

SV GUIDELINES

Decontamination / Spill cleaning

1. Decontamination

1.1. Warning

- Wear safety glasses, gloves and lab coat when handling chemical products or decontaminating solutions.
- Be aware that some decontaminating solutions may leave chemical residues that could be an impediment for the proper functioning of the instrument to be treated or could interfere with biochemical reactions or cell growth (typically in incubators).
- Use the products according to the dilution factors recommended by the manufacturer. If too much concentrated, some products can react with some floor composites (the floor becomes porous and absorbs dirt) or be corrosive for the material to be treated. If too much diluted, the product is not efficient and may elicit resistance mechanisms among microorganisms.

1.2. Products for the decontamination of surfaces (floors, benches, instruments)

The market provides a large choice of decontaminating products. The selection of a specific product will depend on the type (or on the population) of organisms one wants to get rid of, on toxicity, on material compatibility, on product stability, on temperature or on the time required for effective decontamination.

The chemicals efficient for microbiological decontamination belong to aldehydes (formaldehyde and glutaraldehyde), hypochlorite [Sodium hypochlorite (bleach)], peroxydes (hydrogen peroxide), quaternary ammoniums, alcohols, phenols, acids and alcalis. (see Appendix 1). The efficiency of those products will depend on the nature of the agents to be treated (see Appendix 2).

Among the products that are often used, one can find 70% Ethanol, Meliseptol rapid, Biocidal ZF, Mikrobac forte, Perform Q-plus, Biosanitizer, Lysetol, Trigen, or others.

Some of those products show limitations for treating non-enveloped microorganisms and are rarely efficient against spores.

▪ 70% Ethanol

60% to 95% alcohol solutions are common decontaminating solutions. Preferably use 70% EtOH or a mixture of 70 % EtOH with 5% isopropanol (100% EtOH is completely ineffective as a decontaminant). EtOH solutions are flammable: do not spray alcohol solutions towards an open flame or a hot surface. Protect your eyes and do not breathe aerosolized solutions.

▪ Biosanitizer

Activated hydrogen peroxide solution (< 2% H₂O₂ with anionic surfactant compounds), nontoxic (does not contain volatile organic compounds), non-corrosive and nonflammable. Exists in different formulation for floor, bench surface and technical devices. Especially good for decontaminating biosafety cabinet, hoods, laboratory devices.

Biosanitizer is active against non-enveloped viruses. Good cleaning agent for AAV.

▪ Biocidal ZF™

A mixture of 0.1% didecyldimethylammonium chloride and of 0.2% benzalkonium chloride. This quaternary ammonium-based compound does not leave any residual chemical activity and is therefore particularly recommended for decontaminating incubators, biosafety cabinets or sensitive instruments. However, it is a costly product to be used in floor or bench decontamination.

▪ Meliseptol rapid®

A Propan-1-ol-based solution (aldehyde-free and alkylamine-free) ideal for the decontamination of small work surfaces (incubators, benches), waste bags, tube-holders, etc. Not indicated for floor cleaning.

▪ Deconex SURFAX®, Hexaquart® plus, Melsitt®

Among others, those products are especially formulated for floor cleaning.

Note1: The above mentioned solutions are for cleaning or treating surfaces. They are often not suitable for treating liquids containing biological materials, because of the volumes that would be required to get the correct dilution factor. Therefore, consult the manufacturer's specifications for the correct use of those products.

Note 2: For treating biological liquids, other products might be more adequate. For example:

