What is disinfection?

Disinfection generally refers to the use of chemical agents to destroy the potential infectivity of a material. It does not imply the elimination of all viable micro-organisms but rather the reduction of the microbial population to a level which makes the disinfected object safe to handle (1, 2). A disinfectant is a chemical agent which, under defined conditions, is capable of disinfection.

Disinfection is commonly used where sterilisation (i.e. rendering free from all living micro-organisms) is considered to be unnecessary or impractical, e.g. due to an object’s size, or because it may be damaged by sterilisation. **Disinfection is not an alternative to sterilisation.**

The effectiveness of the disinfection process depends on:

- a) the concentration of disinfectant (which can be greatly reduced on standing, and by contact with organic material). Effectiveness often bears a non-linear relation to concentration.
- b) contact between the disinfectant and the material to be sterilised. This depends on both the time of contact, and factors such as air bubbles on the submerged articles that discourage intimate contact. For example, intracellular viruses can be more resistant to disinfectants than free viruses.
- c) the activity spectrum of the disinfectant (see Table 1).
- d) pH and water hardness.
- e) temperature – increases can markedly improve the rate of killing.
- f) nature of the microorganisms present. Use a wide-spectrum disinfectant when the type(s) of organisms present are unknown.
- g) miscellaneous conditions, including the presence of organic material, incompatible soaps or detergents, other chemicals present, the nature of the surface material being treated.

**Table 1: Activities of some common classes of disinfectants.**

*(For guidance only – sensitivities must be assessed on a case by case basis)*

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Vegetative Bacteria eg E.coli</th>
<th>Bacterial spores</th>
<th>Fungi</th>
<th>Lipid-coated viruses</th>
<th>Non-lipid viruses</th>
<th>Myco-bacteria</th>
<th>TSE &amp; prions</th>
<th>Incompatibilities</th>
<th>Inactivation by organic matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypochlorites NaDCC</td>
<td>+</td>
<td>+</td>
<td>limited</td>
<td>+</td>
<td>+</td>
<td>limited</td>
<td>+</td>
<td>-</td>
<td>Both corrode metal and damage rubber Cationic reagents</td>
</tr>
<tr>
<td>Alcohols</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Depends on individual virus</td>
<td>+</td>
<td>-</td>
<td>Flammable, Low but penetrates slowly</td>
<td></td>
</tr>
<tr>
<td>Aldehydes Eg gluteraldehyde, formaldehyde</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Surface-active agents eg Hibitane, MedDis/Trigene</td>
<td>+</td>
<td>-</td>
<td>Limited</td>
<td>+</td>
<td>Depends on individual virus</td>
<td>Depends on individual virus</td>
<td>-</td>
<td>-</td>
<td>Anionic, Ca,Mg,Fe</td>
</tr>
<tr>
<td>Peroxygen compounds Eg Virkon</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Depending on method of use</td>
<td>-</td>
<td>-</td>
<td>Virkon -Mild steel, brass, strong alkalis</td>
</tr>
</tbody>
</table>

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Mechanisms of action:

Disinfectants must be carefully chosen for effectiveness in use. Their properties and typical applications are described below (see also Summary Table 1).

(a) **Hypochlorites** - (eg bleach, HazTabs)

<table>
<thead>
<tr>
<th>Effectiveness</th>
<th>Microorganism/Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly effective</td>
<td>Vegetative bacteria, viruses, fungi</td>
</tr>
<tr>
<td>Not so effective</td>
<td>Bacterial spores, <em>Mycobacterium spp</em></td>
</tr>
</tbody>
</table>

Compatible with anionic and non-ionic detergents, but are inactivated by organic matter (serum, blood, sputum or faeces) particularly at concns below 1000ppm available Cl and may corrode metals and damage rubber.

Commonly available as solutions of sodium hypochlorite and as powdered or tableted sodium dichloroisocyanurate (NaDCC). Chlorine is the chemical recommended by the Advisory Committee on Dangerous Pathogens (1990), The Public Health Laboratory Service (1993) and the Department of Health (1998), for the safe disinfection of blood and body fluid spills and for general environmental disinfection. However, hypochlorite solutions are unstable (Hoffman et al, 1981), bulky to store, may be hazardous (DHSS 1978) and are readily inactivated by organic matter such as blood. By contrast chlorine tablets made with NaDCC are stable, compact and the solutions made up from them are more effective in the presence of organic matter. NaDCC is stable whilst dry but stock solutions decay with time, light and temperature and should be stored in cool and dark conditions. Working solutions need to be changed frequently because of decay caused by addition of organic matter. Concentration of hypochlorite solutions is expressed as parts per million available chlorine (ppm av Cl).

