Title: Decontamination using disinfectants
Purpose: To provide an overview of the requirements relating to decontamination using chemical disinfectants, as applied to work with genetically modified organisms (GMOs), microorganisms and samples/organisms containing these.

Research groups are responsible for the preparation of Safe Operating Procedures (SOPs) and Risk Assessments incorporating these requirements.

Overview:

Chemical disinfectants are used in physical containment (PC) facilities to inactivate or destroy microorganisms present on surfaces, or (under limited circumstances) in liquid waste.

The disinfection of surfaces is a standard requirement in PC facilities to reduce the number of microorganisms to acceptable levels, to manage risks associated with personnel exposure, and cross-contamination, and to prevent release of biological materials from containment. However, disinfection does not necessarily kill all microorganisms present, and not all disinfectants work well for all microorganisms or applications.

The purpose of this document is to provide an overview of the typical disinfectants and their recommended use where regulated biological materials such as GMOs, microorganisms and samples containing these are used or stored.

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When is Decontamination Required:

Decontamination is a *routine* procedure in areas where regulated biological materials are used, and in all PC facilities.

Table 1: Decontamination is required in the following circumstances:

Area or item for decontamination	When is decontamination required?
Work area (e.g., bench, biosafety cabinet)	• At the end of each work session • Before any maintenance or servicing occurs within the facility
Equipment used in direct contact with biological materials (e.g., homogeniser)	• After each use
Equipment used with viable biological materials, but not in direct contact (e.g., centrifuge, vortex, microscope, pipettors)	• After any leakage or contamination • Prior to servicing or repair • Prior to removal from PC2 facility
Furniture (e.g., lab stools)	• Prior to removal from facility
Frequently handled areas (e.g., door handles, fridge/freezer handles, light switches)	• At least fortnightly
Facility cleaning (e.g., shelves, outside of cupboards, sinks)	• Periodically, at least twice-yearly
Contaminated laboratory gowns	• Prior to sending to laundry services
Reusable PPE (e.g., safety glasses, visors)	• Between each use
Reusable labware	• After each use
Gaseous decontamination of biological safety cabinets	• Where required by regulations or as recommended for local hygiene procedures (e.g., some animal holding rooms) • Must only be undertaken by a trained professional.
Biological waste	• Prior to disposal, unless procedures in your local area allow disposal directly to biohazard/clinical waste bins for offsite incineration
Areas affected by a biological spill or release of biological material in the area	• After spill or release incident
Whole facility, including all surfaces, equipment, and furniture	• Prior to suspension or decertification of physical containment or biosecurity containment facilities

*NOTE: Sensitive equipment may be damaged by some disinfectants. Always refer to manufacturer's recommendations for decontamination.

When do I use disinfectants for decontamination?

Not all the decontamination activities described above will involve the use of chemical disinfectants. In specific cases, sterilisation in an autoclave may be a more effective or safer method of decontamination (e.g., for large volumes of liquid biological waste, decontamination of soil and for some equipment or reusable labware).

Chemical disinfectants are routinely used for surface decontamination of work areas, equipment*, and furniture. Where approved by the IBC, suitable disinfectants may also be used for the decontamination of liquid waste. You would not use disinfectants to decontaminate solid waste (e.g., agar plates, disposable gloves) before disposal.

Characteristics of Different Disinfectants:

Disinfectants vary in efficacy due to:

1. Effectiveness against different microorganisms
2. Inactivation by organic matter (including proteins, dust and dirt)
3. Inactivation under sub-optimal environmental conditions (e.g., temperature or pH)
4. Chemical compatibility with materials

When choosing a disinfectant for decontamination, you must consider the specific characteristics of:

- the disinfectant
- the microorganisms or biological material you are trying to deactivate
- the surfaces or materials you are trying to decontaminate

Table 2 gives an overview of the characteristics of commonly used disinfectants.

