Work instruction for disinfecting laboratories

Introduction

Disinfection is the chemical or physical process whereby the number of microorganisms on a surface is reduced to an acceptable level. This technique is used in laboratories and rooms in which work with genetically modified organisms (risk to the environment and people), pathogenic microorganisms (risk to people and the environment), laboratory animals (risk of contamination for people and animals), and human or animal material (risk to people and the environment) is carried out.

In the Netherlands, chemical substances for disinfection (disinfectants) must be approved by the Board for the Authorization of Plant Protection Products and Biocides http://ctgb.nl/) as they need to be tested to verify sufficient efficacy for their intended purpose and that they do not pose a risk to people and/or the environment. Disinfectants are not all equally effective on different types of microorganisms. Table 1 outlines a wide variety of disinfectants and the groups of microorganisms that they are effective on/suitable for. http://ctgb.nl/ also contains up-to-date information on all types of disinfectant.

This instruction outlines how disinfection can best be carried out during regular work and work with laboratory animals, after spillages, and after incidents and how disinfection of incubators and laboratory animal rooms can be carried out.

General information:

- The conditions for efficient use of disinfectants are appropriate concentration and contact time and suitability for the application (Table 1).
- A disinfectant used on a soiled surface is less effective. As a general guideline, a surface should be cleaned with tissues and water/soap first, and then disinfected. The potentially contaminated tissues must be disposed of as biological waste (blue WIVA container) or GMO waste (yellow WIVA container).
- Nitrile gloves should always be worn while disinfecting, in view of the irritant nature of disinfectants on the skin.
- If a material is suspected as being contaminated with potentially pathogenic microorganisms, consult Table 1 for the most suitable disinfectants.

Routine work

Disinfect the workplace before commencing and after completing work

The workbench on which work with GMOs, laboratory animals, PMOs, human and animal tissue is carried out must be cleaned and disinfected with 70% ethanol before and after work, ensuring a minimum contact time of 30 seconds (apply ethanol to the workbench with tissues so that exposure is minimized).

Disinfecting glassware (or other reusable materials)

The appropriate disinfectant should be chosen according to the organism used in the glassware (or on other materials): Glassware (or other material) contaminated with:

- GMOs and PMOs: refer to Table 1 for the appropriate disinfectant and corresponding concentration and minimum contact time. Use the concentration and contact time indicated by the supplier. Ethanol and active chlorine solution are the most common.
- Human or animal material: refer to Table 1 for the appropriate disinfectant disinfectant and corresponding concentration and minimum contact time. Use the concentration and contact time indicated by the supplier. Ethanol, active chlorine solution or Virkon S are the most common.

Disinfecting waste containers, including biological waste containers

Before the biological (blue WIVA containers) and GMO (yellow WIVA containers) waste containers are handed over to Logistics employees, their exteriors must be disinfected with a suitable disinfectant.

Disinfecting liquid waste

- Waste collected over the course of a single day, e.g. in an Erlenmeyer flask, must be disinfected by adding a chlorine tablet (see table 1 for the required final concentration). The liquid and chlorine tablet

should be mixed thoroughly and left with the lid on for at least 30 minutes. The disinfected waste can then be disposed of down a sink.

- An alternative method is to have the CGSA autoclave one day's waste. In that case, the waste must be handed over to the CGSA in a double contained, shatterproof and sealed container that can be disinfected.
- Waste that is extracted, e.g. with a vacuum system, is often left standing for more than one day, in which case a different approach is required.
- To start with, add a chlorine tablet and a little water. Once the collecting flask is full, add another chlorine tablet, mix well until the chlorine tablet has dissolved, and leave it for at least 30 minutes. This is required as the efficacy of chlorine at room temperature is considerably reduced after one day and inactivated further as a result of interaction with organic material, which means that waste added on the third day is no longer disinfected sufficiently.
- Autoclaving this waste is not ideal as it means that no chlorine can be added to the collecting flask (autoclaving a chlorine solution is not permitted owing to formation of chlorine vapour in the autoclave). This would mean that waste would remain in the room for several days without being disinfected. This is an unwanted situation.
- As an alternative, liquid waste may be collected using the Serres gel system. With this system, the liquid is converted to a gel in a bag. The bag containing the gel can be deposited in the GMO waste container.

