GOOD LABORTATORY PRAXIS:

Stand 23.12.05

Safety at work (AG Leiter)

Radiation Safety Instructions (BM)

Genetic engineering (MTD)

Virology Lab Manual (AG-Leiter, and for S3-lab: MTD/Roland Kehm)

Diplom/Masters/PhD information

1. PhD Program Information (see homepage)
2. Freitagsseminar-Information
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Safety at work (AG Leiter)

All labs are biosafety level 2 and the rules have to be observed. The most important rules:

- **Strictly** obey rules for appropriate disposal of waste (separate non-contaminated and infectious/'genetic' waste; glass and sharps; phenol). Wash out all dishes with water before putting them onto the 'Spülküchen' carts

- wear lab coat at all times; gloves or goggles when appropriate
- rules for bench cleaning and hand disinfection are posted in each room

- **no food or drink inside the lab!** Eating and drinking is allowed in the offices and in the coffee room

- report lab accidents to group leader or safety officer (Paul Schnitzler)

- the following instruments and rooms **must not** be used prior to instruction by the person in charge: Ultracentrifuge (Christine Goffinet), Fluorescence microscope (Oliver Fackler), microinjector (Oliver Fackler), FACS (Nico Michel), sequencer (Bärbel Glass), FPLC (Vanda Bartonova), pipetting robot (Heike/Bärbel), Fluorescence spectrophotometer and Fluorescence reader, tissue culture, radioactivity lab 1st floor (Barbara). Report all problems with these instruments or document if required.

- Small amounts of radioactivity can be used in the lab when announced and labelled properly. Pay attention to signs indicating radioactivity.
- Report loss of the key card to the administration

- people from outside who want to enter the P2 area have to be admitted by a group leader
- Instructions of safety officers have to be observed (safety: Paul Schnitzler; radiation: Barbara, Matthias, Paul)
- If you feel insecure about a safety issue or use of an instrument: **ASK!!!**

Documentation:

- all experiments (including those that didn't work) have to be documented properly in the lab book. The book and all primary data remain in the lab (copies can be made for your own use).
- Label bottles, boxes and Eppendorf tubes clearly (and readable…).

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Date	signature	signature AG-Leiter

Radiation safety instructions (BM)

There is the possibility of exposure to ionising radiation or accidental incorporation of radioactive substances (ingestion, inhalation, through open wounds) In the Virology department, people work with non-volatile open radioactive substances **(^{32}P, ^{35}S or ^{3}H-labelled)**. There is a '**Kontrollbereich'** for radioactive work on the 1st floor. Samples containing low amounts of radioactivity (i.e. gel samples) may also be handled in the '**Überwachungsbereich'** on 4th floor. To minimize your risk of exposure, you are required to follow these instructions:

1. General:

- Follow all instructions of the local radiation safety officers (B.Müller, M.Dittmar, P.Schnitzler). A radiation safety officer must be notified in case of accidents or problems. You are only allowed to work with radioactive substances if one of the radiation safety officers is accessible.
- All radioactive experiments carried out must be documented in the book present in the radiation lab.
- Before working with radioactivity, an examination by the Betriebsarzt and an instruction about appropriate working procedures are mandatory
- The dosimeter must be worn in the Kontrollbereich (1st floor) at all times at your chest.

2. Incorporation:

- It is absolutely forbidden to eat, drink or smoke. Do not touch your face with your hands. Persons with open wounds at their hands must not work in the Kontrollbereich.
- Upon leaving the Kontrollbereich, you are required to step on the Dosimeter.
- During work: radioactive working areas have to be clearly labelled and shielded
- After the end of work, your hands and the working area have to be measured and – if necessary – decontaminated. All waste connected with the working area is considered radioactive and has to be disposed of immediately in the Kontrollbereich on 1st floor.
- Radioactive samples must not be stored on the 4th floor
- Radioactive experiments have to be documented in the appropriate book

4. For women: There are special safety requirements in case of pregnancy and during breast-feeding. Thus, a radiation safety officer needs to be notified in case you are pregnant.

I have read and understood these instructions.

I have obtained an instruction covering the topics:

- Storage and handling of radioactive substances
- Appropriate shielding
- Use of monitoring devices
- Radioactive waste disposal
- Decontamination
- documentation

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Date signature signature radiation safety officer

Genetic engineering (MTD)

Safety levels according to Genetic Engineering law (§7)

Safety level 1 genetic engineering without a known risk for human health and environment

WORK WITH RECOMBINANT DNA,
WORK WITH SUB-VIRAL PLASMIDS
VIRUSES, THAT DO NOT INFECT HUMAN CELLS

Safety level 2 genetic engineering with low risk for human health and environment

HUMAN PATHOGENIC VIRUSES, NON-AIRBORNE; CELL CULTURE WITH REPLICATION INCOMPETENT HIV

Safety level 3 genetic engineering with moderate risk for human health and environment.

S3-LAB ONLY: REPLICATION COMPETENT HIV, HCV

Safety level 4 gentetic engineering with high risk for human health and environment.