Table 2: Characteristics of commonly used disinfectants

Disinfectant category	CHLORINE	PEROXYGEN BIOCIDES *Characteristics vary widely. Below description is for Virkon S only.*	ALCOHOLS	QUARTERNARY AMMONIUM COMPOUNDS (QACs)	ALDEHYDES	SYNTHETIC PHENOLICS
Common examples	Sodium hypochlorite	Virkon S	Ethanol Isopropyl alcohol	F10SC, Phytoclean, Klercide-CR Biocide X, CaviCide, Trigene II	Ortho-phthalaldehyde, glutaraldehyde	Biogram
Mechanism of action	Denatures proteins	Denatures proteins and lipids	Denatures proteins; cause membrane damage.	Denatures proteins; binds phospholipids of cell membrane	Disrupts proteins; disrupts nucleic acids	Denatures proteins; disrupts cell wall
Characteristics	Corrodes metals, rubber and fabrics.	May damage some metals (e.g., iron, copper, brass, zinc, steel) and concrete. Short shelf-life.	Rapid evaporation. Leaves no residue. Can damage rubber and plastics.	Some have detergent properties and can be used to clean. Sub-lethal concentrations can lead to resistance in exposed microorganisms.	Slow acting. Only use in well-ventilated areas. Noncorrosive to metals, plastics and rubber. Some (e.g., formaldehyde) are highly toxic & carcinogenic.	Can leave residual film on surfaces, and damage rubber and plastic.
Precautions	Mucous membrane irritation. Toxic gas released if mixed with acids or ammonia or if autoclaved. Carcinogenic product released when mixed with formaldehyde.	Powdered form may cause mucous membrane irritation.	Flammable; may be irritating to injured skin.	Some are irritating to skin, eyes and respiratory tract.	Some are irritating to skin and mucous membranes.	May be toxic to animals, especially cats and pigs. Irritation to skin and eyes.
Factors affecting effectiveness	Affected by pH; rapidly inactivated by organic matter; inactivated by UV. Must be made fresh daily as diluted product has limited shelf-life.	Effective in presence of organic matter, hard water, soaps and detergents. Ineffective against HIV in blood or bodily fluids. Short shelf-life once diluted.	Inactivated by organic matter, rapid evaporation makes effective contact time difficult. Ineffective against HIV in blood or bodily fluids.	Inactivated by organic matter, hard water, soaps and anionic detergents, best at neutral or alkaline pH.	Affected by pH and temperature, inactivated by organic matter, hard water, soaps and detergents.	Effective in presence of organic matter, hard water, soaps and detergents.
Typical concentration and contact time	Surface disinfection = 1% available chlorine, 10 min Liquid disinfection = 1-5% available chlorine (depending on presence of organic matter), 60 min *See important note at bottom of page	1% Virkon S (10 g to 1 L water), 10 min contact time.	Ethanol – 70-80% v/v, 10 min contact time. Isopropanol – 60-70% v/v, 10 min contact time.	Per manufacturer's recommendations for different products.	Gluteraldehyde – 2% w/v, 10 min Ortho-phthalaldehyde – 0.55%, 5 min	Per manufacturer's recommendations for different products.
Typical uses	Surface & liquid disinfection. Preferred disinfectant against HIV and hepatitis viruses and for clean-up of blood and bodily fluids.	Surface disinfection only	Surface disinfection only. Good for general lab bench disinfection. May be used for swabbing biosafety cabinets depending on microorganisms present.	Surface disinfection only	Surface disinfection only. Ortho-phthalaldehyde is used for disinfection for certain types of medical instruments.	Surface disinfection only
EFFICACY AGAINST DIFFERENT MICROORGANISM TYPES						
Gram positive bacteria	Generally effective [a]	Generally effective [a]	Effective	Effective	Effective	Effective
Gram negative bacteria	Generally effective [a]	Generally effective [a]	Effective	Generally effective [a]	Effective	Effective
Endospores	Ineffective	Limited efficacy [a]	Ineffective	Limited efficacy [a]	Variable by product	Ineffective
Mycobacteria	Limited efficacy [b]	Ineffective	Generally effective [c]	Ineffective	Effective	Effective
Fungi	Generally effective [a]	Limited efficacy [a]	Ineffective	Limited efficacy [a]	Effective	Generally effective [a]
Enveloped viruses	Effective	Effective	Effective	Effective	Effective	Effective
Non-enveloped viruses	Effective	Generally effective [c]	Ineffective	Ineffective	Generally effective [a]	Ineffective
Prions	Limited efficacy [b]	Ineffective	Ineffective	Ineffective	Ineffective	Variable by product

[a] Indicates some species variation occurs – check to ensure efficacy against the microorganisms you are targeting.

[b] Sodium hypochlorite requires high concentration of available chlorine and long wet contact time (> 60 minutes) to achieve effective level of decontamination.

[c] Ineffective in presence of bodily fluids (e.g., blood, sputum).

- *These tables are informative only – efficacy, required contact time, concentration and conditions can vary within microbiological groups, environmental conditions, or presence of organic matter. Refer to published literature.*
- **Care should be taken when diluting bleach to ensure correct final concentration of active chlorine is achieved. Note that the starting concentration of sodium hypochlorite varies between different bleach products – read your bottle label carefully! Chlorine dilution calculators are available online to assist – e.g.: https://www.publichealthontario.ca/en/health-topics/environmental-occupational-health/water-quality/chlorine-dilution-calculator*
- *Table adapted from The Centre for Food Security and Public Health, Iowa State University College of Veterinary Medicine with information sourced from Australian/New Zealand Standard 2243.3, DAFF Approved Broad Spectrum Disinfectants List, and the Centres for Disease Control and Prevention Guideline for Disinfection and Sterilisation in Healthcare Facilities.*

Pre-operational Checks:	
1	Ensure that you have reviewed any specific procedures relating to the decontamination procedure, and the safety data sheets (SDS) for chemical disinfectants to be used.
2	Ensure that the disinfectant is within the expiry date and at the correct concentration. If not, make fresh disinfectant in a clean bottle.
3	Disinfectants must be clearly labelled with the date of expiry, disinfectant concentration, and formulation.
4	Ensure that the area to be disinfect is free of any sharps or broken glass. Where these are present, use forceps or tongs to pick up the items and dispose in a sharps or broken glass container (as applicable) before cleanup.
5	Ensure that the area to be disinfected is generally clean and free of dirt, dust, or spilt media. As many disinfectants are inactivated by organic matter, you may need to clean the area before disinfection, or apply the disinfectant twice – wiping with disinfectant once to clean, then a second application for decontamination.

