

DISINFECTION GUIDANCE



DOCUMENT HISTORY

Version	Section(s)	Date	Change details
1.0	All	Mmm	E.g. complete reformat from previous document - SPG 23-09 version 2
		уууу	-2009
			Distel replaces Trigene and Medistel replaces MedDis

Document No	HS G028	Version	1.0	Date Issued	Jul 2022
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1 DEFINITIONS

A disinfectant is a chemical agent which, under defined conditions, is capable of disinfection.

Disinfection refers to the elimination of virtually all pathogenic organisms on inanimate objects and surfaces using disinfectants thereby reducing the level of microbial contamination to an acceptably safe level (**tables, 1, 2**).

In contrast, **sterilisation** refers to the destruction of all micro-organisms. This is typically accomplished with heat and steam through autoclaving. **Disinfection is not an alternative to sterilisation**.

2 SELECTION OF DISINFECTANT

The selection on disinfectant will depend on:

- 2.1 **Type of micro-organism**: the spectrum of activity of disinfectants varies against different microorganisms, users must check with the manufacturers' activity data (see Table 1).
- 2.2 **Contact time:** sufficient time is required for the disinfectant to be in contact with the material to enable effective disinfection.
- 2.3 **Concentration:** disinfectants must be used at the concentrations specified by the manufacturer. The effective life varies once diluted, and new dilutions should be dated and disposed of once out of date. Material must be totally submerged and left in contact with the disinfectant for the manufacturers' recommended times.
- 2.4 **Type of surface**: disinfectants can be inactivated by some surfaces, or chemically attack surfaces. Those containing electrolytes or strong acids/alkalis can corrode stainless steel, while those containing organic solvents can affect the surface of plastic materials.
- 2.5 Hazardous properties: disinfectants are hazardous substances. Disinfectants with the lowest risk to human health should be used where practical, in accordance with the principles of COSHH. Liquid or tablet forms should be purchased in preference to powder forms to prevent inhalation.

3 ADVERSE EFFECTS

Disinfectants can be adversely affected by.

- 3.1 Organic material
- 3.2 Other chemicals
- 3.3 Temperature
- 3.4 pH
- 3.5 Hardness of water used for dilution

4 VALIDATION OF DISINFECTANTS

- 4.1 Disinfectants should be used in accordance with manufacturers' instructions. Chemical disinfectants are evaluated for their bacterial, fungicidal, virucidal, sporicidal or mycobactericidal activity using a variety of tests. Manufacturers provide information on the activity of registered products for micro-organisms and recommend appropriate dilutions. These can be used for decontamination of HG1 and HG2 organisms.
- 4.2 However, there may be differences in activity of disinfectants under actual lab conditions,

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particularly if there are very high levels of organic matter present. In this case it may be necessary to validate the disinfectant under the conditions of use.

4.3 For work with higher risk HG2 and all HG3 agents, in-house efficacy testing should be done to evaluate the disinfectants performance under the specific conditions of use.

5 MICROBIAL RESTISTANCE TO DISINFECTANTS

5.1 The sensitivity of each organism to disinfectants depends on the cellular structure, composition, and physiology. The outer surface acts as a barrier to the uptake of disinfectant. In some cases, enzymatic degradation of the disinfectant occurs, particularly in biofilms where micro-organisms can become resistant by multiple mechanisms such as enzyme production or pH gradients within the biofilm. Chlorine-based disinfectants can inactivate biofilm bacteria. More rarely genetic mutations and acquisition of additional genetic factors can lead to resistance via alteration in the cell envelope or chemical efflux pumps. This is more likely to occur after long term exposure.:

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The Antimicrobial Spectrum of Disinfectants

This table provides general information for selected disinfectant chemical classes. Antimicrobial activity may vary with formulation and concentration. The use of trade names does not in any way signify endorsement of a particular product. They are provided as examples.

