Name of Appendix: **Instructions for disinfection and sterilization**

**A. Goal:**

Select disinfection and sterilization methods, and frequency of their use.

**B. Disinfection**

Ensure that there is a sufficient amount of all the disinfecting supplies needed before working with a biohazard source.

1. Selecting disinfectants
   1. The disinfectant must be appropriate for the organism being disinfected, based on information from the literature. General data are specified in Table 1 in Appendix A.
   2. To disinfect surfaces from agents for which special disinfectants have not been defined, use hypochlorite solutions, an iodine solution or formaldehyde (their effectiveness is described in the aforementioned table).
2. The following work surfaces must be disinfected:
   1. At the start of any work
   2. Before each break
   3. Before leaving the laboratory area
   4. At the end of the work day
   5. Before calling a technician or any other employee
3. Instruments will be disinfected in the following cases:
   1. When work with the instrument has been completed
   2. When you suspect an accumulation of organisms that cause infectious diseases
   3. Before removing the instrument from the lab
   4. Before calling a technician
4. Disinfection conditions
   1. Disinfection conditions are derived from the data contained in Table 1 in Appendix A. The duration of disinfection should be at least as noted in the table.
   2. **Personal disinfection**
      1. **Iodine solution of 500 ppm** is recommended for personal disinfection. It is effective against a broad range of bacteria and viruses.
      2. **Ethanol or isopropyl** at a concentration of 70%-85% is effective for bacteria and viruses with an outer fatty membrane.
   3. **General disinfection**
      1. Although **iodine solutions** (Iodophor or Wescodine) are not effective against bacterial spores, they are recommended for general use at a concentration of **75-1600 ppm**.
      2. **Formaldehyde**[[1]](#footnote-1)\* – the action of this disinfectant is based on methylation of the amino acids. It is effective for destroying spores. The effectiveness of the substances is reduced at temperatures lower than 20°. Disinfection takes 8-12 hours. Afterwards, several hours to a day are needed for traces of the disinfectant to evaporate.
         1. **Formaldehyde** gas is created through the depolymerization by heat of paraformaldehyde – this is recommended for disinfecting spaces.
         2. An aqueous solution of formaldehyde with a final concentration of 50% active material should be used to disinfect surfaces.
         3. The use of formaldehyde vapors produced by heating an aqueous solution of formaldehyde (37%-40% formalin) requires a longer contact time than formaldehyde gas. 10 ml. of solution are needed for each cu.m. of space. Make sure there are no sources of water or moisture, the temperature is at least 20°C and a minimum humidity of 70%.
         4. Use personal protective equipment when disinfecting with formaldehyde.
   4. Glutaraldehyde is effective for disinfecting surfaces from viruses. Use an active ingredient concentration of 2%-3% in a solution of 0.3% sodium bicarbonate with a pH level of 7-8. The solution remains active for 14 days. Minimal wait time for disinfecting with a single active ingredient is 30 minutes. For sterilization, soaking for at least 10 hours is required.
      1. When working with glutaraldehyde, you must wear personal protective equipment.
   5. Mixtures of several disinfectants are more effective relative to a single substance (for example, with available formaldehyde or chlorine).
5. Disinfection using UV radiation
   1. Use a low-pressure mercury vapor lamp where, according to the manufacturer’s specifications, 95% of its radiation is within the wavelength range of 253-257µm.
   2. Check the radiation strength of the germicidal lamps once a month, and replace them when the strength is lower than 75% of what is noted.
   3. Clean the dust from the lamps every other week. If there a great deal of dust accumulation, clean the lamp more frequently.
   4. Do not remain in the room when the UV lamp is operating without the following protection:

* When the UV radiation source is weak, use goggles with regular glass lenses. The goggles must be closed, with side protection.
* Other protection for a weak UV source – a glass or Perspex screen, 1-2 mm. thick.
  1. Do not operate the UV source without warning those who are nearby.
  2. When the UV lamp is in use, the following signs must be set up:
* When operating a small UV source, hang a sign at the entrance to the laboratory and on the device: “UV CAUTION”, with a picture of protective goggles, made of PVC.
* In rooms where UV radiation is being used on a large space, attach to the UV lamp switch a warning light as well as a sign on the entrance, “UV CAUTION”, with a picture of protective goggles.
  1. In rooms where UV radiation is being used on a large area, operation of the radiation source must be controlled from outside.

