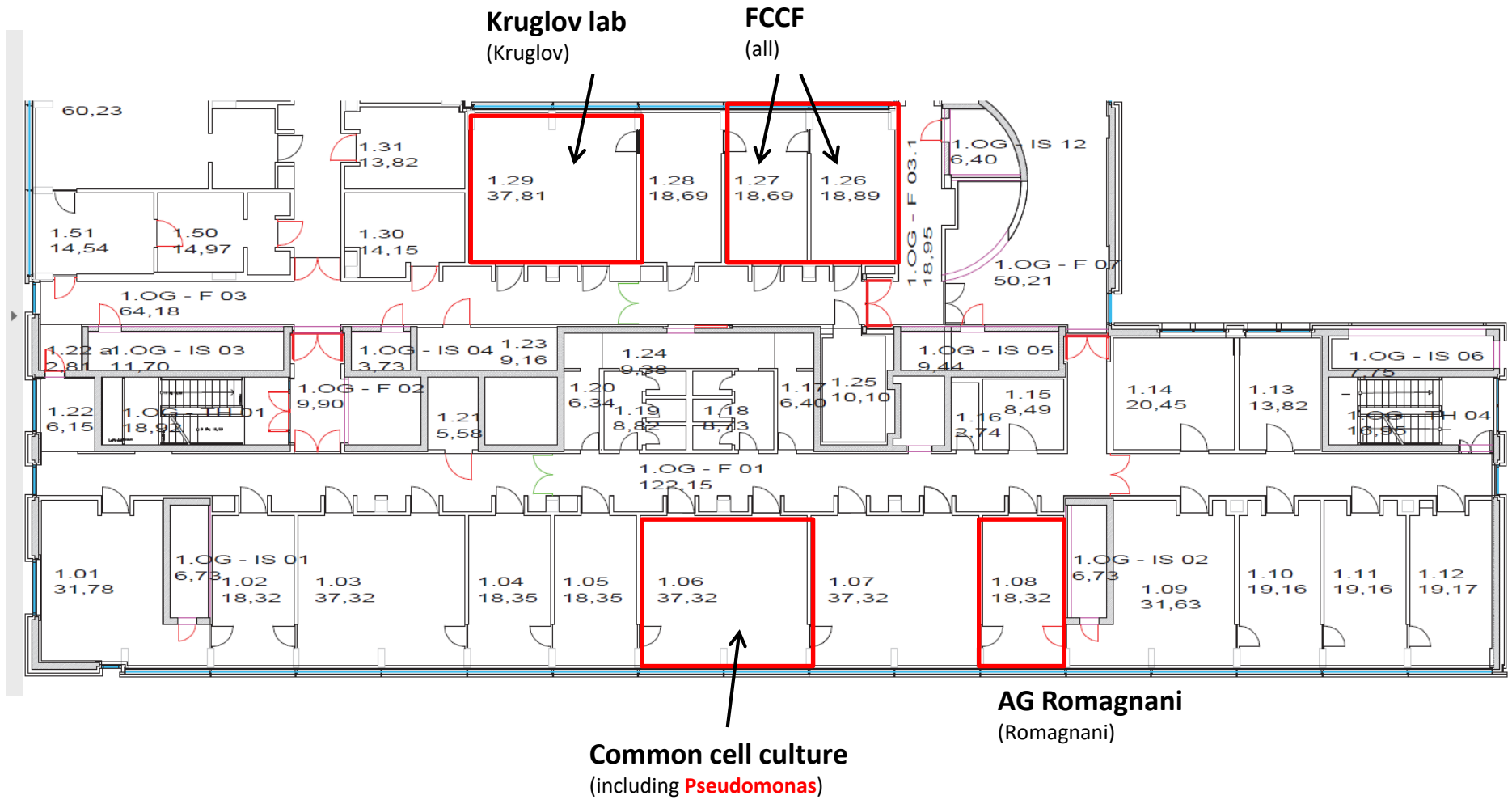
Ground Floor



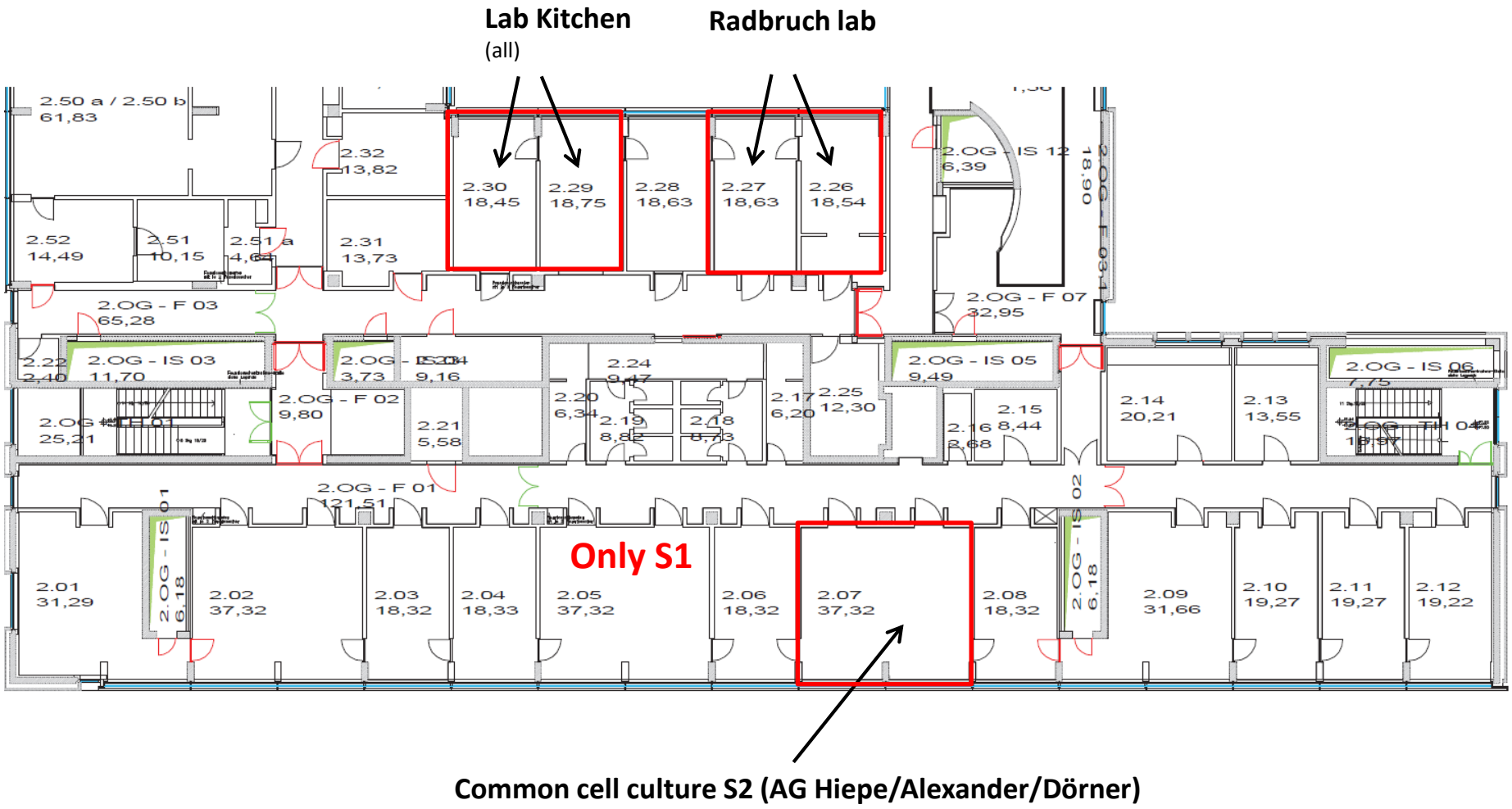
S2 cell culture
(AG Latz)

Devices i.e. Kryotom
(alluser)