- **Bleach**
 - Bleach (also known as Javel or sodium hypochlorite) is a very efficient decontamination product. It is active against most of the microorganisms, but is completely inefficient in the treatment of spores. Bleach is also relatively corrosive, which reduces its use as a general decontamination product.
 - Bleach is highly efficient at a final concentration of 2%. Higher concentrations (up to 5%) are only necessary for the treatment of solutions containing high levels of amino acids (e.g. solutions containing albumin). Some publications claim that as little as 0.5% bleach is active with highly sensitive organisms.
 - Most bleach stock solutions available on the market are 14% concentrated. Dilute 4 to 7 x times (\approx 28 to 15 ml of 14% bleach for 100 ml of solution to be treated) to get the final active concentration.
 - **Bleach treated liquid waste must be eliminated as a chemical waste (OMoD 18 01 02).**
 - Never autoclave a solution containing bleach.
- **Sodium hydroxide**
 - Sodium hydroxide (NaOH) 0.1 M final (or 4% final) is recommended for the treatment of solutions where free nucleic acids or nanoparticles are of concern.
- **Virkon ®**
 - Virkon (60% Potassium peroxomonosulfate, 20% Sodium Dodecylbenzenesulphonate and 10% Sulfamic Acid) is a very effective virucide, bactericide and fungicide.
 - Virkon is particularly efficient against parvoviruses.
 - Ideal concentration is 1% final.
 - Virkon-treated solution can be eliminated down the drain (sink).
- **Vesphene ®:**
 - Vesphene is a product specially developed to inactivate Mycobacterium strains.
 - As Vesphene is highly toxic for the environment, its use will be restricted to laboratory areas where Mycobacterium strains are in culture.
 - Vesphene-treated solutions are eliminated as chemical waste.
- **Lysetol ®:**
 - Lysetol is a glutaraldehyde-based product used mainly for the decontamination of surgical instrument.
 - It is bactericide, virucide, fungicide and active against spores. It can also be used to treat cell culture waste.
 - Lysetol-treated solutions are eliminated as chemical waste.
- **Trigene™:**
 - Trigene contains n-alkyl dimethyl benzyl ammonium chloride (5%), didecyl dimethyl ammonium chloride (7.5%) and poly (hexamethylene) biguanide hydrochloride (0.1%).
 - It is bactericide, virucide, fungicide and efficient against spore. Used in veterinary hospital and animal accommodation. It has also be proven to be active against parasite.
 - Suitable for general disinfection of all surfaces (floors and walls), kennel areas, examination tables, surgery walls, food and water bowls and bedding.

All decontaminant products, once diluted, have a **short shelf-life** and **are unstable**. Some might be corrosive. Please, refer to the manufacturers' instructions of use before selecting a product.

Please consult the document [BIO_BSR_MB_Liquid_Biological_waste_\[E\]](#) for the elimination of P1 and P2 liquid biological waste.

Other decontamination products are available on the market. Select them accordingly to your needs and be always aware of potential toxicity and material compatibility.

2. Decontamination of laboratory ware.

Spoiled glassware or plastic ware has to be soaked in a decontaminating solution (e.g. Deconex 11, Gigasept AF, Lysetol, etc.) before being processed for cleaning.

As a basic rule, let soak at least overnight in the decontamination solution before washing. Cleaned glassware can be sterilized by dry-heat.

3. Cleaning biological spills

3.1. Warning

Always remember that spills can generate droplets and aerosols that can spread widely. Therefore, a spill, even small, can contaminate large surfaces without leaving evident trace (no visible liquid) of the contamination spread.

3.2. Spill kit

When growing regularly larger volumes (~ >100 ml) of cell or bacteria, it is recommended to have a spill kit available. A typical spill kit contains

- A supply of fluid-resistant disposable gloves (EN304-2).
- A tyvek suit or a disposable lab coat.
- Safety glasses
- Shoe or boot covers
- FFP2 respiratory masks must be available if there is a significant risks of aerosols.
- An appropriate disinfectant or decontaminant product. Virkon is a product of choice, since it is stable when stored as a powder; prepare aliquots of Virkon in 50 ml Falcon tube (typically 10 or 50 g of powder for 1, respectively 5 l of decontaminant; final concentration must be 1%).
- A large beaker or a bucket for dissolving the Virkon.
- Absorbing materials such as paper, pads or tissue towels.
- Autoclavable biohazard bags
- Dustpan and brush
- Forceps to pick up glass debris and a disposable box for the elimination of sharps.
- For large volume spills or when large surfaces must be covered beach towels might be handy.
- Laminated clean-up procedure card.
- Laminated “No entry – Spill cleaning” card

3.3. Managing the spill

3.3.1. Small spill (less than a few ml)

When working with small containers of biological materials at the bench or in the biosafety cabinet, disinfectant, paper towels, and a biohazardous waste receptacle should be readily available in order to clean and disinfect the area at the end of the procedure. Spills at the bench or inside a biosafety cabinet (BSC) are not likely to involve broken glass. In most cases, you should have everything you need to clean up a spill. The basic steps for cleaning up this kind of spill safely include:

A. Inside a biosafety cabinet

1. Stop your procedure and assess the scene. Determine what was spilled and where did the contamination go.
2. Assess your gloves and lab coat for contamination. If these items got contaminated, replace them before proceeding with cleanup.
3. Wet a paper towel or an absorbing pad with disinfectant and then carefully blot up the visible contamination. Discard towel as biohazardous waste.
4. Discard contaminated items that cannot be effectively surface-disinfected or decontaminated by autoclaving as biohazardous waste.
5. Apply disinfectant to all surfaces impacted by the spill; wait the prescribed contact time before removing disinfectant residues and resuming procedure.
6. If the spill enters the grille of the BSC, this will require cleaning and disinfection of the containment pan underneath the work surface. This procedure does not need to be done immediately but generally requires 2 people and some amount of cabinet disassembly.