**C. Sterilization**

1. Sterilization using an autoclave
   1. Conditions for sterilization in an autoclave are as follows:

The duration of sterilization depends on the type of agent, its quantity and the temperature achieved (and also depends on the pressure and air exchange). The minimum temperature required for sterilization is 121°C at a pressure of 15 psig, for a minimum duration of 20 minutes. When there is a vacuum pre-cycle, sterilization can be at 132°C for 10 minutes.

The duration of sterilization of the agent being treated depends on its nature. When there is no pre-cycle, sterilization times are as follows:

* Liquids, at 121°C, depending on the maximum quantity in one vessel, calculated at one hour for every 4 liters.
* Animals, their droppings and pads – 121°C for 8 hours, except for small animals with abdominal incisions – for 2 hours.
* Transgenic plants - 121°C for 1 hour.
* Biological waste - 121°C for 1 hour.
  1. Each bag placed into the autoclave shall have a physical marker attached. Once a week an inspection must be conducted using a Bacillus stearothermophilus biological marker.
  2. The autoclave should be operated only by employees who are trained to do so. Workers should undergo training, or a refresher course, once a year as described in Appendix C.
  3. Specific operating instructions for the autoclave sterilization process should be stated clearly and concisely, and must be available at all times. These instructions should be written in addition to the general instructions (Section 7.5) and in accordance with the manufacturer’s requirements.
  4. General instructions for using the autoclave:
     1. Release all of the covers.
     2. Fill the bags to at most 2/3 full.
     3. Do not tie the bag tightly. Ensure that vapor can escape as the pressure rises.
     4. Make sure there is liquid in all of the containers.
     5. Attach a chemical marker to each bag and container.
     6. Containers with liquids should be filled up to 2/3 full.
     7. Sharp objects should be placed in the autoclave in hard and durable containers.
     8. Ensure that vapors can flow freely around the containers.
     9. At each operation check the pressure and temperature data during sterilization, and its actual duration.
     10. Operate according to the device’s instructions.
     11. Use heat-proof gloves and protective goggles.
     12. Open the autoclave only after it reaches atmospheric pressure and a temperature of 60°C.
     13. Open the door gradually.
     14. Do not stand in front of the autoclave opening when unlocking the door.
     15. Allow the contents to cool for 20 minutes.
     16. Tighten the covers and fasteners of all containers.
     17. In the event that the sterilization cycle was not completed as required (due to a power outage, malfunction, etc.), another sterilization cycle should be performed.
     18. Once a week, or after something spills inside, the autoclave should be thoroughly rinsed.
  5. In addition to these instructions, biological waste must be treated and sterilized in accordance with Directive 07-329.
  6. During a sterilization cycle in the autoclave, do not mix biological waste with clean instruments and materials.
  7. **Periodic inspection** of the autoclaves must be performed according to manufacturer’s instructions, and at least every 14 months for a steam-producing autoclave, and every 26 months for an autoclave with steam produced externally. The inspection should be performed by a tester who is certified by the Ministry of Labor and Social Affairs.
  8. **Recording and saving autoclave sterilization data**
     1. The following parameters must be recorded in a table: Date, source of the material, duration of sterilization, temperatures and pressure during sterilization, inspection of marker and special remarks. See Appendix B for an example. The data shall be recorded separately for each autoclave. Additionally, all periodic inspections, malfunctions and repairs to the device should be noted on a page attached to the table.
     2. The completed tables for each autoclave must be saved for at least three years.