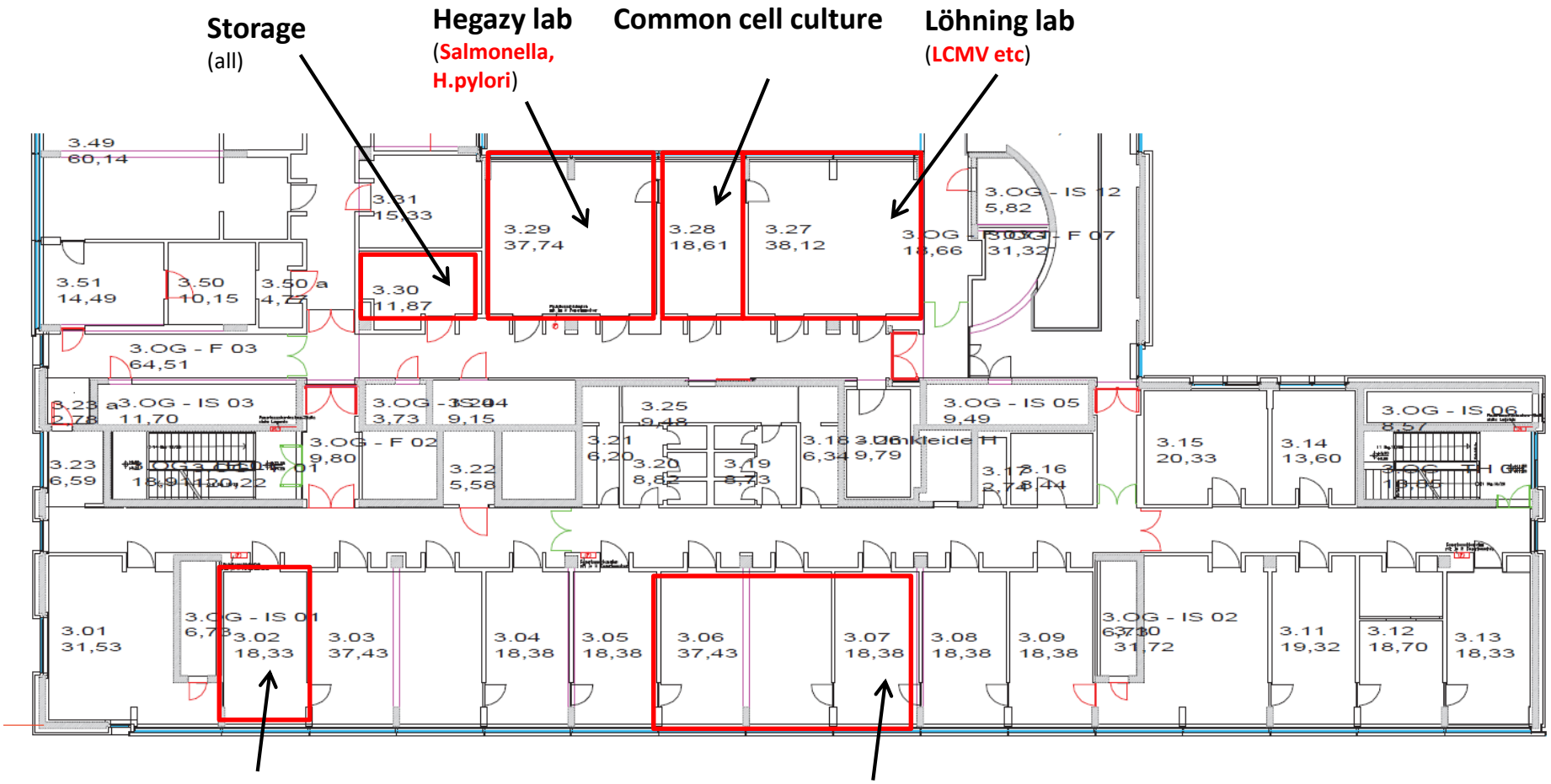
1st Floor


Kruglov lab
(Kruglov)
FCCF
(all)
60,23
1.31
13,82
1.29
37,81
1.28
18,69
1.27
18,69
1.26
18,89
1.OG - IS 12
6,40
1.OG - F 03.1
18,95
1.OG - F 07
50,21
1.51
14,54
1.50
14,97
1.30
14,15
1.OG - F 03
64,18
1.22
2,81
a1.OG - IS 03
11,70
1.OG - IS 04
3,73
1.23
9,16
1.24
9,38
1.OG - IS 05
9,44
1.OG - IS 06
7,75
1.OG - F 02
9,90
1.20
6,34
1.17
6,40
1.25
10,10
1.15
8,49
1.14
20,45
1.13
13,82
1.22
6,15
1.21
5,58
1.19
8,82
1.18
8,73
1.16
2,74
1.OG - F 01
122,15
1.01
31,78
1.OG - IS 01
6,73
1.02
18,32
1.03
37,32
1.04
18,35
1.05
18,35
1.06
37,32
1.07
37,32
1.08
18,32
1.OG - IS 02
6,73
1.09
31,63
1.10
19,16
1.11
19,16
1.12
19,17
Common cell culture
(including Pseudomonas)
AG Romagnani
(Romagnani)

