Guidelines for use of the Laboratory for Environmental Pathogen Research (LEPR)

Wolf Hall 2227

Von Sigler, Ph.D.

Associate Professor of Microbial Ecology
Department of Environmental Sciences
University of Toledo

von.sigler@utoledo.edu

Ph: 419 530.2897 (office)
419 530.4581 (lab)

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Important:

In case of emergency dial 2600 (campus police) from any campus phone, or 419 530.2600 from any off campus phone. Do not dial 911 unless directed by the campus police.

Research in the Laboratory for Environmental Pathogens Research (Sigler Lab, WO 2227) is dependent on several factors to ensure efficient, accurate and productive use of lab space. Among the most important of these are:

1. Cleanliness
2. Upkeep of inventory
3. Respect for other researchers

Below is a list of guidelines that I expect all who use this lab space to abide by and to consider when setting up- and performing experiments in the LEPR.

Cleanliness - Our work in this laboratory involves sensitive, nucleic acid-based- and microbiological techniques. Such techniques are absolutely dependent on sterility and minimization of contamination. Therefore, cleanliness is a top priority.

1. Wash your hands before leaving the LEPR. It is an advisable practice to wash your hands when entering the lab as well if you are expecting to perform assays.

2. Keep all bench tops AND desk tops organized, clean, and dust-free. This includes not only the main bench-tops, but also the upper shelves where bottles, boxes, and papers are often placed. Use 70% ethanol for this purpose.

3. Gloves must be worn for ALL PROTOCOLS. I will not look at anyone's data if I know that they have not been wearing gloves while working.

4. Keep the areas around the sinks clean and free of dirty and/or clean glass- and plastic ware. This means that clean/dry glass- and plastic ware will need to be put away regularly. DO NOT LET THESE THINGS ACCUMULATE AROUND THE SINKS.

5. If you see trash on the floor, a bench, in the hood, etc., THROW IT AWAY.

6. The LEPR is a BSL2 laboratory and we work with several potentially infectious agents including pathogenic *Escherichia coli*, *Staphylococcus spp.*, *Salmonella spp.*, and others associated with human infections. Please be aware of the risk involved with being in close contact with these agents. If you desire background literature, please see Dr. Sigler.

7. All biohazardous materials (gels stained with ethidium bromide, pathogenic bacteria, spent syringes and pipet tips used in pathogen cultures, etc.) must be disposed of in the red biohazard containers.

Sharp materials (syringe needles, glass pipets, etc.) must not be disposed of in the normal refuse containers. Conversely, do not use biohazard bags or totes (labeled and red in color) for non-biohazardous refuse. These items are to disposed of in designated sharps containers. When sharps containers become full, place them (fully closed) in the larger biohazard bins, and notify me (or Safety and Risk Management) that we need another sharps container.

8. To avoid accumulating biohazardous materials, make sure that the department of Safety and Risk Management (dial 3600) is notified in a timely manner to remove the biohazard totes.

9. In the event of a chemical or biological spill, a spill kit is located in the laboratory. Be familiar with its location and how the spill kit works *before you need it*. Following cleanup of a biological spill with the spill kit, prepare a 10% bleach solution and wash the effected area to kill any remaining bacteria. Prepare bleach solutions <u>fresh</u> when needed and dispose of unused bleach solutions following use.

10. Keep equipment clean, especially the:
    a. Centrifuges
    b. Fast-Prep
    c. Image analysis unit
    d. Electrophoresis units
    e. PCR hood

11. Keep pipettes clean and in the racks located on each bench. Remove empty tip boxes from the bench-tops and replace them with full ones.

<u>A few words about equipment:</u>

1. Fast-Prep – DNA/RNA is extracted in this machine – KEEP IT CLEAN.
    a. Wipe out the inside after each use, especially the underside of the lid.

2. Image analysis unit
    a. Keep the outside housing of the unit clean and free of dust and fingerprints.
    b. When you are finished capturing an image, be sure to TURN THE UV LAMP OFF IMMEDIATELY. These bulbs have a limited life-span and are expensive to replace.
    c. Always remove your gel when you are finished, clean the transilluminator surface THOROUGHLY with 70% ethanol and leave the unit door open a few inches to allow ventilation into the housing. Do not use anything other than Kimwipes when cleaning the transilluminator surface.

