

Guidance on Decontamination

Guidance

1. Introduction

Sterilization using pressure and steam i.e. by autoclaving, is the most reliable means of decontamination and where possible should be used in preference to chemical disinfection.

However, this method is not applicable in all situations and chemical disinfection is often the only practical method of decontamination for large spaces, fixed items or large surface areas and for heat labile materials or equipment. Where time permits, heat-labile materials and equipment may be sterilized by gaseous chemicals such as formaldehyde or by ionizing radiation.

2. Definitions:

2.1 Sterilization

Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms. An item is either sterile or not and a sterilization process is one that kills all microorganisms, including spores. Sterilization can be accomplished by heat, gasses such as ozone, ethylene oxide, hydrogen peroxide and formaldehyde or by radiation (in industry). From an operational standpoint sterilization is defined as a process, after which the probability of a microorganism surviving on a treated item is less than one in one million (10^{-6}). This is sometimes referred to as the "sterility assurance level".

2.2 Disinfection

Disinfection is generally a less lethal process than sterilization. Disinfection is used to reduce the number of micro-organisms present to an acceptable level so that the item being disinfected is safe to handle. Disinfection should not be confused with sterilization, a process that renders an object free from all viable organisms.

2.3 Decontamination

Both disinfection and sterilization are methods of decontamination as this is the general term used to reduce microbial contamination to render an item "safe". Cleaning may also be regarded as a decontamination method as it too can remove micro-organisms from a soiled surface.

3. Chemical Decontamination (significant content taken, with permission, from the University of Edinburgh policy on Disinfectants –

<http://www.safety.ed.ac.uk/resources/Bio/Guidance/General/Disinfectants.shtm>.

3.5.7-3.5.9 is derived with modification from the Australian/NZ microbiological laboratory standard AS/NZS 2243)

The use of chemical disinfectants, normally in the form of proprietary products specifically for use in laboratories, is a widespread and important control technique in biological

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laboratories. Where micro-organisms and other potentially hazardous biological materials, such as clinical samples are handled, chemical disinfection should be routinely used to decontaminate surfaces and equipment, and prevent microbial growth in spent culture fluids etc.

Wherever possible the use of disinfectants should be consistent throughout a department rather than varying between laboratories, although different types of work may need particular types of disinfectant because of differences in the susceptibility of agents. If possible, the number of different disinfectants used in a laboratory should be reduced to a minimum to avoid mistakes in application.

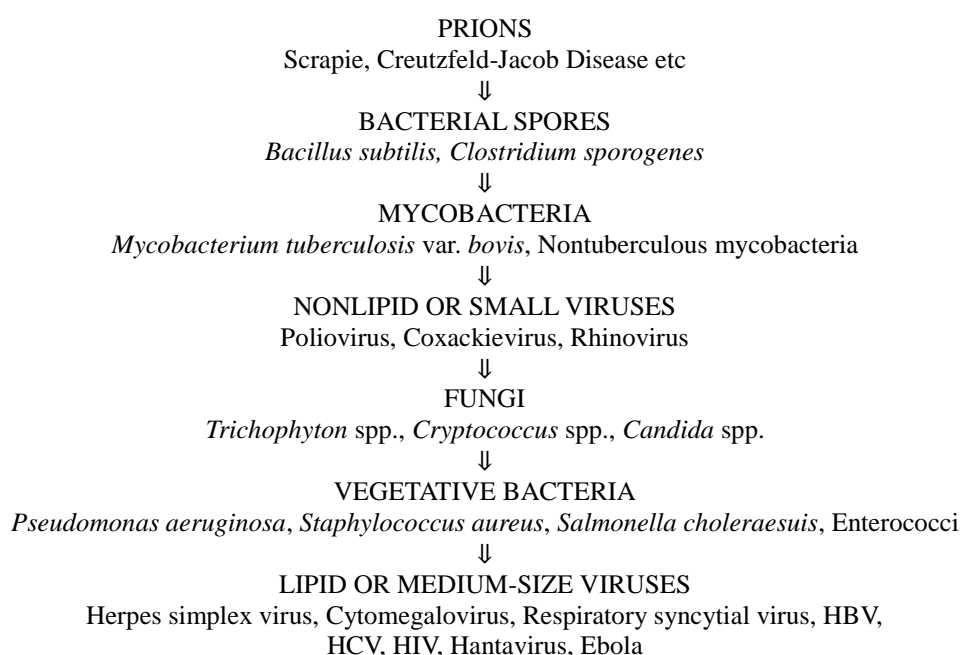
3.1 Factors Affecting the Choice of a Disinfectant