2nd Floor


Lab Kitchen
(all)
Radbruch lab
2.50 a / 2.50 b
61,83
2.32
13,82
2.30
18,45
2.29
18,75
2.28
18,63
2.27
18,63
2.26
18,54
2.OG - IS 12
6,39
2.OG - F 03
65,28
2.OG - F 07
32,95
2.52
14,49
2.51
10,15
2.31
13,73
2.OG - IS 03
11,70
2.OG - F 02
9,80
2.21
5,58
2.24
9,17
2.20
6,34
2.19
8,82
2.18
8,73
2.17
6,20
2.25
12,30
2.OG - IS 05
9,49
2.15
8,44
2.16
8,68
2.14
20,21
2.13
13,55
2.OG - IS 06
2.OG - F 01
121,51
Only S1
2.01
31,29
2.02
37,32
2.03
18,32
2.04
18,33
2.05
37,32
2.06
18,32
2.07
37,32
2.08
18,32
2.09
31,66
2.10
19,27
2.11
19,27
2.12
19,22
Common cell culture S2 (AG Hiepe/Alexander/Dörner)

3rd Floor


Storage
(all)
Hegazy lab
(Salmonella, H.pylori)
Common cell culture
Löhning lab
(LCMV etc)
3.49
60,14
3.51
14,49
3.50
10,15
3.50 a
4,75
3.31
15,33
3.30
11,87
3.29
37,74
3.28
18,61
3.27
38,12
3.OG - IS 12
5,82
3.OG - F 07
31,32
3.OG - F 03
64,51
3.OG - IS 03
11,70
3.OG - F 04
9,15
3.OG - F 02
9,80
3.22
5,58
3.25
9,48
3.21
6,20
3.20
8,82
3.19
8,73
3.OG - IS 05
9,49
3.15
20,33
3.14
13,60
3.OG - IS 06
8,57
3.23
6,59
3.01
31,53
3.OG - IS 01
3.02
18,33
3.03
37,43
3.04
18,38
3.05
18,38
3.06
37,43
3.07
18,38
3.08
18,38
3.09
18,38
3.OG - IS 02
31,72
3.11
19,32
3.12
18,70
3.13
18,33
Mashreghi lab
Lab (bacteria, S2 Gen)
and Influx AG Chang
(Chang)

Safety rules

Work with biological material or gene modified organisms of risk group 2/3**

- only allowed in S2 labs

Access

- **No access to S2 labs without prior introduction by the project leader/group leader**!
  - Ute Hoffmann PI for work with S2 GVOs and untested human material
  - Ahmed, Chiara, Max and Ute for pathogens used in their groups
- Meet the occupational health physician before starting to check vaccination status against Hepatitis A+B

Transport

- *In house:* in closed, leak-proof, unbreakable falcon tubes or FACS tubes with lid or boxes
- *To other institutes:* in closed, leak-proof, unbreakable tubes etc within another closed, leak-proof, unbreakable box containing absorbent material

Safety rules

Clothing

- Closed and robust footwear
- Gloves (eco Nitril from ecoShield)
- Safety glasses and face mask if necessary

Lab coats

- Closed lab coats mandatory
- Labelled lab coats only for S2 labs
- Don´t use S2 lab coats in S1 labs
- After wearing for 2 weeks:
  put in bin for S2 lab coats (in room 3.30)
- Contamination:
  put immediately into an autoclave bag
  and store in an empty autoclave bin in the lab kitchen

Safety rules

Disinfectant

- All labs: B15 2% - 5 min incubation
- Labs with spore forming bacteria: OxyFoamS - 60 min incubation
- Contaminated dishes and instruments: Sekusept aktiv 7% bath - 60 min incubation

Disinfection

In case of contamination and before you finish working:

- Disinfect the sterile bench or other workplaces
  by squeezing bottle and paper towel
- Disinfect contaminated lab dish by dipping it into a bath
- Exchange B15 2% daily, unused solution is fine for 28d
  (stock solution in room 0.01, 1.05, 2.29 and 3.30)
- Disinfection of incubator once a month by wiping with
  B15 2% or OxyFoamS and then start sterilization program
  In case of contamination same procedure

Safety rules

Sterile bench

- Use the sterile bench **for all S2 work** (aerosol formation could be possible i.e. during spilling, plating etc)
- It has to be clean, tided up, not overcrowded
- Disinfection of work space after finishing your work wiping with B15 2%
- Cleaning once a month by using B15 2% (work space, below the plates) and also the top wiping with B15 2%