Commonly used dilutions are
- 1,000 ppm av Cl for general wiping of equipment/benches
- 2,500 ppm av Cl for discard containers
- 10,000 ppm av Cl for spillages
- 20,000 ppm av Cl for work surfaces, incl cabinets where prions/TSE have been handled (not NaDCC)

NaDCC granules are recommended for spillages

(b) **Alcohols** (e.g. 70% ethanol, 60% isopropanol)

<table>
<thead>
<tr>
<th>Effectiveness</th>
<th>Microorganism/Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective</td>
<td>Bacteria, <em>Mycobacterium spp</em> (not when dried to surfaces), some viruses (not non-enveloped viruses), fungi</td>
</tr>
<tr>
<td>Not effective</td>
<td>Bacterial spores</td>
</tr>
</tbody>
</table>

They have poor penetration of organic matter, particularly proteinaceous material. Alcohols give a very rapid kill of bacteria and some viruses but they are not long-acting because of their relatively volatility. Because they are flammable, do not use them near naked flames or...
equipment liable to generate sparks. The most effective strength is 70-80% (v/v). This is possible due to the mode of action of alcohols which is to denature proteins, these denature more readily in the presence of water. A surface wipe is a convenient method of disinfection, but due to evaporation has limited effect and should therefore be confined to surfaces with no visible contamination.

(c) **Aldehydes** (e.g. formaldehyde, glutaraldehyde)
Effective against all major groups of micro-organisms except prions

However they are respiratory irritants or sensitizers for which Occupational Exposure Limits (OELs) apply.
Use of formaldehyde or aqueous solution (formalin) is restricted to fumigation of MSCs and cryostats as per manufacturer’s instructions and may only be carried out by staff that have been trained in this procedure.
Gluteraldehyde may only be used for non-disinfectant uses such as fixation of cells or tissues and then only if no other method can be used.

Carry out appropriate risk assessments and draw up Standard Operating Procedures (SOPs) before using any of these materials.

(d) **Surface Active Agents** (e.g. the quaternary ammonium compounds – MedDis/Trigene)
four main groups -
  anionic
  cationic
  non-ionic
  amphoteric

Effective against bacteria, fungi, viruses, Myobacteria and spores

They are used as detergents, disinfectants and solubilisers, activity as a disinfectant will depend on the formulation and method of use. Trigene advanced is based on nano-emulsion technology enabling the active ingredients to be carried rapidly through cell walls of micro-organisms.
These are relatively non-toxic and non-irritant but are inactivated by organic matter and soap. They are autoclavable.

(e) **Peroxygen compounds**, (includes Virkon)
Effective against -
  bacteria
  Fungi
  Viruses
Variable effectiveness -
  bacterial spores
  *Mycobacterium spp*

Peroxygen compounds do cause some corrosion, this varies with individual products, but is less than that noticed with hypochlorites.
Virkon is a fast-acting oxidising system based on potassium monopersulphate combined with a surfactant to aid penetration of the oxidant and allow simultaneous cleaning and disinfection. It contains potassium peroxymonsulfate, sulphamic acid, malic acid, sodium hexametaphosphate, and sodium dodecyl benzene sulphonate which work synergistically to attack the key structures within the organism, resulting in inhibition of enzymes systems and loss of cell wall integrity. It is effective against all 17 virus families affecting man and animals including HIV1 and Hepatitis A & B.
It is active against *Mycobacterium spp* when used at 3% concentrations for 20-30 mins.

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Virkon contains a pink indicator that fades when the solution is ready for replacement. However, in practice autoclavable jars and pots are rarely transparent and the colour depends on what has been discarded, for example, the volume of waste liquid in the pipettes and its organic content.

Virkon gives off SO$_2$ when autoclaved and can liberate molecular halogens from halide salts

**What micro-organisms may be present?**

Listed below are the main microorganisms of concern that may be present. Spore-bearing organisms are not a cause of respiratory infection. This list is not exhaustive and is for indication only.

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Micro-organisms of main concern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteria incl. Mycobacteria</td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
</tr>
<tr>
<td></td>
<td>Enveloped virus</td>
</tr>
<tr>
<td></td>
<td>Non-enveloped virus</td>
</tr>
<tr>
<td>Human Blood</td>
<td>Hepatitis B, C, HIV, other adventitious organisms</td>
</tr>
<tr>
<td></td>
<td>Hepatitis A, E, other adventitious organisms</td>
</tr>
<tr>
<td>Other Human material</td>
<td>TB, Streptococcus, Staph. Aureus, other adventitious organisms</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B, C, HIV Common cold virus, other adventitious organisms</td>
</tr>
<tr>
<td></td>
<td>Hepatitis A, E, other adventitious organisms</td>
</tr>
<tr>
<td>Animal</td>
<td>Prions included but do not fall into any of these categories</td>
</tr>
<tr>
<td></td>
<td>Retroviruses</td>
</tr>
<tr>
<td>Mammalian cell lines</td>
<td>Hepatitis B, C, HIV, retroviruses other adventitious organisms</td>
</tr>
<tr>
<td></td>
<td>Hepatitis A, E, other adventitious organisms</td>
</tr>
<tr>
<td>Micro-organism Cultures (GM)</td>
<td><em>E. coli</em> derivatives</td>
</tr>
<tr>
<td></td>
<td>Yeast</td>
</tr>
<tr>
<td></td>
<td>Campylobacter</td>
</tr>
<tr>
<td>Viral cultures (GM)</td>
<td>Retroviruses</td>
</tr>
<tr>
<td></td>
<td>Derivative of λ phage Adeno-associated virus</td>
</tr>
</tbody>
</table>