3.1.1 The Microorganism to be Inactivated

Microorganisms vary in their susceptibility to chemical disinfectants. Lipid-containing viruses and the vegetative forms of most bacteria are relatively susceptible. Acid-fast bacteria (*Mycobacterium* spp.), non-lipid-containing viruses and Fungi, are less susceptible while bacterial spores are resistant to the action of many chemical disinfectants. The agents of scrapie, Creutzfeldt-Jakob disease and other prions are extremely resistant to chemical disinfection.

To put it another way, disinfectants do not generally kill all the organisms with which they come into contact and do not disinfect all organisms or forms of an organism (e.g. spores) equally well.

Table 1. DESCENDING ORDER OF RESISTANCE TO CHEMICAL INACTIVATION (after BMBL 5th Edition)



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There are exceptions to this list, for example *Pseudomonas* species are sensitive to some disinfectants, but if they form biofilms protected cells can approach the resistance of bacterial spores.

3.1.2 Whether the disinfectant is going to be used in a clean or dirty situation:

The presence of other materials in or on items or surfaces to be disinfected can have an effect on the activity of the disinfectant. The presence of organic material such as blood or serum in cell culture medium, other chemical agents including soaps and detergents and the pH and temperature can all reduce the effectiveness of the disinfectant.

The concentration of disinfectant to be used is likely to vary depending on whether it is used in "dirty" or "clean" conditions, used routinely or in the event of accidents, etc.

3.1.3 The Nature of the Surfaces and Equipment to be Disinfected:

Some disinfectants will chemically attack items being disinfected. Stainless steel can be pitted by strong acids and (in some conditions) halogen active disinfectants. Concern over the use of active chlorine based disinfectants on rotors, rotor buckets and centrifuge bowls has some merit and alternative disinfectants should be employed. Plastics can be affected by disinfectants containing organic solvents while various metals may be attacked by strong acids or alkalis, halogen active substances or disinfectants containing electrolytes.

3.1.4 The Problems Associated with Using a Product:

Most disinfectants have toxic properties and some are also highly corrosive, causing damage if they come into contact with skin or eyes. Some disinfectants e.g. glutaraldehyde and hypochlorites may also have irritant properties and so cause respiratory problems if used in poorly ventilated areas. Some disinfectants may react with other chemicals causing the release of hazardous gases e.g. bleach and acids or bleach and cleaning agents containing ammonia.

Precautions should be given for handling both concentrated disinfectants and made up in-use dilutions. When handling concentrated disinfectants care should be taken to avoid splashing, and goggles or a full-face visor and gloves should be worn. A safety data sheet should be readily available. When selecting a disinfectant both the efficacy of the products available and the hazards associated with their use must be taken into account.

Some products have cleaning properties in addition to their disinfecting capacity. There can be significant benefits in the use of these types of products, for example when wiping down a bench as it will both be cleaned and disinfected at the same time. Furthermore, when using a disinfectant such as Virkon for disinfecting instruments or equipment that have been in contact with blood and tissue, the cleaning effects allow for better overall disinfection.

3.2 Making Up Working Dilutions

Disinfectants are usually provided in concentrated form and have to be diluted in water to the working strength for use. The manufacturers' instructions should be followed to ensure that the required concentrations are achieved. Over dilution will render the

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disinfectant ineffective. Once made up the disinfecting capacity of diluted products tends to deteriorate rapidly with time. Manufacturers should recommend how long a diluted solution can be stored and this be noted. Some products contain coloured indicators to show effective disinfecting capacity. If the disinfectant in use does not contain an indicator then a "use by" or expiry date should be clearly marked on the bottle when the solution is made up.

3.3 Contact Times

Chemical disinfectants need to be applied to the item they are disinfecting for sufficient time to enable the disinfection to be effective. Manufacturers should recommend contact times (in combination with concentrations) for various applications. Objects should be fully immersed and air pockets should not be present. Disinfectants should always be used in accordance with the manufacturer's instructions.