Ren mus of a mo	nov sta ny st	al of organic materia lways precede the us disinfectant. susceptible	se Se	Acids hydrochloric acid, acetic acid, citric acid	Alcohols ethanol, isopropanol	Aldehydes formaldehyde, paraformaldehyde, gluteraldehyde	Alkalis sodium hydroxide, ammonium hydroxide, sodium carbonate	Biguanides chlorhexidine, Nolvasan*, ChlorHex*, Virosan*	Halog sodium hypochlorite	ens iodine	Peroxygens accelerated hydrogen peroxide (Rescue [*]), potassium peroxymonosulfate (Virkon-S [*]), peroxyacetic acid, (Oxy-Sept [*] 333)	Phenolic Compounds (Lysol [*] , Osyl [*] , Amphyl [*] , TekTrol [*] , Pheno-Tek II [*])	Quaternary Ammonium Compounds (Roccal, Zepharin DiQuat, Parvosol [®] , D-256 [®])
		mycoplasmas		+		•••	•••			•••			•
		gram-positive bacteria	1	•		•••	+		•	+	•		•••
		gram-negative bacteri	а	•		•••	•		+	+	•	•••	•
IS		pseudomonads		•		•••	+	2	•	٠	•		
nisn ts		rickettsiae		2	+	•	+	2	•	٠	•	•	±
orgal		enveloped viruses		•	+	•••	+	2	•	+	+	± a	2
croc		chlamydiae		2	1	•	+	2	•	+	+	2	
f mi		non-enveloped viruses	s			•	±		•	1	2		
ty o lical		fungal spores		2	1	•	+	2	•	+	2	+	2
hem		picornaviruses (i.e. FM	D)	•	N	•	+	N	N	N	•	N	N
cept to c		parvoviruses		N	N	•	N	N	•	N	1	N	- 6-
sus		acid-fast bacteria			+	•	+		•	+	2	2	
		bacterial spores		2		•	±		•	+	+ ^b		
		coccidia					+ c					+ d	- 6-
_		prions											- 6-
ma	ost	resistant	LEGEND	highly effective effective limited activity	─ no a N info	activity rmation not available		a–varies with o b–peracetic ao c–ammonium d–some have a	composition cid is sporicidal hydroxide activity against	coccidia		ی Foo هاک	enter for d Security blic Health

Table 1: Activities of some common classes of disinfectants.

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

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6 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).

- 6.1 Hypochlorites (e.g., bleach, HazTabs)
- 6.1.1 Hypochlorites highly effective against vegetative bacteria, viruses and fungi. They are not so effective against bacterial spores, Mycobacterium spp



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

Commonly available as solutions of sodium hypochlorite (domestic bleach) and as powdered or tableted sodium dichloroisocyanurate (NaDCC) (e.g., Chemgene).

(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 fluidspills and for general environmental disinfection).

- 6.1.3 Hypochlorite solutions are unstable (Hoffman et al, 1981), bulky to store, are 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 so 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).
- 6.1.4 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 NaDDC)
- 6.1.5 NaDDC granules are recommended for spillages

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

- 6.2.1 Effective against bacteria, Mycobacterium spp (not when dried to surfaces) some viruses (not nonenveloped viruses) fungi
- 6.2.2 Not effective against bacterial spores
- 6.2.3 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 possibly due to the mode of

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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.

6.3 Aldehydes (e.g., formaldehyde, glutaraldehyde)

- 6.3.1 Effective against all major groups of micro-organisms except prions
- 6.3.2 These 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.
- 6.3.3 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.
- 6.3.4 Carry out appropriate risk assessments and draw up Standard Operating Procedures (SOPs) before using any of these materials.

6.4 Surface Active Agents (e.g., the quaternary ammonium compounds – Medistel /Distel)

- 6.4.1 Four main groups:
 - anionic
 - cationic
 - non-ionic
 - amphoteric
- 6.4.2 Effective against bacteria, fungi, viruses, Myobacteria and spores
- 6.4.3 They are used as detergents, disinfectants and solubilisers. Their activity as a disinfectant will depend on the formulation and method of use. "Distel" 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.

6.5 Peroxygen compounds, (includes Virkon)

- 6.5.1 Peroxygen compounds do cause some corrosion, this varies with individual products, but is generally less corrosive than hypochlorites.
- 6.5.2 Virkon is a fast-acting oxidiser based on potassium monopersulphate combined with a surfactant to aid penetration and to allow simultaneous cleaning and disinfection. It contains potassium peroxymonosulfate, sulphamic acid, malic acid, sodium hexametaphosphate, and sodium dodecyl benzene sulphonate which work synergistically to attack the key structures within the organism, resulting in inhibiton of enzymes and loss of cell wall integrity. It is effective against all 17 virus families affecting man and animals including HIV1 and Hepatitis A & B.
- 6.5.3 It is active against *Mycobacterium spp* when used at 3% concentrations for 20-30 mins.
- 6.5.4 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.
- 6.5.5 Virkon gives off SO₂ when autoclaved and can liberate molecular halogens from halide salts

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7 WHAT MICRO-ORGANISMS MAY BE PRESENT

Listed below (**Table 2**) 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.

