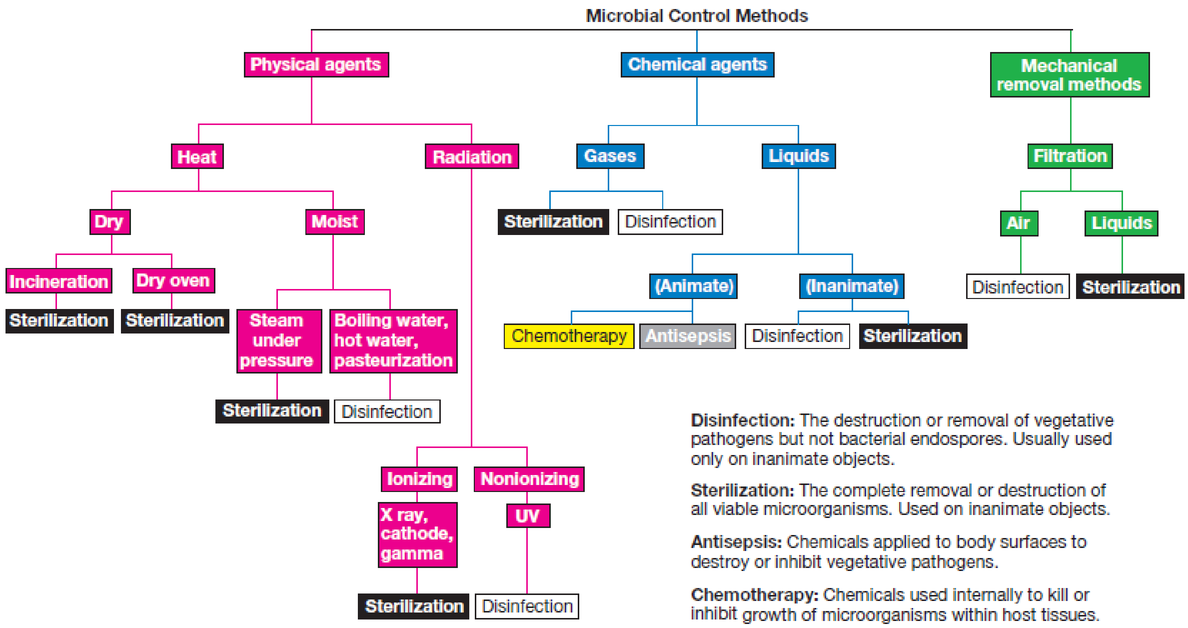
## ANTISEPTICS AND DISINFECTANTS

**Disinfection** refers to the use of physical or chemical methods for the destruction of microorganisms, especially potential pathogens, on the surfaces of inanimate objects or in the environment**.** This process destroys vegetative pathogens but not bacterial endospores. **Antiseptics** are applied directly to the exposed body surfaces (e.g., skin and mucous membranes), wounds, and surgical incisions to destroy or inhibit vegetative pathogens.




### Properties of an ideal disinfectant / antiseptic

An ideal disinfectant or antiseptic has the following characteristics:

1. Ideally, the disinfectant should have a wide spectrum of antimicrobial activity. It must be effective against a wide variety of infectious agents (bacteria, including spores, fungi, and viruses)
2. It should possess high penetrating power.
3. It should not corrode metals during sterilisation of instruments
4. It should not cause irritation or sensitisation when applied on the body
5. It should not be toxic in case it is absorbed into circulation
6. Other properties include: it should be active even in presence of organic matter, effective in acid/alkaline medium, should be stable, compatible with other disinfectants / antiseptics and inexpensive.

**Factors influencing activity of disinfectants / antiseptics**

Various conditions influencing the efficiency of disinfectant are as follows:

1. Temperature: Surge in temperature increases the efficiency of disinfectants
2. Type of microorganism: Vegetative cells are more susceptible than spores.
3. Physiological state of the cell: Young and metabolically active cells are more sensitive than old dormant cells. Non-growing cells may not be affected.
4. Environment: The physical or chemical properties of the medium or substance influence rate as well as efficiency of disinfectants, e.g., pH of the medium and presence of extraneous materials.

**Types of disinfectants / antiseptics**

They include phenolic compounds, halogens, alcohols, aldehydes, gases, surface active agents, oxidizing agents, dyes, heavy metals, as well as acids and alkalis.

**PHENOLIC COMPOUNDS**

The use of phenol has been restricted, but certain derivatives of phenol are widely used. Phenolics are protoplasmic poisons at higher concentrations, but at lower concentration they inactivate cellular enzyme systems. Phenolic compounds are broad-spectrum antiseptics and act by denaturing proteins and disrupting cell membranes. They are effective in the presence of organic material and remain active on surfaces long after application. **Cresol** (methyl phenol): it is used as solutions of cresols in soaps (Lysol). It is toxic to skin or tissues and mainly used for preliminary sterilisation of infected glasswares in laboratory, disinfection of excreta, cleaning floors of wards and operation room in hospital.