3.4 Discard Jars

A discard jar is a container of disinfectant into which contaminated items are placed to disinfect them prior to final disposal. Wherever possible, autoclaving of dry discard should replace the use of discard pots with disinfectant. If this is not possible then a suitable disinfectant should be chosen depending on the nature of the work and appropriate dilutions prepared. It is usually convenient for the discard jar, when freshly made up, to be approximately half full to allow for increased fluid levels when items are added. If liquid waste is to be added to the disinfectant then the initial concentration should be proportionately increased to ensure the final concentration after additional waste liquids are added does not drop below the effective disinfection concentration. If liquid waste is

aspirated into a container then the amount of concentrated disinfectant added should allow for dilution to the final volume of the full receptacle. Ensure vacuum lines are protected from potential contamination (See Appendix 1).

Items placed in discard jars must be completely submerged in the disinfectant. All surfaces of the item should come into contact with the disinfectant. Items must remain in the disinfectant for at least an hour. The disinfectant can then be washed down a sink (not a hand wash basin).

3.5 Types of Disinfectant

The following types of disinfectants are recommended (in no particular order) for use in laboratories. Key points to be taken into account when selecting the disinfectant are given. Manufacturers' instructions should be consulted for suitable concentration and contact times and further details of applications. Risk assessments should provide clear guidance on handling precautions needed, particularly when using concentrates.

3.5.1 Hypochlorites

Examples: Sodium hypochlorite, Chlorox, Presept

Hypochlorites are widely available as solutions of sodium hypochlorite or tablets of sodium dichloroisocyanurate (NaDCC). NaDCC can also come in a powdered form.

They have:-

- Wide range of bactericidal, virucidal, and fungicidal activity
- Limited activity against bacterial spores
- Rapid action

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- Inactivated by organic matter, particularly if used in low concentration
- Corrosive to some metals and may damage rubber
- Compatible with anionic and non-ionic detergents
- Incompatible with cationic detergents
- Irritant
- Chlorine gas released when mixed with strong acids
- Carcinogenic products produced when mixed with formaldehyde
- One of disinfectants of choice for use against HIV and hepatitis B viruses
- Not very effective against *Mycobacterium* spp.

Commonly used dilutions (expressed in parts per million available chlorine):

- 1,000 ppm for general wiping of equipment and benches
- 2,500 ppm for discard containers (if required)
- 10,000 ppm for spillages
- 20,000 ppm for work surfaces, including microbiological safety cabinets, where material containing prions/TSE agents has been handled (NaDCC not effective in this context). Appendix 2 abstracted from a WHO document gives recommended dilutions.

Care should be exercised if departments buy domestic bleach solutions for use in the laboratory. They commonly have additives such as perfumes and may change formulations without warning. Recently it was reported by a DSR that a batch of domestic chlorox contained only 2.5% hypochlorite i.e. 25,000 ppm

available chlorine where at least 5% hypochlorite had been expected.

3.5.2 Clear Soluble Phenolics

Examples: Hycolin, Stericol, Clearsol

- Wide range of bactericidal activity
- Good fungicidal activity
- No activity against spores
- Variable virucidal activity - usually poor against non-enveloped viruses
- Compatible with anionic and non-ionic detergents and metals
- Not readily inactivated by organic matter
- May be inactivated by rubber and some plastics
- Contain detergents
- Concentrates are stable but stability is reduced on dilution
- Agent of choice for *Mycobacterium* spp.

3.5.3 Peroxygen Compounds

Example: Virkon

- Wide range of bactericidal, viricidal and fungicidal activity
- Variable activity against bacterial spores and *Mycobacterium* spp. There is a report that it is inactive against HIV in serum.
- Corrosivity varies with different products, but less so than hypochlorites
- Made up dilutions have very low toxicity and no irritancy but powders are irritants and should be weighed in a fume hood.
- Built-in colour indicator
- Good detergent properties combines cleaning with disinfection
- Stable for seven days on dilution

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Due to its wide spectrum of activity, suitability for use in most applications, the pink indicator to show disinfectant capacity and the high degree of safety to users, Virkon is recommended as the disinfectant of choice for most University laboratory applications.