**Which disinfectant to use, when to use it and how**

All disinfectants should be used with care, and staff should wear laboratory coats, gloves and protective eye wear when using them. Disinfectants must not be used in combination.

1. *Alcohol – 70% ethanol*

   A bottle of clearly labeled 70% ethanol should be kept by all Category II cabinets

   Use for swabbing down work surfaces, particularly Category II cabinets, at the end of use or if very small amounts for contamination have occurred ie less than 100µl.
2. Hypochlorite – supplied as Haz-Tabs or granules

It is important that an accurate strength of chlorine is made up for disinfection purposes, ie 10,000ppm for blood spills, 2,500ppm for discard jars and 1,000ppm for general environmental cleaning.

4 Haz Tabs/1L water = 10,000ppm available chlorine
1 Haz Tab/2.5L water = 1,000ppm available chlorine

Small spills of blood ie < 100μl should be cleaned with solution of 10,000ppm av Cl, mopped up with disposable tissues, the area should be then wiped with 70% alcohol.

Major spillages (includes breakage of centrifuge tubes) < 7ml of any human biological material should be covered in Haz Tab granules.

3. Surface active compounds supplied as MedDis/Trigene

MedDis/Trigene demonstrates activity similar to 2% alkaline gluteraldehyde. It is used at a concentration of 5% and will be active for 21 days at this concentration. It is intended for use with medical equipment and contains a mixture of corrosion inhibitors. MedDis is sometimes used in hospitals as it is effective against TB and will not corrode stainless steel.

Working solution made by adding 25ml concentrate to 500ml water.
At the end of each day
- all instruments are soaked in 5 % MedDis for at least 30 minutes and then rinsed in 70% ethanol.
- Cabinet and tray saturated with 5 % MedDis and wiped with blue roll. They are then wiped with 70% ethanol
- Protective sleeves should be sprayed with MedDis taking care to prevent inhalation and then wiped with 70% ethanol.

Trigene is sold as concentrate or as ready to use solution:
- General, intermediate risk applications to be used at dilution of 1:200
- Heavy soilage and High risk areas 1:100
- For use with tissue cultures and discard jars 1:10

4. Peroxygens compounds supplied as Virkon

Virkon powder is an irritant. Weigh it out in a fume hood or other suitable enclosure, use eye protection, gloves and laboratory coat when using Virkon powder.

Virkon solution is active for 7 days

a) Discard jars – routinely contain 2% aqueous Virkon. Items placed in discard jars must be completely immersed in the disinfectant so that both inner and outer surfaces are covered. Leave to soak for at least 24 hours. Autoclave items before sending for incineration. Do not allow discard jars to become reservoirs of infection. Wash them thoroughly and preferably heat-treat them before refilling.

Care must be taken when using with blood as Virkon coagulates with blood.

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b) Disinfection of surfaces and equipment - Use a 1% aqueous Virkon solution. Wipe surfaces with excess Virkon solution using a paper towel or apply by spraying ensuring precautions are taken to prevent inhalation. Leave for 10 minutes. Do not soak metal items in Virkon for prolonged periods as even clean water can initiate corrosion of aluminium alloys and deterioration of anodized protective coatings if immersion is prolonged. Use a water-moistened paper towel to remove any white deposit that remains on the surface after drying. Discard towels and gloves into a bag for autoclavable waste.

For routine decontamination of buckets, trunnions, angle heads, bowls, etc, leave for 30 seconds, rinse with water, and wipe off with a paper towel.

c) spills – Wear a facemask and single-use gloves when using the powder to clean up spillages. Cover spillage with Virkon powder, leave for 3 minutes. Scrape powder spillage mixture into a safe receptacle and send for incineration. Wash and disinfect area with 2% Virkon

**Summary**

Use hypochlorites (Haz Tab) for pathology work and spills of pathological material

Use ethanol for swabbing work surfaces or for very small pathological spills (less than 100μl)

Use Virkon for discard jars, disinfection of benches or pathological spills

Use formalin for fumigating containment cabinets/cryostat or in the case of serious biological spills only, or before any maintenance work is carried out on the equipment.