Disinfecting solid waste during viral work

- Cultivation flasks/well plates/petridishes, etc.: use a pipette to insert fresh active chlorine solution (see table 1 for the required final concentration) and allow to stand overnight in a biosafety cabinet or deposit the waste in a plastic bag, seal (button or tape), and deposit in the GMO waste container.
- Disposable pipettes/pipette tips: place a beaker containing fresh active chlorine solution (see table 1 for the required final concentration) in a biosafety cabinet. After use, place the disposable pipettes in the beaker and suck up the chlorine solution. Leave the pipettes in the beaker. Carefully drop the pipette tips into the chlorine solution and leave them there.
- Once the work is complete, remove the disposable pipettes from the beaker and deposit them in a plastic bag. Remove the pipette tips from the beaker using tweezers, deposit them in the plastic bag, and place them in a GMO waste container.
- Tissues: soak tissues in the appropriate disinfectant (to kill any viruses) and deposit them in the GMO waste container. If the tissues have not been soaked, they can be deposited in a plastic bag, the bag sealed (button or tape), and deposited in a GMO waste container.

Disinfecting biological safety cabinets and equipment

- Biological safety cabinets must be disinfected before and after work. Choose the appropriate disinfectant according to the microorganism (see Table 1). In addition, all equipment placed inside or outside the biosafety cabinet must also be disinfected.
- In most cases, disinfection with 70% ethanol, 30 seconds wet surface is sufficient (apply the ethanol using a spray bottle and tissue). If working with, e.g. adeno-associated virus or adenovirus, 70% ethanol is insufficient and another disinfectant must be used, e.g. chlorine or Virkon S (see Table 1).

Work with laboratory animals

- Surfaces: disinfect with 1% Virkon S, contact time 10 minutes. Then, rinse everything with water to remove Virkon S residue. To thoroughly disinfect large surfaces, the disinfectant is best applied with well-soaked micro-fibre cloths.
- Rooms: laboratory animal rooms should be disinfected by instructed employees (CPV) using hydrogen peroxide. This method is only suitable if the room can be sealed gas-tight. The room will be released once the concentration of hydrogen peroxide is lower than 1 ppm and the indicators have been read off.

Spillages (small quantities)

ML-I

- Cover the contaminated surface or material with a tissue and allow the liquid to soak in.
- Spray 70% ethanol onto the tissue.

- Pick up the tissue with tweezers and deposit the tissue in the GMO waste container.
- Disinfect the work surface or material again with 70% ethanol, minimum contact time 30 seconds.

ML-II

- Cover the contaminated surface or material with a tissue and allow the liquid to soak in.
- Spray a suitable disinfectant onto the tissues (see Table 1).
- Pick up the tissue with tweezers, move it to the biosafety cabinet in a plastic bag, button it tight, and deposit it in the GMO waste container.
- Disinfect the work surface or material again with the correct disinfectant and contact time.

Disinfecting incubators

- Switch off the incubator promptly so that it is at room temperature when disinfected; this prevents exposure to alcohol vapours.
- Wash the incubator (including shelves) on the inside using tissues soaked in H_2O + 1% SDS. Deposit the tissues in the GMO waste container.
- Wipe down the inside of the incubator with wet tissues to remove the SDS residue.
- Then, disinfect the entire incubator (including the shelves again) with 70% ethanol.
- All loose parts can be handed over to the CGSA for autoclaving after disinfection.

Incidents, accidents and spills

Disinfecting after breakage or spillage

ML-I

- Wear gloves and safety goggles (to protect against the chlorine solution)
- Prepare a fresh chlorine solution (see table 1 for the required final concentration)
- Ensure that the liquid is unable to spread further by pouring the fresh active chlorine solution around the spillage site
- Cover the spillage using tissues/absorption cloths soaked in active chlorine solution and leave them to work for 10 minutes
- Pick up the contaminated tissues/cloths with tweezers and deposit them in the GMO waste container; exercise caution in case of glass shards and other sharps
- Cover the surface and materials with tissues to absorb the liquid and repeat until all liquid has been absorbed. Deposit all waste (including glassware) in the GMO waste container
- Disinfect the spillage site with tissues soaked in fresh chlorine solution, working from the outside to the inside. Deposit the tissues in the GMO waste container
- Wash the spillage site with water and soap
- Report the incident.