B. Spill on the floor or outside a BSC

As above, assess the situation and check your equipment for potential decontamination. Then:

1. If required, remove the breached container. If breached container was glass, remove glass pieces using tongs or disposable broom/dust pan. Place glass in sharps container for disposal. If

container was not glass, place it in a biohazard bag for disposal or appropriate secondary container.

2. Treat, absorb and remove the spill contamination. Cover spill with disinfectant saturated towel and allow to treat spill for several minutes. Absorb and remove spill contamination. Place absorbed spill materials and associated wastes in biohazard bag. Some surfaces and spill materials will require more "cleaning". This is when you should use a small brush saturated with disinfectant to gently loosen and remove surface contamination.
3. Disinfect all impacted surfaces. Apply disinfectant to all surfaces impacted by the spill (including those in the "splash zone"); wait the prescribed contact time before removing disinfectant residues.
Use care to limit contact with contaminated surfaces when removing your Personal Protective Equipment (PPE)! Place all used spill response materials (including mechanical tools and disposable PPE) in the biohazard bag for treatment as biohazardous waste.

3.3.2. Large spill

Large spill are unlikely to happen in a biosafety cabinet. In most of the cases, they consist generally in large tissue culture vessels breaking on the floor, spreading liquids on large surfaces, projecting droplets and generating aerosols.

1. In the case of a large spill, stop immediately what you are doing and leave the room. Just before leaving the room, remove your personal protective equipment and leave it inside.
2. Wait at least 30 minutes to allow the aerosols to settle or to be removed by the ventilation exhaust system. In the meantime, alert the BSO in charge of the lab and/or the Biosafety coordinator. Restrict the entry to the contaminated zone (affix on the door a warning panel, see appendix 3).
3. Organize the cleaning. Prepare fresh decontamination solutions and assess how much material you might need to clean the floor. If a spill kit is available, use its content. After use, do not forget to refill the kit with the missing material.
4. Don the appropriate PPE and enter the lab. Progress towards the spill by laying on the floor absorbing paper pads or towels soaked in decontaminant. During progression, remove any debris and discard them a biohazardous waste.

Large spills must be reported in the incident manager program on <http://sv-safety.epfl.ch>

4. Versioning

20.01.2015	version 1.0	

Appendix 1

Categories of product used as decontaminant

Peroxygen compounds

Hydrogen peroxyde
Peracetic acid
Potassium peroxymonsulfate
Butanone-2 peroxyde

Halogen-releasing compounds

Sodium hypochlorite
Calcium hypochlorite
Di and Trichloroisocyanuric acid
Chloramine T
Chlorine dioxide

Phenolics

Chlorocresol
Vesphene
Biphenyl-2-ol
O.Benzyl
p.chlorophenol

Aldehydes

Formaldehyde
Glutaraldehyde
Glyoxal
Polymer of formaldehyde

Iodine

Iode
PVP-I

Tri-amine

(aminopropyl-N-dodecyl prepanediamine)

Quaternary ammonium compounds

Benzalkonium chloride (BAC)

Acids

Formic acid
Lactic acid
Benzoic acid
Salicylic acid
Glycolic acid

Alkalis

Sodium hydroxyde
Cyanamide
Ca(OH)₂
CaO
Ca, Mg Oxide

Alcohols

70% Ethanol
Isopropyl alcohol
Phenoxyethanol

Appendix 2 Biocidal activities of various chemical product categories

Type of disinfectant	Spectrum	Gram +	Gram -	L Virus	NL Virus	Fungi	Algae	Parasites	Spores	Advantages	Disadvantages
Phenols	+	x	x	x						Surface disinfectant	Possible skin irritation due to phenol residues
Quaternary ammoniums	+	x	(x)	x						Cleaning products	Contact sensitization (when long exposure time) Hard to degrade
Alcohols	++	x	x	x		x		(x)		Surface disinfectant	Pure alcohol (100%) does not work (needs water to permeate the membrane)
Hypochlorite	++	x	x	x	x	x	x		x	Very efficient	Corrosive
Hydrogen peroxide	++	x	x	x	(x)	x			x	Increased efficiency when mixed with quaternary ammonium solutions	
Glutaraldehyde	++ +	x	x	x	x	x	(x)	x	x	Active in presence of limited amounts of organic material	Extremely toxic
Formaldehyde	++ +	x	x	x	x	x	(x)	x	x	Excellent disinfectant	Carcinogen
Per-acetic acid	++ +	x	x	x	x	x	x	x	x	Sporocidal also at low temperatures, i.e. RT Efficient in the presence of organic substances	Corrodes steel, bronze, galvanized iron, copper. Unstable, especially when diluted.



SPILL CLEANING

