ב"ה יום שלישי כ"ח אב תש"ף 18 August 2020

Procedure for working with viruses, blood and tissues of human origin – August 2020

The following document will present the procedure for working with pathogens and human samples, and below they both will be refer as 'pathogen'.

The defined safety level (Biosafety level, BSL) is 2+, ie, work in safety structure of level 2, while adopting some of the work procedures of safety level 3.

Working with a pathogen that is genetically engineered to produce an oncogene or toxin requires special approval from the Committee for Pathogens.

All the instructions below, add to the existing work practices in biological laboratories in general, and in safety level 2 laboratories in particular.

New employees / students should work under supervision at the beginning of their work and will receive clear instructions regarding the nature of the work.

Personal protection

- Work with regular personal protection, ie, lab coat, gloves, protective glasses and closed shoes.
- In addition, work at level 2+ requires the use of double gloves and coverage of the space between the sleeve and the glove (sleeves or long gloves).
- Separate lab coats should be assigned to work with the pathogens, and care should be taken to remove the lab coats upon completion of the work.
- Do not work with exposed wounds.
- Any work involving exposure to blood, blood products, body fluids or tissues of human origin, requires vaccination against Hepatitis B, before starting work.

Equipment

- Work with the pathogen will only be performed in a biological hood. Work outside the hood is only permitted when the pathogen is neutralized or fixated.
- It is recommended to specify a separate hood for work with the pathogen, but it is also possible to work in a common hood for work that is not related to the

pathogen. In this situation, the hood should be thoroughly disinfected at the end of the work with the pathogen.

- It is advisable to dedicate specific pipettors for work with the pathogen. Pipettors can be shared with other procedures provided that they are thoroughly cleaned after working with the pathogen (rinsing with 70% ethanol), and provided that these pipettors are used exclusively for work in the biological hood.
- Do not use a suction device - do not suction by public air pipes or a local pump. All pipetting will be performed by a mechanical pipettor. Use of a suction device will only be possible if a HEPA filter is installed on the suction device.
- As much as possible, disposable laboratory equipment (pipettes, test tubes, etc.) should be used to reduce risks.
- Do not grow the pathogen in plates but only in flasks, and preferably in flasks with a filter. Growing pathogen in plates will only be permitted after a risk assessment has been performed by an authorized person.
- Do not use sharp tools (needles, scalpel, etc.) when working with a pathogen. Use of sharp equipment is permitted only after a risk assessment by an authorized person and provided that the required experiment cannot be performed without the use of the sharp device.
- Use of an incubator - preferably a separate incubator. Using a common incubator is possible under these conditions:

Plates with pathogens on a separate shelf (preferably bottom)

Clear marking of the incubator as a "biological hazard"

Anyone working with the incubator will be notified of the existence and location of the pathogens.

Work Methods

- Do not transfer fluid containing pathogen by pouring from vessel to vessel. The liquid should be transferred by pipetting only.
- While shifting from working with a pathogen to working / using equipment that is also used for work without pathogens, remove the top gloves (inside the hood) and throw them in the container inside the hood.

Tubes / boxes / freezers / refrigerators / incubators and any other equipment containing the pathogen must be clearly marked.

- Avoid growing unnecessary large volumes of pathogen and in any case do not load a large volume of liquid in a single vessel.
- Before removing the flask from the incubator, be sure to close the cap.
- In a protocol that involves the formation of viruses by plasmid transfection, the flask can be transferred to the BSL2+ incubator, without the limitations of safety level 2+, and henceforth the flask will be considered to contain a pathogen.
- A decision on the number of cell passages required after viral infection, in order to move to work at biosafety level 2, will be made after a risk assessment by a qualified person. The risk assessment will address the survival of the virus in culture, the likelihood of recurrence, the presence of episodes, etc. (e.g., after lentivirus infection, three passages must occur before returning to being considered a safety-level cell line 2). Using those cells in experiment performed on public device, require permission from the device responsible person.
- At the end of the work with the pathogen, the hood must be disinfected with 70% ethanol or bleach (fresh solution diluted 1:10).
- Wash hands with soap and water after work.
- A careful record must be kept, in a special notebook: of the sources of blood and body fluids arriving at the laboratory, the names of the employees who treated them, the date, manner of use and place of work.
- In any case of malfunction / effluent, the following factors must be reported:

Laboratory Head / / Laboratory Director

Security Center: 03-531-7777 Tzfat: 072-264-4910

Garbage treatment: All contaminated material remains inside the hood!