1. **Sterilization using glutaraldehyde**
   1. See Section 5.4.
2. **Selecting indicators**
   1. Use Bacillus stearothermophilus to sterilize with steam at least once a week.
   2. Use Bacillus subtilis subsp. Globigii to sterilize with dry heat or ethylene oxide.

**Appendix A – Table 1 – Common Disinfectants: Dilutions for use, characteristics and possible applications**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Substance** | **Dilution for use (L/gm)** | **Contact time (min.)** | | **Disinfection** | | | | **Primary characteristics** | | | | | | | | **Possible uses** | | | |
|  |  | **Viruses with fatty membrane** | **Broad spectrum** | **Bacteria in vegetative state** | **Viruses with fatty membrane** | **Viruses without fatty membrane** | **Bacterial spores** | **Shelf life more than one week** | **Corrosive** | **Sediment** | **Destroyed by organic substances** | **Damage to skin** | **Eye irritant** | **Respiratory irritant** | **Toxic** | **Work surfaces** | **Contaminated glass containers** | **Disinfecting equipment surfaces** | **Disinfecting liquid waste** |
| Warm, dry oven 160-180° |  | 240-180 | | + | + | + | + |  |  |  |  |  |  |  |  |  | ± |  |  |
| UV radiation (253.7 n) | Cm2/W 40µ | 30-10 | | + |  | ± |  |  |  |  | [[2]](#footnote-2)\*\*\* | + | + |  |  |  |  |  |  |
| Quaternary ammonium compounds | 20-1 | 10 | NE | + | + |  |  | + |  |  | + | + | + |  | + | + | + | + |  |
| Phenol compounds | 50-10 | 10 | NE | + | + | ± |  | + | + | + |  | + | + |  | + | + | + | + |  |
| Hypochlorite[[3]](#footnote-3)\* | 10-5 | 10 | 30 | + | + | + | + |  | + | + | + | + | + | + | + | + | + | + | + |
| Iodophorm 0.47%\* | 16-0.075 | 10 | 30 | + | + | + | + | + | + | + | + | + | + |  | + | + | + | + |  |
| Ethanol or isopropyl | 850-700 | 10 | NE | + | + | ± |  | + |  |  |  |  | + |  | + | + | + | + |  |
| Formaldehyde solution | 80-2 | 10 | 30 | + | + | + | + | + |  | + |  | + | + | + | + | + | + | + |  |
| Glutaraldehyde | 20 | 10 | 30 | + | + | + | + | + |  | + |  | + | + |  | + | + | + | + |  |
| Ethylene oxide 35-60° | 0.8-0.4 | 240-105 | | + | + | + | + |  | + |  |  | + | + | + |  |  | ± | ± |  |

According to: WHO Laboratory Biosafety Manual and Laboratory Safety, Fleming et al., Eds.

**Appendix B – Sterilization cycle data**

Faculty Room Autoclave

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Date** | **Source of material** | **Type of material** | **Sterilization time** | **Temperature** | **Pressure** | **Marker** | **Remarks** |
|  |  |  |  |  |  |  |  |
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**Name of person in charge Telephone Signature**

**Appendix C – Training for sterilization**

All training will include the following topics:

1. Familiarity with all the functions of the device.
2. Safely operating and using personal protective equipment.
3. Loading and unloading the device.
4. Actions in case of a malfunction.
5. Ensuring sterilization effectiveness.
6. Pressure, temperature and sterilization time needed.
7. Recording.
8. Familiarity with safety instructions.
9. Practical experience.

1. \* This substance is included in the list of substances that are proven carcinogens in animals. [↑](#footnote-ref-1)
2. \*\*\* Does not penetrate through soil and other materials [↑](#footnote-ref-2)
3. \* Available Halogens

   NE Not effective

   ± Results vary according to virus [↑](#footnote-ref-3)