3.5.4 Alcohols

Examples: Ethanol, Isopropanol, Methanol, Industrial Methylated Spirits (IMS))

- Good bacterial and fungicidal activity
- No activity against spores
- Variable activity against viruses (ethanol less effective against non- enveloped viruses, propanol not effective against viruses)
- Only recommended for limited use (such as on clean surfaces and for flaming forceps etc - seek alternative wherever possible)
- Poor penetration into tissues
- Should only be used on physically clean surfaces as poor penetration of organic matter
- Rapid action
- Alcohols must be diluted to 70-80% before use (100% alcohol is not an effective disinfectant)
- Highly flammable
- Effective against Mycobacterium spp.

3.5.5 Aldehydes

Examples: Formaldehyde, Glutaraldehyde e.g. Cidex

Chemicals such as formaldehyde and glutaraldehyde have irritant and toxic properties and can be extremely hazardous. Therefore these types of chemical disinfectants should NOT be used as a general disinfectant in the laboratory and should only be employed only for

specialized uses when no suitable alternative is available.

Glutaraldehyde based disinfectants are commonly used for disinfection of instruments that cannot be heat sterilized. Australian guidelines also suggest it is suitable for cleaning a centrifuge bowl in the event of a spill. Consideration should be given to using an alternative such as Virkon although there are reports that under some circumstances Virkon can be corrosive. If glutaraldehyde is used then extreme care must be exercised in its use.

The use of formaldehyde should be limited to gaseous fumigation for disinfection of, for example, microbiological safety cabinets and Class 3 laboratories. Departments should not undertake this type of work without first discussing the issues with the Safety Office. Suitable alternatives are available for most other applications.

3.5.6 Quaternary Ammonium Compounds

Quaternary ammonium compounds (QACs) are cationic detergents with powerful surface active properties. They are effective against Gram-positive bacteria and lipid-containing viruses, e.g. herpes and influenza, but are less active against Gram-negative bacteria and non-lipid-containing viruses. They are inactive against Mycobacterium spp. and bacterial spores. QACs tend to be inactivated by protein adsorption, anionic soaps and detergents, and cellulosic and synthetic plastics materials. However, they are non-toxic, inexpensive, non-corrosive to metals and non-staining. Because of their detergent properties, they have been used mainly in formulations of cleaning agents in the food industries.

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3.5.7 Chlorhexidine

Various formulations of chlorhexidine (as chlorhexidine gluconate) with compatible detergents and ethyl alcohol, or ethyl and isopropyl alcohols, are used as skin disinfectants.

The alcoholic formulations have shown to be effective against HIV. In general, aqueous chlorhexidine is active against Gram-positive bacteria, only moderately active against Gram-negative bacteria and inactive against sporing bacteria, Mycobacterium spp. and non-lipid-containing viruses. Alcohols in the skin disinfectant formulations extend the spectrum of activity of chlorhexidine. Chlorhexidine is of low toxicity, except for neurological tissues, and rarely causes hypersensitivity. It is compatible with quaternary ammonium compounds but is incompatible with soap and anionic detergents. Chlorhexidine is widely used in skin disinfectant formulations, but is not recommended as a general disinfectant.

3.5.8 Acids and Alkalis

All acids are corrosive and care needs to be taken with their use. Acids are effective against a wide range of microorganisms. Hydrochloric acid solution of 2% concentration can be used in places contaminated with urine, blood, faeces, and in sewage collection areas.

Acetic and citric acids are effective for general use against many viruses. A solution of 0.2% citric acid is recommended for personal decontamination. Phosphoric and sulphamic acids are used in food processing areas.

Alkalis have activity against a wide range of microorganisms even in the presence of heavy organic loads in such places as drains and areas contaminated by sewage.

Alkalis are disinfectants of choice for many animal holding areas or animal facilities. 1M sodium hydroxide is a very effective and readily available decontaminant. It retains a high level of activity in the presence of organic matter and is recommended in many situations, such as decontamination of drains and animal houses. Sodium carbonate 4% solution can be used as a wash for animal cages and animal transport vehicles. Sodium metasilicate 5% solution is used as a wash for aircraft and air transport crates.

3.5.9 Other Commercially Available Disinfectants.

There is a plethora of commercially available disinfectants and the choice can be confusing.

