Work and safety procedures for working with pathogens / agents with a biological safety level of BSL-2

The work procedures are aimed at work involving agents with a moderate risk level to humans and the environment.

Microorganisms/agents defined at BSL-2 safety level are those that can cause illness in humans (e.g., salmonella, listeria, human cell lines, HBV, work with blood / bodily fluids, lentivirus vectors, and more).

The pathogen can enter the body in the following ways:

- Through the skin – as a result of puncture or injury from a sharp object, or penetration through a cut or wound
- Through mucous membranes – eyes, nose, mouth
- Through/by means of drops or aerosol

Work procedures

Work with pathogens must be carried out in a laboratory rated for a biological safety level BSL-2 or BSL-2+ according to the decision by the University's Safety Unit.

Work will be performed in a dedicated departmental lab. This lab can also be used as a general tissue culture room.

1. The tissue culture room designated for working with pathogens must be equipped with infrastructures (see Section 2 below) and approved by the biological safety supervisor.
2. **Essential equipment in the dedicated room:**
    - Biological fume hood (Class II)
    - Centrifuge and closed centrifuge test tubes
    - Incubator
    - Microscope (preferred)
    - Biological waste receptacles
    - Pipettes and tips with filters
    - Work equipment to be used in this room only

3. **Signs** on the entrance door must indicate:
    - Work with name of pathogen
    - Entrance restricted to authorized personnel only
4. **Prior to starting work:**
    - Wear a lab coat (disposable lab coat is preferable), **two pairs of gloves** and sleeves (to protect the area between the coat and the gloves).
    - Protective goggles
    - Closed shoes
    - Respiratory protection (mask)
    - Prepare a disinfectant solution
    - Prepare a bucket for "biohazard" waste with a pair of biohazard bags (one inside the other), and a biohazard bag inside the fume hood.
    - It is recommended that you avoid using sharp instruments. If this cannot be avoided, prepare a receptacle for disposal of "sharps."
5. It is preferred, and recommended, when working with cultures containing pathogens that you work with filtered flasks.
6. Use filtered pipettes and tips. Collect all of the used tips into a plastic bottle (a clean and empty medium bottle) and transfer the bottle with a little bit of water and not hermetically sealed to a waste bag.
7. Single use equipment that has been contaminated with pathogens should be thrown into a biological waste receptacle inside the biological fume hood. The biological receptacle found outside the fume hood is used for waste that is not contagious.
8. Keep the door to the cultures room closed at all times when working with pathogens.
9. Do not bring vectors or other pathogens into the cultures room any time you are working with a specific pathogen.
10. Do not leave solutions containing pathogens in the fume hood or the centrifuge without supervision.
11. Personal protective equipment, the disposable lab coat, gloves and sleeves should be put into a biohazard waste bag inside the room approved for working with BSL-2 pathogens.

12. Do not go from a BSL-2 lab into another lab wearing the clothes you used to work with a pathogen. You may leave the lab only wearing a clean lab coat and clean gloves.
13. Flasks should be placed in the incubator on a tray.
14. Please be sure to avoid squirting or creating spray.
15. After working in the fume hood, change your contaminated gloves for clean gloves. Do not touch equipment or surfaces outside the fume hood with contaminated gloves.
16. At the end of the work, close the biological waste bag, even if it is not full. The next day, the bag may be reopened until it is 2/3 full, before sending it to the autoclave.
17. A. To examine cells under a **microscope**, take the following steps:
    - Carefully close the flasks.
    - Bring the flasks to the microscope on a dedicated tray (with an absorbent pad).
    - If the microscope is located on another floor or in a different building, the samples must be housed in a sealed, unbreakable container.
    - At the end of the work, clean the surface of the microscope with 70% ethanol.
18. B. To examine cells under a **confocal microscope**, take the following steps:

    If the object being examined under the microscope has a liquid phase, with the possibility that the pathogen is located in the liquid medium, then:

    Before approaching the microscope, collect the medium (to keep it in the incubator, if necessary), rinse the cells carefully 3 or 4 times with PBS or a work medium, and replace the medium containing the pathogen with a fresh medium.

    All of the aforesaid is in addition to the provisions of paragraph 17A, that is: Storing the samples in an unbreakable sealed container if it must be moved from the lab, a tray with an absorbent pad, a "kit" for dealing with spills, etc.
19. The biohazard bags should be closed (not tightly, but loosely); you should add a little bit of water:

For a dry bag, add up to 250 ml. The exterior bag should also be closed (loosely) and sent to the autoclave for purification.