ML-II

- Leave the room immediately and wait a minimum of 15 minutes until the aerosols have precipitated
- Wear a lab coat, gloves, and safety goggles when entering the room (to protect against the chlorine solution)
- An FFP2 mask should be worn, according to the type of microorganism
- Prepare a fresh chlorine solution (see table 1 for the required final concentration)
- Ensure that the liquid is unable to spread further by pouring a ring of fresh active chlorine solution around the spillage site
- Lay tissues/absorption cloths soaked in chlorine on the spillage site and leave to work for 10 minutes
- Pick up the contaminated tissues/cloths with tweezers and deposit them in the GMO waste container; exercise caution in case of glass shards and other sharps
- Cover the surface and materials with tissues to absorb the liquid and repeat until all liquid has been absorbed. Deposit all waste (including glassware) in the GMO waste container
- Disinfect the spillage site with tissues soaked in fresh chlorine solution, working from the outside to the inside. Deposit the tissues in a GMO waste container
- Wash the spillage site with water and soap
- Report the incident

Disinfection after a puncture, cut or splash incident

- Thoroughly press out the wound immediately to ensure that it bleeds well, then rinse with tap water under a fast-flowing tap or with physiological salt
- Disinfect the wound or skin with a skin disinfectant, such as 70% ethanol, chlorhexidine, or iodine tincture
- Rinse contaminated eyes for 10 minutes with the eye rinse available in the laboratory
- Rinse a contaminated mouth with water
- In the event of heavy bleeding, consult the company emergency response team by calling 1333 (UM)/1000 (MUMC+) or +31(0) 433875566 (mobile)
- If there has been blood contact with a needle or material, the person in question must be taking to the emergency department of the MUMC+
- Report the incident via the internal UM notification system or Iris (MUMC+)

Table 1: Overview of application disinfectans

Naam	Approval number	Bacteria	Enveloped virus	Non enveloped virus	Mould and yeast	Mycobacteria/ spores	Hand disinfectans	Contactti me	Manufacturer
Virkon S	13676 N	++	++	++	++	-	N/A	10 min	DuPont De Nemours
Suma tab D4 **	7321 N	++	++	++	++	+	N/A	5 min	Diversey B.V.
Stafilex Chlorine tablet	6706 N	++	++	++	++	+	N/A	5 min	Diversey B.V.
Halamid	8242 N	++	-	-	++	-	N/A	10 min	Axcentive SARL
Medicarine**	9451 N	++	++	++	++	+	N/A	5 min	Ecolab BV
Halamid d	8241 N	++	-	-	++	-	N/A	20 min	Axcentrive SARL
Alcohol 70% met 10% IPA	14193 N	++	+		++	-	N/A	30 sec	Added Pharma
Actisan 5l **	8960 N	++	++	++	++	+/-*	N/A	5 min	Veip BV
H_2O_2	14094 N	++	++	++	++	-	N/A	5-30 min	Brenntag
Sterillium med	13451 N	N/A	N/A	N/A	N/A	N/A	++	30 sec	Bode Chemie

* *only suitable for mycobacterium and not for spores*

** *for chlorine solutions: cleaning/releasing floors minimum 100 ppm solution (1 tablet/15 litres of water); cleaning up spills and then releasing the surface minimum 2000 ppm (1 tablet/750 ml water); disinfecting contaminated liquid minimum 2000 ppm (1 tablet/750 ml liquid)*

Abbreviations

BSC	Biological safety cabinet
CPV	Central Laboratory Animal Facility
Ctgb	Board for the Authorization of Plant Protection Products and Biocides
FFP	Filtering Facepiece Particle
GMO	Genetically Modified Organism
MO	Microorganism
ML-I/II	Microbiological Laboratory Class I/II
MUMC+	Maastricht University Medical Centre+
PMO	Pathogenic microorganism
Ppm	parts per million
UM	University Maastricht

References

- Decree and Regulation on Genetically Modified Organisms, Environmental Management 2013, IenW April 2014.
- Board for the Authorization of Plant Protection Products and Biocides: http://ctgb.nl/
- Website HSBM Maastricht

Further information

For further information, please contact the BSO.