‚PROJEKTLEITER' Department of Virology (December 2005)

Prof. Kräusslich	S3:	Expression of recombinant proviral DNA of HIV Cloning of HCV cDNA
	S2:	Expression of recombinant HIV DNA (incomplete proviruses) HIV Genes in MuLV(ampho) lentiviral gene transfer systems (HIV, Maedi/Visna Virus)
PD Dr. Dittmar	S2:	gene transfer systems (Retroviruses, Lentiviruses, Pararetroviruses)
Dr Keppler	S2:	HIV Genes in Adenoviral vectors; Export of HASPB
Dr Michel	S2:	gene transfer systems for HIV-Proteins (nef)
PD Dr. Schnitzler	S1:	Storage of recombinant measles-GFP Virus

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Date signature signature ‚gentechnik' safety officer

Virology Lab Manual (AG-Leiter, for S3-lab: MTD/Roland Kehm)

Biosafety level for HIV
HIV belongs to biosafety level 3**. HIV transmission occurs via blood, saliva and breast milk, but not through aerosols.

HIV causes AIDS and there is no vaccine available.

For the classification into biosafety levels it is important to distinguish
replication competent viruses (always S3 lab),
replication incompetent with the probability to recombine (also S3 lab for safety reasons) and
replication incompetent viruses without the risk to recombine (S2 lab possible).

In addition, recombinant viruses with deletions in RT and/or integrase are not able to integrate into the host genome (infected cell lines) and are classified to biosafety lavel 1.

Bacteria S2/S3

- bacteria containing S3-plasmids are classified as S3-organisms. Therefore, transformation and growth of S3-bacteria has to take place in the S3-lab.
- Storage of S3-bacteria in the S3-lab
- After lysis of the grown bacteria, the subsequent steps for plasmid purification can be performed ion the S2-laboratories.
- bacteria containing S2 or S1 plasmids are grown in the S2-laboratories.

Newly constructed recombinant plasmids need to be classified together with the group leader before using them.

Examples for plasmids often used in the laboratories (AG Keppler)

S2-Plasmids in Bacteria	**S2-Virus**
• HIV-$_{1NL4-3}$E$^-$R$^-$luc (env-, vpr-, nef-)	VSV-G HIV-$_{1NL4-3}$E$^-$R$^-$luc
• HIV-1$_{NL4-3}$E$^-$R$^-$gfp (env-, vpr-, nef-)	VSV-G HIV-1$_{NL4-3}$E$^-$R$^-$gfp
• HIV-2$_{ROD}$E$^-$R$^-$gfp (env-, nef-)	VSV-G HIV-2$_{ROD}$E$^-$R$^-$gfp
• Delta R8.91 („Trono“ Vektor)	VSV-G, Delta R8.91, pHR-based transfer vector
• MuMLV gag-pol oder stable PLAT-E cells (293T-based)	VSV-G MuMLV and transfer vector
• SIV$_{mac239}$luc (env-, nef-)	VSV-G SIV$_{mac239}$ luc
• „Akari“ Plasmids (env-, vpr-)	VSV-G Akari“ Viruses

S3-Plasmids in bacteria	**S3-Virus**
• „Kirchhoff“-Plasmids pBR HIV-1$_{NL4-3}$ IRES-GFP (Δ*nef*, HIV-1$_{NA-7}$ *nef* oder HIV-2$_{BEN}$ *nef*)	„Kirchhoff“-Virus with or w/o VSV-G
• HIV-1 R7/3-YU-2 gfp (nef-)	HIV Env NL4-3E$^-$R$^-$luc
• HIV-1 R7/3 gfp (nef-)	HIV Env NL4-3E$^-$R$^-$luc

Working in the S3-lab:

Requirements:

- Experience in cell culture and working with viruses (S2) are needed, before the introduction into the S3-lab
- Introduction through either Dr. Roland Kehm or S3-safety officer of the Dept. of Virology
- Important!: At first, all experiments are performed in the presence of an experienced colleague before working alone..

Need to know:

1. How to define biosafety level 2 and 3 for plasmids, bacteria and cells
2. Important phone numbers, Who to contact in case of an emergency
3. Security gate area: changing into S3-lab coats, import/export of material
4. Rules for working with glassware, sharp objects
5. How to autoclave disposed material (solid/liquid)
6. How to keep working area clean, during experiments and after finishing
7. Storage of cells and virus stocks (-80°C, liquid N2-tank)
8. documentation of experiments, use of fax machine

Documentation for working in L3/S3-lab

(Regular working hours and ‚after work' rules)

Regular Working Hours *(Mo – Fr., 8:00 – 16:30):*
While entering the S3-lab, inform Dr. Kehm. If he is not present, call him or inform S3-safety officer before starting the work. If both can't be reached, the rules for 'after work hours' apply.
You need to sign in, including time and proposed experiment, before starting the work.

‚After work' hours*: (Mo-Fr., 16:30 – 8:00, weekends and holidays):*
To enter the S3-lab (INF 344b) you need a key to open the first door. (the key-card can be used to open the security gate). After signing out, the front door has to be locked again.