Centrifugation

- Use only tight and closed tubes

Pipettes and needles

- Single use **plastic pipettes for all human material**
- Use only safety needles and scalpels
- Glass pipettes could be used for work with S2 GVO material

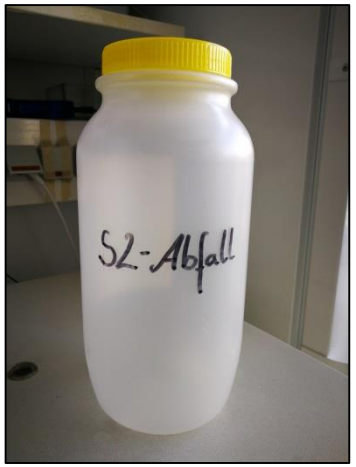
Pipette disposal

Safety rules

Waste

Glass pipettes

- Collect in Sekusept Aktiv 7% filled containers
  Bring to lab kitchen when it´s full



Liquid waste

- Collect in closed, unbreakable bins
- Bring to lab kitchen (2.29/30) for autoclaving every evening

Solid waste

- Collect in S2 labelled autoclave bins (also pipette tips)
- Spore forming bacteria waste: Please label for a different sterilization program
- Fill them only up to 2/3 and bring them to lab kitchen
- Don´t store this waste over weekend

Sharp items

- Collect in yellow multi-safe bins
- Bring them to lab kitchen

Safety rules

Handling of -80°C freezers I

- Wear a lab coat & thermo gloves

Boxes

- Use ONLY plastic boxes to store samples or reagents (with or w/o grid, available at lab manager lab 2.08)
- label boxes with appropriate tags and permanent marker before storage (mandatory: workgroup, name and Box ID)

Storage

- store your boxes only in the space dedicated to your workgroup
- storage of lose tubes or bags is NOT ALLOWED

Materials not meeting this criteria will be disposed!

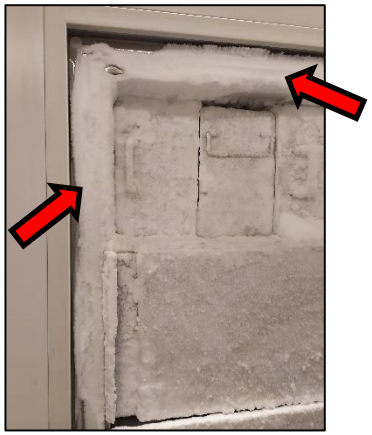
By the end of the year, please reduce and inventory your samples!

In General

- Refer to senior staff / supervisors in case of questions or un-clarities BEFORE „just trying out“
- Keep proper inventory of your freezer content – at vial and box level – to reduce time to search & find

Safety rules

Handling of -80°C freezers II



Open/Close

- Open the freezer only as short as possible to remove a box or a complete rack
- Make sure that the freezer door is properly closed after use!
- Do NOT open the freezer, if the current temperature is already to warm (> -70°C)
- Remove ice at the door sealing regularly to ensure a proper closure

In case of technical or mechanical problems

- please inform:
  the lab managers (labmanager@drfz.de)
  or the in-house technicians (Betriebstechnik@mpiib-berlin.mpg.de)

Act responsibly!
Be aware that all your colleagues rely on proper functionality and use of the freezers.

Safety rules

In case of an unexpected event

Release of large amounts of micro organisms or GVOs (i.e. spilling)

- Alert colleagues, close the area, inform the project leader

Waste disposal

- Wear lab coat, glasses, nitril gloves mandatory
- filtering half mask in case of aerosols (FFP2)

Liquids

- Take up liquids with an B15 2% dry lab tissue
- Transfer to S2 autoclave bins
- Disinfect contaminated area
  (no entering of other colleagues during this time, keep doors and windows closed)

Contaminated items including lab coats

- Transfer in an extra S2 autoclave bin (except sharp items – multi-safe bin)
- Autoclave immediately

Safety rules

Cleaning your hands

Before you start working

- Washing with Baktolin first (2-3 shots, induce foam, dry with paper tissue)
- Desinfect with Sterillium classic pure before using gloves
  (2-3 shots, 30 sec incubation time, drying)
- Use Prolind Hautschutzcreme sensitive (blue) – size as large as a cherry stone

In case of contamination

- Disinfect with Sterillium classic pure first
- Washing with Baktolin during lab work not required
- Use Descolind Protect (red)

After finishing your work

- Disinfect with Sterillium classic pure first
- Wash with Baktolin
- Use Descolind Expert (blue)

Before you start and recording of work

Before new group members start to work with S2 pathogens

- They have to be introduced by the project leaders!
- The Betriebsarzt (occupational health physician) has to be informed

Before new group members start to work with untested human material

- They have to be introduced by the group leaders!
- The Betriebsarzt has to be informed and vaccination against Hepatitis A+B required

When you work with S2 GVOs

- All gene technical work and storage has to be recorded in Formblatt Z separate from that for S1!