Biological sample	Micro-organisms of main concern						
	Bacteria incl. Mycobacteria, Fungi	Enveloped virus	Non-enveloped virus				
Human Blood		Hepatitis B, C, HIV, other adventitious organisms	Hepatitis A, E, other adventitious organisms				
Other Humanmaterial	TB, Streptococcus, Staph. Aureus, other adventitious organisms	Hepatitis B, C, HIV Common cold virus, other adventitious organisms	Hepatitis A, E, other adventitious organisms				
Animal	Prions included but do not fall into any ofthese categories	Retroviruses					
Mammalian cell lines		Hepatitis B, C, HIV, retroviruses other adventitious organisms	Hepatitis A, E, other adventitious organisms				
Micro-organismCultures (GM)	E.coli derivatives Yeast Campylobacter	Retroviruses					
Viral cultures (GM)			Derivative of λ phageAdeno- associated virus				

8 WHICH DISINFECTANT TO USE WHEN TO USE IT AND HOW

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

8.1 Alcohol – 70% ethanol

- 8.1.1 A bottle of clearly labeled 70% ethanol (with a flammable hazard warning sticker) should be kept by all Class II cabinets
- 8.1.2 Use for swabbing down work surfaces, particularly Class II cabinets, at the end of use or if very small amounts for contamination have occurred i.e. less than 100 μ l

8.2 Hypochlorite – supplied as Haz-Tabs or granules

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

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- 8.2.2 4 x Haz Tabs/1L water = 10,000ppm available chlorine1Haz Tab/2.5L water = 1,000ppm available chlorine
- 8.2.3 Small spills of blood i.e. < μ 00 l should be cleaned with solution of 10,000ppm av Cl, mopped up with disposable tissues, the area should be then wiped with 70% alcohol
- 8.2.4 Major spillages (includes breakage of centrifuge tubes) spillages of any human body fluids should be covered in Haz Tab granules.

8.3 Surface active compounds supplied as Medistel /Distel

- 8.3.1 Medistel /Distel 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.
- 8.3.2 Medistel is sometimes used in hospitals as it is effective against TB and will not corrode stainless steel.
- 8.3.3 Working solution made by adding 25ml concentrate to 500ml water.
- 8.3.4 At the end of each day:
 - all instruments are soaked in 5 % Medistel for at least 30 minutes, then rinsed in 70% ethanol.
 - cabinet and tray saturated with 5 % Medistel and wiped with blue roll, then wiped with 70% ethanol.
 - protective sleeves are sprayed with Medistel taking care to prevent inhalation, then wiped with 70% ethanol.
- 8.3.5 Distel is sold as concentrate or as ready to use solution and should be used at the following dilutions

General, intermediate risk applications	1:200
Heavy soilage and High-risk areas	1:100
For use with tissue cultures and discard jars	1:10

8.4 Peroxygen 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 solution is active for 7 days

8.4.1 **Discard jars** – routinely fill with 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 higher risk items (HG2 and above) 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.

8.4.2 **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

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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.

8.4.3 **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

9 SUMMARY

- 9.1 Use hypochlorites (Haz Tab) for pathology work and spills of pathological material
- 9.2 Use ethanol for swabbing work surfaces or for very small pathological spills (less than 100µl)
- 9.3 Use Virkon for discard jars, disinfection of benches or pathological spills
- 9.4 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.

10 REFERENCES/ RESOURCES

Decontamination-and-disinfection (EHS.MIT.EDU)

Safe working and the prevention of infection in clinical laboratories and similar facilities(HSE 1991)

Disinfectant Selection and Use | Environment, Health and Safety (Cornell.edu)

The Center for Food Security and Public Health at Iowa State University offers an <u>excellent quick reference</u> <u>chart</u> for disinfectant selection.

Management and operation of microbiological containment laboratories (ACDP) (Mar 2019)

Biosafety unit - GOV.UK

Practical Disinfection Guidance for the Clinical Laboratory (APHL.org)

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