Web-cam surveillance is installed to allow supervision from the Dept. of Virology. (Laptop/Monitor is located in room 459)

Either two people enter and leave the S3-lab together, or another person in the Dept. of Virology overlooks the work using the laptop/monitor. This person has to be named when signing in (special forms for 'after hours work' are located at the S3-ab entrance).

Important phone numbers L3/S3-Labor:

- **Dr. Roland Kehm** — Tel: **56-32372 (mobile)** oder **56-5024 (office)**
  Head of L3/S3-Labors — oder 0170-7622037
  oder 0179-8858600 oder 0170-7622038
- **PD Dr. Matthias Dittmar** — Tel: **56-1322** oder 0170-8928848
  1. Stellvertreter
- **Dr. Thomas Pietschmann** — **Tel: 56-6449**
  2. Stellvertreter
- **PD Dr. Paul Schnitzler** — Tel: **56-35016** oder 0170-7622039 oder
  3. Stellvertreter — 06203-406138 oder 0170-7622040

In the case of an emergency:

Technical lab failure (security gate, pressure, autoklave):

Zentrale Leitwarte Tel: 56-444 oder 56-7272

Exposition (contaminated eyes, mouth, nose, needle stick accidents):

1. Immediately use antisepticum „Oktinisept“ and de-contaminate areas (antisepticum is stored in fridge in the security gate area)

2. Contact S3-safety officer, or colleagues

3. Contact ‚Dienstarzt’ in the Dept. of Virology: Tel: 56-35021 (Mo – Fr, 8:00 – 16:30), or 0171-634 00 89 (Diensthandy)

4. If contaminated by wild type HIV variants (without known resistance)

   Use HIV-PEP accordingly (information how to use PEP can be found in the security gate area at the fridge door)!
   - Contact „Needle stick-Hotline“ der Hautklinik Ambulanz (THE HIV-experts in Heidelberg): Tel: 56-1606..
   - Contact ‚Durchgangsarzt (D-Arzt)’ at the Chirurgische Ambulanz. The accident needs to be documented by the University.

5. Inform the group leader

lab contamination

- Spills can be de-contaminated according to the rules. If larger volumes or areas are contamited contact Roland Kehm or S3-safety officer of the Dept. Of Virology.

  Wear respiratory mask (stored in each lab, first drawer near the door)
  Use paper towels to soak up the liquid spill, decontaminate paper/liquid with 3% Incidin perfekt-solution. Dispose papers and decontaminate surface again using 3% Incidin perfekt-solution

Ad 1.

Safety at work:

- NO food NO drink, NO smoking in the S3-lab.

Own Clothes

- Important: don't expose to much skin while working in the S3-lab. Wear long trousers/skirts and shoes supplied by the university (closed at front, no sandales).

Ad 2:

Transfection of proviral DNA:

Using Calcium-Phosphat oder Lipofection to ‚activate' the proviral DNA can be performed in the S2-lab. The closed tubes containing the DNA shall be transported into the S3-lab and only there added to the cells

Using Electroporation und Nucleofection (only possible in S2-lab) the closed tubes containing cells and proviral DNA shall be immediately transferred into the S3-lab.

Ad 3:

Export of HIV-positive samples:

Only 4 ways are possible to export samples from the S3-lab:

1. Autoclave

2. Fixing the cells with Methanol/Aceton (1:1) for 10 min (e.g. for *blue cell assay*)

3. Fixing the cells/virus pellets with 2% PFA in PBS for min. 90 min (e.g. for FACS) or with 2% Glutaraldehyde in PBS for 60 min. Tranfer the fixed cells into fresh tube before leaving the S3-lab

4. Cell culture supernatants (e.g. for p24 Ag-ELISA): add 1/10 Volume of 5% Triton X-100 (final concentration 0,5% Triton X-100) (e.g.. 200 µl supernatant + 20 µl 5% Triton X-100)

- Before leaving the S3-lab: decontaminate the surfaces of tubes and plates with 3% Incidin Plus

Important:

Once processing exported material in the S2-lab, take special care. Use gloves, especially when using the FACS.

Ice in S3-lab:

Ice or thawed ice shall be disposed in the security gate area sink after decontaminating it with 3% Incidin Plus.

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Date	signature	signature S3-lab safety officer

Diplom/Masters/PhD Information

1. PhD Program Information: see homepage

2. Freitagsseminar-Information

Every FRIDAY, 9.00am-10.30am, Seminarraum. Presentation in english.

Two researchers present their recent work and results:

Short introduction,
summary of last presentation
Progress since last presentation,
Outlook

Diploma/Master students present their results after submission of written thesis.

Unpublished details about all projects in the lab including everything presented at lab meeting, group meeting and virus club are strictly confidential!

3. Journalclub-Information

Every TUESDAY, 11.45 a.m. (Lunch-Seminar). Presentation in english.

- Only papers with high impact factor (. **IF > 10**).
- Inform colleagues by email about your choice (Friday before you are scheduled). Include pdf-file.
- Introduction, Results of the paper, summary, and critical evaluation (methods, conclusion) are required to allow for discussion.