For example what is the most appropriate anti-mycobacterial agent? The phenolic based disinfectants mentioned previously or say TRISTEL, a product based on the activity of Chlorine dioxide which has proven mycobactericidal activity and is marketed in quantities that are not only practicable for hard surface laboratory usage but also for proteinaceous spillages that may contain various agents. Chlorine dioxide products are listed as non-corrosive, do not affect plastics or bench surfaces, and are low hazard to users requiring general ventilation for use. Chlorine dioxide is sporicidal, bactericidal, virucidal and fungicidal. The Tristel products in question are as follows:-

Hard surface biocide: Tristel Duo Foamer / D at £7.50 per bottle giving 125 squirts of foam

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surface biocide each squirt covering 1 sq.m., contact time 30 seconds and stated to be biocidal against (for e.g.) spores of C diff, bacillus; mycobacteria inc. TB, Acinetobacter, VREF, HIV, Hep, Herpes, norovirus, etc

Spillage: Tristel Fusion (Fragrance) - FUS/SUR/Fragranced - at £20.00 per box, 40 sachets per box - once activated can dilute up to 5 litres, contact time of 30 seconds required, activity lasting for up to 5 hours. Unactivated sachets (two part - sodium chlorate on one side, citric acid on the other separated by a membrane barrier), and have a shelf life of 2 years. (Same biocidal range)

Another disinfectant, Trigene, which a number of departments in the University use, also claims a wide spectrum of activity including anti-mycobacterial activity. This agent has yet another set of chemicals as the active agent (halogenated tertiary amine – Hexamethylenebiguanide Hydrochloride Alkyldimethyl Benzyl Ammonium Chloride). It appears to be less effective in the presence of a high organic load but still claims activity against for example Pseudomonas aeruginosa in the presence of a 50% blood loading.

The question of which disinfectants are most appropriate for a specific department will

involve all of the factors discussed above and ideally would be subject to an in use validation test. However in practice these validation tests are difficult to establish, particularly where viral agents are concerned. It is also worth noting that manufacturer's claims of activity are generally based on idealized tests without organic loading, in solution. Consequently activity claims should be scrutinized carefully when establishing which disinfectants to use. If a department wishes to use a disinfectant that is new to them it would be appropriate to discuss the matter with the University biological safety officer.

3.6 Contamination of Disinfectants

Working solutions of disinfectants should be frequently replaced with freshly prepared dilutions from stock solutions. This applies particularly to those disinfectants which are subject to inactivation by organic or other materials, loss of stability or significant dilution through the introduction of wet instruments. Otherwise, the inactivated, exhausted or diluted disinfectants may become contaminated and may even support the growth of the bacterial contaminants. The containers or dispensers used should also be emptied and decontaminated between batches and their contents not merely 'topped up'.

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APPENDIX 1

(Modified from <http://www.yale.edu/ehs/Documents/Bio/VacuumSYSProtection.pdf>)

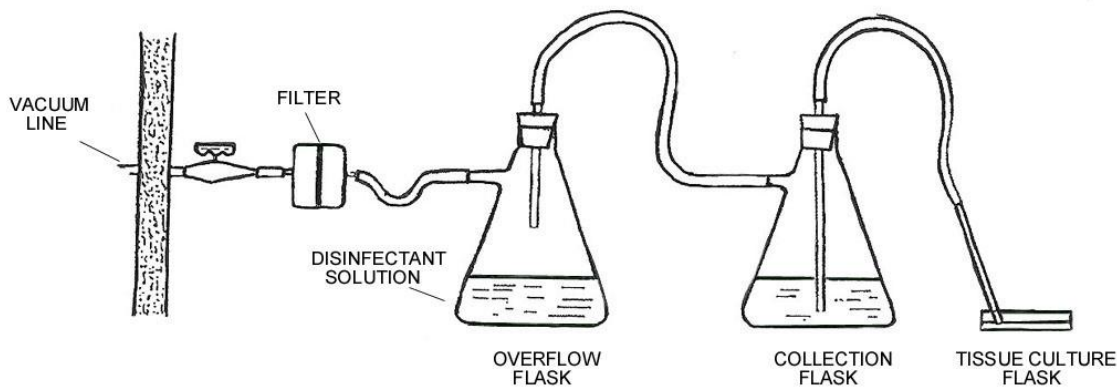
PROTECTION OF VACUUM SYSTEM FROM CONTAMINATION

CDC/NIH Guidelines for Biosafety Level 2 (BSL-2) now state that vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required. CDC/NIH Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 3rd Edition (2007).



Vacuum line traps and filters prevent suction of potentially infectious materials into the vacuum lines. The trap system also prevents vacuum lines from clogging with noninfectious materials.