20. Liquid waste with a volume of less than 500 ml. can be disinfected inside the fume hood. Fill the bottle with a tenth of the volume of the liquid waste of 10% sodium hypochlorite. Wait one hour and then you may pour the disinfected liquid into the sink in the room, while running the faucet.
21. To use the **pumping system** inside the fume hood use a double trap that sits inside the spill containment pallet. Do not use glass pipettes. For pumping we recommend using a disposable sterile tip of 1 ml. connected to the pumping tube, through a 5 ml. plastic pipette cut to a length of 10 cm. You can also use a single use Pasteur pipette. The collection bottle should contain a 1% sodium hypochlorite solution at a ratio of 1:10 of the bottle's volume.
22. When pumping is completed, rinse the rubber tube with a clean hypochlorite solution.
23. Centrifugation should be performed on closed test tubes to prevent splashing. The centrifuge must be in a room that is rated BSL-2.
24. When the work has been completed, clean the fume hood and the incubator handle, as well as all of the equipment you used, with 70% ethanol.
25. If you wish to use an **ultracentrifuge** located in another room, you must follow these instructions:
    - Display a sign indicating work with the name of the pathogen, duration of the work, your name and your cell phone number;
    - Fill the plastic test tubes not more than 75% of the volume of the tube;
    - Balance the test tubes when weighing inside the fume hood in the BSL-2 room;
    - Carefully place the test tubes inside the metal container, taking care to avoid splashing;
    - Close the metal container with the help of an appropriate metal plug;
    - Clean the outside of the container with 70% ethanol, switch to clean gloves and a clean lab coat (leave the contaminated personal protective gear in the BSL-2 room);
    - Bring the closed test tubes to the department ultracentrifuge in a stand;

- At the end of centrifugation, disinfect the rotor with 70% ethanol, even if you did not notice any spills;
- The test tubes should be opened inside the fume hood in the BSL-2 room;
- Clean the centrifuge containers with 70% ethanol.

26. When work is over, after removing your gloves, wash hands with soap and water.

Decontamination

Standard decontamination methods use sodium hypochlorite found in the University's chemicals storeroom as a 10% solution.

00049661	**Sodium Hypochlorite solution 10%**	**LT**	**1 LT**	**Bio-lab**	**19330201**	**Pure**	**10.57**

- Keep a bottle containing a fresh 0.6% sodium hypochlorite solution (diluted 1:16 of the original) in the fume hood – it can be kept for a week.
- Prepare a spray bottle containing 70% ethanol
- Prepare a face mask in the BSL-2 laboratory

Decontaminating liquid waste

Liquid waste, such as a medium and samples containing pathogens, must be decontaminated with sodium hypochlorite until the final concentration in the bottle of waste is 1% for one hour.

Decontaminating a small spill

Cover the spill with absorbent paper/paper towels, and carefully pour onto the paper a 0.6% sodium hypochlorite solution. Collect the paper into a biohazard bag. Repeat the procedure a second time.

Decontaminating a large spill

Wear a face mask to avoid spray during the decontamination process. Cover the spill with absorbent paper and carefully pour onto the paper a 0.6% sodium

hypochlorite solution. Collect the paper into a biohazard bag. Repeat the procedure a second time.

Decontaminating drops of spray

Cover the area with absorbent paper/paper towels, and carefully pour onto the paper a 0.6% sodium hypochlorite solution or 70% ethanol. Collect the paper into a biohazard bag.

Decontaminating solid waste

Used pipettes should be soaked for 30 minutes in a dedicated tank with 0.6% sodium hypochlorite solution. **The volume of solution in the tank must be sufficient to cover the edge of the pipette.** Afterwards, gently shake and transfer to a biohazard bag to remove the biological waste.

Dishes, flasks, etc. should be transferred to a biohazard bag (double) and sent to the autoclave.

Recently, the University's storeroom has received a disinfectant called "Virusolve." The disinfectant comes in a spray bottle, in a container (5 liters) that is ready to use and as a container (5 liters) of concentrate:

Item no.	Description	Measurement unit	Quantity	Manufacturer	Manufacturer Cat. No.	Clean	Price (NIS)
10008317	Virusolve +trigger spray	Each	750 ml.	Medistock	J30TP-1		83.33
10008316	Virusolve + RTU	Each	5 L.	Medistock	J30C		192.29
10008313	Virusolve + concentrate	Each	5 L.	Medistock	J29C		384.59

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