Hazard Management:

Disinfectants are effective and safe chemicals for decontamination when used properly. If unsafely managed, they can be hazardous to workers or cause damage to laboratory equipment or surfaces.

When selecting a disinfectant, consider the hazardous properties of the chemical. All staff and students who will work with the disinfectant should read the Safety Data Sheet (SDS) and be familiar with the safe intended use of the disinfectant.

Where possible and appropriate, a hazardous disinfectant should be substituted for a less harmful disinfectant (e.g., ortho-phthalaldehyde is a safer choice than glutaraldehyde). Alternative decontamination processes such as autoclaving or dry-heat sterilisation may also be considered.

Common hazards

- Alcohols are flammable. Keep away from heat sources, sparks and flames such as Bunsen burners and motorised equipment.
- Mixing disinfectants with other chemical substances can produce hazardous vapours. For example, toxic chlorine gas is rapidly released when sodium hypochlorite is mixed with acids or ammonia and can cause serious injury or death to exposed workers.
- Many disinfectants are irritants to the skin, eyes and mucous membranes. Some are toxic (e.g., phenol) or known carcinogens (e.g., formaldehyde). Ensure that you consult the SDS, follow safe operating procedures, work in a well ventilated area and wear all recommended PPE when handling disinfectants. Consider substituting for safer alternatives where possible and appropriate.
- Heating or autoclaving disinfectants, or applying disinfectants to hot surfaces can produce hazardous vapours. Do not autoclave materials that have residual disinfectants present. Ensure that you store disinfectants at the recommended temperature. Take care and consult the SDS before using disinfectants on hot surfaces.
- Prolonged or repeated exposure to some disinfectants can cause skin sensitisation and/or allergic contact dermatitis. Ensure that you consult the SDS, follow safe operating procedures, and wear all recommended PPE when handling disinfectants.

General precautions

- Keep all disinfectants in correctly labelled containers with the contents, concentration and expiry date clearly displayed.
- Do not mix disinfectants with other chemicals, disinfectants or cleaning products.
- Do not breath vapour or spray. Prepare and use disinfectants in a well-ventilated area.
- When diluting, only use clean water, and only at room temperature (unless otherwise specified in manufacturer's directions).
- Wear personal protective equipment as described in the SDS, risk assessment and/or SOP.
- Store disinfectants in a cool, dry area protected from sunlight.
- Wash hands with soap and water after use.
- Other than commercially supplied hand sanitisers, do not use chemical disinfectants to decontaminate your hands (e.g., do not spray hands with 70% ethanol).
- Always follow any specific instructions from the manufacturer and all precautions on the product packaging or the SDS.
- When using corrosive disinfectants (e.g., bleach) on metal surfaces, wipe the surface with clean water following disinfectant application to remove any corrosive residues.
- Follow all safety requirements when working in areas with electrical devices.

Legislation, Guidelines and Standards:

- Australian/New Zealand Standard 2243.3 Safety in Laboratories – Part 3: Microbiological Safety and Containment
- DAFF Broad Spectrum Disinfectants list : https://www.agriculture.gov.au/biosecurity-trade/import/arrival/arrangements/requirements/disinfectants
- OGTR Guidelines for the Transport, Storage and Disposal of GMOs
- Therapeutic Goods (Standard for Disinfectants and Sanitary Products) (TGO 104) Order 2019

Abbreviations and Definitions:

Cleaning: Removal of obvious dirt, dust and spilt materials using a detergent, or disinfectant with detergent properties.

Disinfection: The process of removing pathogenic microorganisms using chemical disinfectants. Disinfection does not necessarily kill all organisms, and is less effective than sterilisation.

Decontamination: A general term used to refer to chemical or physical processes for removal of microorganisms.

Genetically Modified Organism (GMO): In this document, GMO refers specifically to microbiological GMOs, or GMOs that may be inactivated by chemical disinfection.

- Any genetically modified (GM) microorganism, cell or viral vector.
- Any non-GM cell, or microorganism that contains genetically modified microorganisms or genetically modified material.

Microorganism: protozoa, fungi, archaea, bacteria, unicellular algae, virus, viroid, parasite, or prion.

Physical Containment (PC) facilities: Facilities used for the containment of regulated biological material or organisms (e.g., GMOs, microorganisms).

Sterilisation: Refers to any process that removes and kills or deactivates all microorganisms.

This guidance document is supplied to specify requirements under relevant legislation, guidelines and standards relating to the compliant handling of regulated biological materials including but not limited to GMOs, microorganisms and samples/organisms containing these.

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