1. The collection flasks should be monitored and emptied or replaced before it is filled. If using the vacuum line in the biological safety cabinet, place collection flasks inside the unit. The second (overflow) flask can be outside the biological safety cabinet. Never use a glass flask at floor level unless it is adequately shielded by a plastic container.
2. Add the chemical disinfectant to the collection flasks in full strength. Allow the aspirated fluids to complete the dilution. (For example: Start with 100ml bleach, aspirate 900ml fluids and discard).
3. Replace vacuum filter when it is clogged or if liquid makes contact with the filter.
4. Check that all connections or seals are tight to assure the vacuum is adequate.



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APPENDIX 2

Two different dilutions of household bleach are used for disinfection in field work.

Abstracted from a WHO document on influenza surveillance

<http://www.who.int/csr/resources/publications/surveillance/Annex7.pdf>

- 1:10 bleach solution (which contains 5,000 ppm chlorine concentration), a strong disinfectant that is used to disinfect:
 - Excreta
 - Bodies
 - Spills of blood/body fluids
 - Vehicles and tires
 - It can also used to prepare 1:100 bleach solution

- 1:100 bleach solution (which contains 500 ppm chlorine concentration) can be used to disinfect:
 - Surfaces
 - Medical equipment
 - Bedding
 - Reusable protective clothing before it is laundered

Also recommended for:

- Rinsing gloves between contact with different patients (if new gloves are not available)
- Rinsing gloves, aprons, boots before leaving a patient's room
- Disinfecting contaminated waste before disposal

To prepare 1:10 bleach solution add one volume of household bleach (e.g. 1 litre) to nine volumes of clean water (e.g. 9 litres).

To prepare 1:100 bleach solution add one volume of 1:10 bleach solution (e.g. 1 litre) to nine volumes of clean water (e.g. 9 litres).

Note: 1:100 bleach solution can also be prepared directly from household bleach by adding 1 volume of household bleach to 99 volumes of clean water (e.g. 100 ml of bleach to 9.9 litres of clean water) but making it up from 1:10 bleach solution is much easier!).

There are some other products containing chlorine that can be used to make up disinfectant solutions. These are not as suitable as household bleach for this purpose. A table for the preparation of chlorine solutions from some of these compounds is given below.

Preparing chlorine solutions using products other than household bleach

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Chlorine product	1:10 solution	1:100 solution
Calcium hypochlorite powder or granules (70%) (High Test Hypochlorite -HTH)	7 g (0.5 tablespoonful) per 1 litre of water	7 g (0.5 tablespoonful) per 10 litres of water
Bleaching powder (Chlorine of Lime) with 30% active chlorine	16 g (1 tablespoonful) per 1 litre of water	16 g (1 tablespoonful) per 10 litres of water

Disinfection

All objects that have come in contact with potentially infectious materials should be decontaminated.

Decontamination of surfaces

Wear an apron, heavy-duty gloves and other barrier protection if needed. Disinfect surfaces by wiping clean with a 1:100 chlorine solution, then incinerate all absorbent material in heavy-duty garbage bags. The surfaces must be rinsed with clean water after disinfection.

Disinfecting surfaces in laboratories where PCR work is undertaken

Disinfect surfaces with a 1:100 chlorine solution (more dilute solutions are not effective). The chlorine must subsequently be removed as it is caustic and may damage equipment. This may be done either by wiping the surfaces with clean water or with 70% alcohol (which also has a useful additional effect against most bacteria [not against bacterial spores] and vegetative fungi).

Decontamination of blood or body fluid spills

For spills, use 1:10 chlorine solution to inactivate pathogens before soaking up the fluid with absorbent materials. These absorbent materials must then be incinerated.

Disinfection of hands

The principal means for disinfecting hands is by washing with soap and water. If available, a commercial hand disinfectant containing alcohol, chlorhexidine or polyvidone iodine could be used. The use of strong chlorine solutions (such as a 1:100 chlorine solution) should be avoided as it is dangerous.

Sterilization and reuse of instruments and materials

In field outbreak situations, sterilization and reuse of any instruments or materials is not generally advisable. However, it may be necessary to reuse instruments etc. and these should first be disinfected with chlorine, cleaned and then sterilized. Items such as instruments used for autopsy should be disinfected with 1:10 chlorine solution or 70% ethanol.

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