The rule is: Removal of a pathogen substance outside the hood will be carried out only after either the pathogen has been completely neutralized, or it is in a closed vessel which is transferred for autoclaving.

Here are some options for treating waste in a hood, and any lab can choose the convenient option for them.

- Insert a high container (which can also be closed when contains pipettes), and dispose of all waste in it: pipettes, tips, gloves, plates, liquids (aerosol should be avoided as much as possible), etc. At the end of the work the container will be

closed with a lid, inside the hood, including closing the panel, and took out when it is completely closed.

- Insert a medium container, with a bag inside. All the disposable equipment is thrown into it. At the end of work the bag will be closed inside the hood and took out to a biological container for sterilization. Do not use this way to collect contaminated liquids as there is a chance that the liquid will accidentally spill to the hood through the folds of the bag. Do not forget to add 100 ml of water to the bag before tying it.
- Similar to the previous option, the bag can be hanged on a dedicated bag holder.
- Use a small liquid collection tool (medium bottle, for example). At the end of the work the vessel will be closed inside the hood and thrown in a biological container for sterilization.
- Similar to the previous option, but you can add (in the beginning of the procedure or before removing from the hood) bleach to a final concentration of 0.5%, wait about twenty minutes and then pour the liquid into the sewer. Note: In the nano building, it is forbidden to pour any chemical waste into the sink.

During sterilization in the autoclave, the Biohazard program (a program that includes sterilization of the emitted air) must be used. The duration of the program should be 40 '121oC or 30' 134oC.

Using a centrifuge:

Centrifuge with a fixed angle: Opening and closing of the rotor will be done inside a biological hood.

Centrifuge with variable angle: Cups with a lid should be used. Opening and closing of the cups will be done inside the hood only.

Do not fill the tubes to the end. After centrifugation, make sure that no liquid is spilled from the tubes inside the rotor and centrifuge, and disinfect with a detergent or 70% ethanol if any spilled occurred.

Centrifugation in a centrifuge that does not belong to the parent laboratory: Coordinate with the person in charge of the centrifuge, and thoroughly disinfect the rotor and the walls of the centrifuge after use.

Airborne-infecting pathogen: Two of the following three barriers must be used: cupped caps, rotor lid, and a rubber gasket around the capsule lid. If any of these demands

are missing - all people present in the room are required to wear a respiratory during the centrifugation.

Observation under a microscope:

It is advisable to wrap the cling film / plate in plastic before placing it on the microscope. Alternatively, if it is a pathogen dedicated microscope, the work surface should be disinfected after use.

Observing the pathogen under a microscope that is not part of the parent laboratory equipment requires the approval of the person in charge of the microscope.

Mobility of tools containing pathogen between rooms in the university:

When transferring pathogen-containing vessels within the university, the vessels should be transported within a tightly closed secondary vessel, made of non-fragile material. The gloves should be replaced after placing the tube/flask with the pathogen in the vessel, so as not to contaminate the vessel from the outside. On the external vessel there will be a clear marking of biological risk. The vessel should be disinfected with ethanol after use.

Receipt and delivery of pathogens outside the university:

Pathogens must not be introduced and / or shipped from the university without the approval of a safety officer.

The pathogen will be transported in three packages, ie, 1) a test tube, inside 2) a tightly closed vessel made of non-fragile material 3) an outer closed package (cardboard or styrofoam) with a "biological hazard" marking.