3. DGGE and agarose electrophoresis units
    a. Always keep the outside of the units clean, as well as the working space surrounding them.
    b. Good electrophoresis results depend on non-depleted running buffer. Maintain the buffer levels in the units and replace the buffers as necessary (approximately every 5000 V·h). We use 1X TAE exclusively and keep an inventory of 50X stock, so there is no excuse for not having plenty of buffer on reserve for replenishing depleted buffer.
    c. DO NOT store DGGE glass plates in the dish strainer at the sink. Dry them on the bottle-holder above the sink and place them in the drawer under the DGGE machine when they are dry.
    d. DO NOT store the DGGE internal unit in the dish strainer at the sink. Dry it (lying down, not standing-up) on blue bench paper on the center bench.

4. Microplate reader
    a. Keep the dust cover on the reader when not in use.

5. Spectrophotometer
    a. Be sure that the cuvettes are clean before placing them into the machine for reading.
    b. When not in use, keep the lid closed to avoid dust accumulation inside the reading chamber.

6. Power supplies
    a. Routinely wipe dust from the housings and be sure to keep the area around the cooling fans clear of obstructions.

7. Autoclave
    a. Instructions for the autoclave are on the side of the autoclave. Please follow them. If you have any questions, please see me or a senior graduate student.

8. PCR Hood
    a. To decontaminate the hood prior to use, press the UV light button with the door closed. The UV light will come on for 15 minutes, degrading any DNA/RNA in the hood. The light will automatically shut off after 15 minutes.
    b. If an alarm sounds on the hood, look at the readout to see what it says. If necessary, replacement air filters are located in a drawer beneath the PCR machines. Otherwise, you should contact me to take care of the problem.

If the laboratory becomes untidy, will expect a mandatory cleaning session. This should never have to happen if the above guidelines are followed.

Upkeep of Inventory - There is nothing more frustrating than planning a day of research, only to be stopped by a shortage of tubes, tips, reagents, etc. Therefore it is EVERYONE'S personal responsibility to make sure that the lab's inventory of all "community items" is kept at reasonable levels. These responsibilities include preparing reagents and consumable stocks, THEN AUTOCLAVING THEM if necessary. If you do not know how to make the solutions that we use, then ask someone in the lab or refer to the Molecular Methods folder on the bookshelf. Do not rely on anyone else to perform these tasks. This is YOUR research, rely on yourself.

1. There are several buffers that need to be on hand at all times. These include:
   a. 50X TAE buffer, pH 8
   b. 1X TAE buffer, pH 8
   c. 10 mM PO_4 buffer, pH 7 (autoclaved)
   d. 50/50/50 nucleic acid extraction buffer, pH 8 (autoclaved)
   e. 50/50/50 nucleic acid extraction buffer, pH 9.5 (autoclaved)

2. There are several consumables that need to be on hand at all times. These include:
   a. 1000, 200, 50, and 10 μl pipet tips. (boxed and autoclaved)
   b. 2.0, 1.5, and 0.5 ml microcentrifuge tubes (autoclaved)
   c. Toothpicks (autoclaved)
   d. 10% (w/v) PVPP in water (autoclaved)
   e. Spin-filter tubes (autoclaved)
   f. 200 ml milk dilution bottles containing 45 ml 10 mM PO_4 buffer (autoclaved)
   g. Agarose gels (1%)
   h. 6X gel loading dye
   i. Filter funnels with 0.45 μm membranes (ethanol dipped and dried on blue bench paper).

3. Please designate a lab member to compile a list items (with the date, number needed, supplier, and catalog number) that need to be ordered and send them to me every Thursday so I can order them on Fridays. Do this BEFORE you run out. I will not have much sympathy for you if you realize you are in need of an item after you have exhausted your supply.

Respect for Other Researchers – Since you are not the only one using this laboratory, you MUST consider the needs of others if you are going to use the LEPR.

1. Keep the lab doors locked WHENEVER you are not in the lab.

2. PLEASE TAKE PHONE MESSAGES. These need to be detailed and clear. Please note:
   a. Caller's name
   b. Time of the call
   c. Return phone number

   Additionally, if it is a call for Dr. Sigler, please find out the reason for the call. Many different people try to reach him and it is important know the nature of the business before returning a phone call.

3. We go through cycles of use on our equipment, especially the thermalcycler and DGGE apparatus. Please sign up in advance if you plan to use these machines. Before you go through the set-up of PCR reactions or the pouring of DGGE gels, ALWAYS be sure to check the sign-up sheet to be certain that no one else plans to the machines. Also be cognizant of the timing of your experiments. Do not sign up for a three-hour PCR program if someone else is expecting to use the thermalcycler two-and-a-half hours later.