

## Safety rules

Pathogenic strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* fall into Risk Group 2 of microorganisms and associated with human diseases that are rarely serious and prophylactic intervention often available. Thus biosafety level 2 (BSL2) guidelines are required for handling of microbes at our lab.

### Pathways of infection transmission:

- 1) Direct contact of mucosal membranes with contaminated surfaces and/or object
- 2) Inhalation of infectious airborne secretions
- 3) Ingestion

Thus, our goal is **to prevent any contact between us/our belongings and bacteria**. If your stuff is contaminated bacteria can get to you easily.

There are three areas at our lab: Shiri's office and main room divided into microbial zone and computer zone. All bacteria must stay at microbial zone, while your belongings are at computer zone. These rules are intended to clarify risks and minimize them.

### Strongly prohibited

- 1) To eat at the laboratory main room.
- 2) To chew a gum or eat candies during the experimental work.
- 3) To touch face, hair, apply cosmetics and adjust contact lenses during the experimental work.
- 4) To work when you are ill.
- 5) To run, to throw thing and to shout at the lab.
- 6) To make parties at the laboratory main room.

### What to do with private things

- 1) Items that will leave the laboratory must be not contaminated. **Do not touch them during the work!**
- 2) **Do not use cell phone or personal electronic devices during the laboratory session.**
- 3) All personal belongings, e.g., backpacks, purses, books, etc., should be stored way from the work area. Papers that will be taken home should be protected from contamination during the lab work.
- 4) During the work all notes should be written with laboratory-use-only pens and pencils. These items should never leave the laboratory.

### Training practices

- 1) Everybody have to handle microorganisms safely and responsibly.
- 2) Students should demonstrate competency with Risk Group 1 microbes before working with Risk Group 2 pathogens.
- 3) Students should be informed of safety precautions relevant to each exercise before beginning the exercise.
- 4) Immune-compromised people (including those who are pregnant or may become pregnant) and people living with or caring for an immune-compromised individual should consult to laboratory director and additionally physicians to determine the appropriate level of participation in the laboratory.

## Document practices

1) All students have to sign safety agreements explaining that they have been informed about safety precautions and the hazardous nature of the organisms they will handle throughout the course.

## Personal Protection Requirements

1) Prepare yourselves to work:

- Wear **closed-toe shoes** that cover the top of the foot. Hard-sole shoes without open toes are required in all laboratories to protect against heavy objects, hot liquids, or broken glass. The closed-toe style is necessary due to the additional risk of contamination in the microbiology lab.
- **Tie back long hair.** Put off dangling jewelry. Bracelets and wrist watch must be covered with gloves or put off.
- Wear **laboratory coats**. The sleeves of the lab coat should completely cover the sleeves of street clothes. **Laboratory coats must be used at the microbial zone only! Do not enter computer zone in coat!**
- Wear **gloves** when handling microorganisms or hazardous chemicals:

<b>Gloves on (Coat on)</b>	to work in sterile conditions to work with microorganisms prefer to use nitrile gloves working with chemicals
<b>Change gloves</b>	as soon as gloves are damaged in case of a suspicions of bacterial contamination to take something from stock in cupboards to bring bacteria from stocks at -80C to work with devices out of the microbial zone or out of the lab
<b>Gloves off (Coat off) +Hand wash</b>	in the end of the laboratory session to leave the lab for a break to check something at the non-microbial zone

- 2) Prefer to work with bacteria in a biological safety cabinet
- 3) A/C must be turned off if you work on the bench at microbial zone.
- 4) Work with evaporating chemicals in a fume hood at the student lab downstairs.

## Standard Laboratory Practices

- 1) Wash hands after entering, after handling viable materials, after removing gloves, and before leaving the laboratory.
- 2) Keep the door closed while the laboratory is in session.
- 3) The area for culturing and working with microorganisms should be as separate as possible from the area for taking notes.
- 4) Try to organize and prepare all required materials before handling bacteria.
- 5) Perform all procedures carefully to minimize the creation of splashes or aerosol.
- 6) Students should notify instructors of all spills or injuries.
- 7) Label all containers clearly.
- 8) Do not handle broken glass with fingers; use a dustpan and broom or any analogues.
- 9) Clean all surfaces used during the work with bacteria on completion of work or at the end of the day and after any spill or splash of viable material. Use 70% EtOH.

## **Removal of Biological Waste**

- 1) Remove all cultures, stocks and other regulated wastes used during the work with bacteria to autoclavable bag. Seal the bag with a autoclave tape, cover the first bag with another autoclavable bag and seal it too for transport wastes from the laboratory.
- 2) Autoclavable glass vessels should be sterilized by autoclavation at program 5 (120°C 20min) at the same day or during the next day after use, when rinsed, washed in dish washer and dried.

## **Removal of Hazardous Waste**

- 1) Liquids containing SDS, ethanol, isopropanol, etc. (the full list can be found here: <https://www.uwgb.edu/safety/envpolicies/#04>) should be diluted with 4V of water and then flush down the drain of a chemical sink with 20 volumes of water. If pH of liquids is <5 or >9, solutions should be neutralized before flushing down.
- 2) All cases, there is any suspicion that chemically hazardous waste is contaminated with bacteria, should be referred to Shiri to get a final decision how to utilize it.

## **EtBr Handling**

- 1) Work on the special bench only.
- 2) Use nitrile gloves. Change gloves every time you finish to work with EtBr or any contact with EtBr containing gel and switch to applying samples, preparing TAE buffer, etc. Wash your hands later on.
- 3) One drop of EtBr is added directly to cooled (45-50°C) agarose gel pured in a special tray.
- 4) Do not open a bath when an electrophoresis has just finished its run. EtBr is volatile and can be spread with aerosol. Wait 5 min.
- 5) Transfer a gel on a special stand to prevent dropping on the floor.
- 6) After taking a picture of the gel discard the gel into EtBr can in the equipment room and clean UV-visualizer with paper towel.

## **Stock Culture Requirements (additionally)**

Obtain fresh stock cultures of microorganisms annually (e.g., purchased, revived from frozen stock cultures, etc.) to be certain of the source culture, minimize spontaneous mutations, and reduce contamination. Keep stock cultures in a secure area.

### These rules are based on:

Emmert, E. (2013). Biosafety Guidelines for Handling Microorganisms in the Teaching Laboratory: Development and Rationale. Journal of Microbiology & Biology Education, 14(1). doi:10.1128/jmbe.v14i1.531

Appendix a: cdc guidance and information on microorganisms. DOE/EA-1442R; Final Revised Environmental Assessment for The Proposed Construction and Operation of a Biosafety Level 3 Facility at Lawrence Livermore National Laboratory, Livermore, California, Issued: December 2002, Revised: January 2008

Emmert's paper is printed and could be found on the notice board.

Lab members have an access to full versions of these and several more documents in the folder "Safety rules" on our Dropbox.