When you plan to use new S2 pathogens or S2 GVOs

- Please contact Ute first!
  - S2 gene technical work has to be notifed at LAGeSo
  - S2 work with pathogens has to be notified an LAGetSi and Gesundheitsamt

Attention: All experiments with gene modified infectious pathogens have to be notified at Gesundheitsamt, LAGetSi and LAGeSo! Please contact Ute!

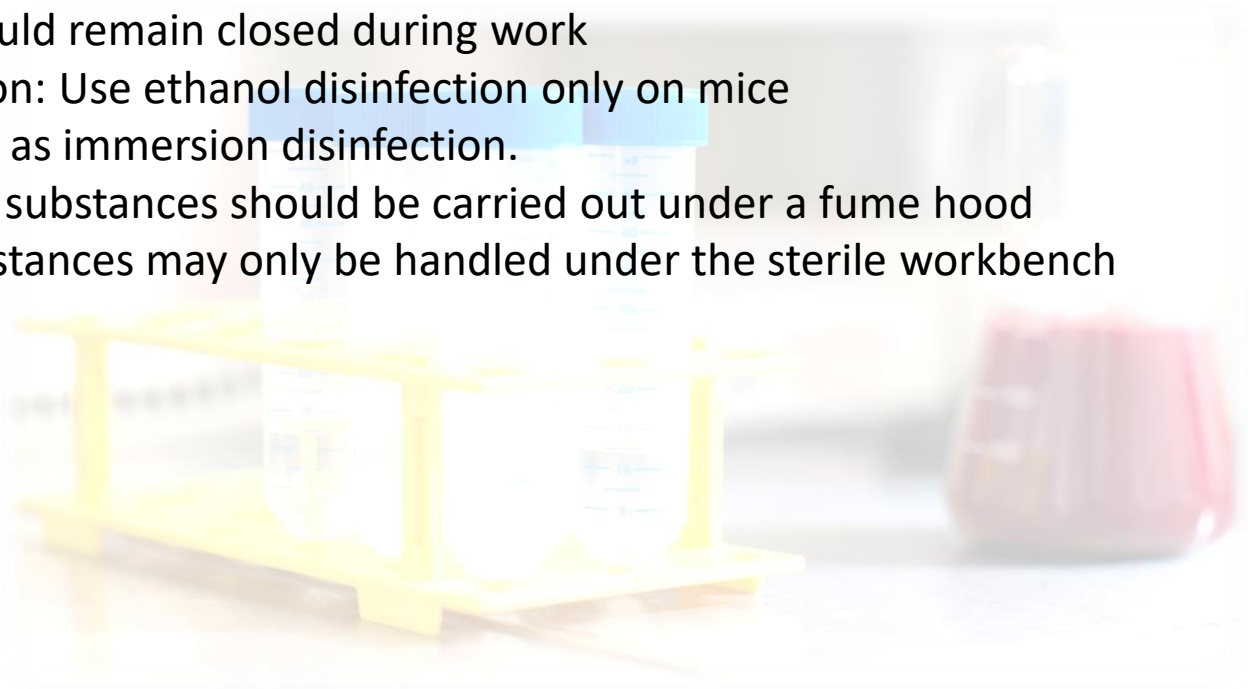
Reduction of room air exchange rate

Room air exchange rate (RLT):

- is reduced

Follow the previous rules even more strictly than before

- Windows and doors should remain closed during work
- Avoid ethanol disinfection: Use ethanol disinfection only on mice and also only in beakers as immersion disinfection.
- All work with hazardous substances should be carried out under a fume hood
- S2 GMOs and S2 biosubstances may only be handled under the sterile workbench



Final remarks

In case of an accident/injury:

- Please fill out the PDF document (intranet at Job safety – Verbandbuch/First Aid book) and bring it to personnel department

In case of considerations and questions

- Don´t hesitate to contact Robert and Ute

Signatures

- Please sign our list **at the lobby**
  and cross the material(s) you are working with on this list

Thank you for your attention!