**Chloroxylenol** (dimethyl phenol): it is active ingredient of **Dettol** and it is not effective against Gram-negative bacteria.


**Chlorohexidine**: it is bactericidal at high dilution for a wide of microorganisms including *Pseudomonas aeruginosa*. Chlorohexidine is used extensively in teat dips for control programmes in dairy cattle. It is less toxic and effective in presence of blood or pus.

Disinfectants containing phenolic compounds are potentially toxic for workers, domestic animals and wildlife. In circumstances where substantial amounts of these disinfectants are used in farm buildings, run-off should not be discharged into ponds, lakes, rivers or streams. Fluid containing phenolic disinfectants should be collected in slurry tanks or other suitable holding facilities and applied to arable land remote from water courses.

**Savlon** is a combination product of chlorohexidine with cetrimide. Savlon is widely used in burns, wounds, for surgical instruments and preoperative antisepsis of skin. A solution containing 5 ml of Savlon in a litre of clean water can be put on wounds whereas a disinfecting solution can contain 30 ml in a litre of clean water.

**HALOGENS**

Chlorine and iodine are the only two routinely used halogens because fluorine and bromine are dangerous to handle. These agents are highly effective disinfectants and antiseptics, because they are microbicidal, they are also sporicidal with longer exposure.

**Chlorine**

Chlorine kills not only bacterial cells and endospores but also fungi and viruses. Chlorine and its compounds are commonly used for disinfecting water supplies, swimming baths, and food and dairy industries. In the laboratory, **hypochlorites** are used as laboratory disinfectants on surfaces of bench and in contaminated spots; however hypochlorite solution decays rapidly and should be prepared freshly and daily. Hypochlorites are broad-spectrum chlorine-realeasing disinfectants of choice against viruses. Aqueous solution of sodium hypochlorite (5.25%) is called household bleach; it is used in concentration of 0.2% to 1% depending upon the circumstances. Hypochlorite reacts with formaldehyde and one of the products is found to be carcinogenic. Hypochlorites are inactivated by organic matter, and corrode metals.

**Iodine**

Iodine rapidly penetrates the cells of microorganisms, where it apparently disturbs a variety of metabolic functions. It kills all types of microorganisms if optimum concentrations and exposure times are used. Iodine activity is not as adversely affected by organic matter and pH. The two primary iodine preparations are free iodine in solution and iodophors.

**Iodine tincture** is a 2% solution of iodine and sodium iodide in 70% alcohol that can be used in skin antisepsis. Because iodine can be extremely irritating to the skin and toxic when absorbed, strong aqueous solutions and tinctures (5–7%) are no longer considered safe for routine antisepsis.

**Iodophores**

Iodophores are complexes of iodine and a neutral polymer, such as polyvinyl alcohol. This formulation permits the slow release of free iodine and increases its degree of penetration. These compounds have largely replaced free iodine solutions in medical antisepsis because they are less prone to staining or irritating tissues. **Betadine, povidone,** and **isodine** are the common iodophors compounds that contain 2-10% of available iodine. Iodophores are widely used for skin, mucosa, and wounds antisepsis including treating burns. A 2.5% ophthalmic solution containing an iodophore is a useful prophylactic against neonatal conjunctivitis; it can replace ophthalmic antibiotics. Povidone iodine can be used in teat dips for mastitis control programmes in dairy cattle.

**ALCOHOLS**

Alcohols are bactericidal and fungicidal but not sporicidal, viricidal. **Ethyl alcohol** (ethanol) and **isopropyl alcohol** (isopropanol) are the two most popular alcohol germicides. They are effective at a concentration of 60-70% in water. They act by denaturing bacterial proteins and possibly by dissolving membrane lipids. They are used as skin antiseptics. Isopropyl alcohol is used for disinfection of clinical thermometers. A 10-15 minute soaking is sufficient to disinfect thermometers. **Methyl alcohol** is an effective disinfectant against fungal spores and is used for disinfecting cabinets and incubators.

**ALDEHYDES**

Formaldehyde and glutaraldehyde are the two most commonly used aldehydes that are used as disinfectants. They are highly reactive molecules that combine with nucleic and alkylating molecules. They are sporicidal and can also be used as chemical sterilants.

**Formaldehyde**

Formaldehyde is usually dissolved in water or alcohol before use. In aqueous solution, it is bactericidal, sporicidal, and also effective against viruses. Commercial formalin contains 40% formaldehyde gas in water to which 10% methanol is added to prevent polymerisation. A 10% solution of formalin is used for preserving fresh tissue specimens, killing bacterial cultures and suspensions, cleaning contaminated surface. Formalin is used to sterilise bacterial vaccines, and in preparation of toxoid from toxins.

**Glutaraldehyde**

A 2% buffered solution of glutaraldehyde is an effective disinfectant. It is less irritating than formaldehyde and is used to disinfect hospital and laboratory equipments. Glutaraldehyde usually disinfects objects within time frame of 10 minutes but may require as long as 12 hours to destroy all spores. Glutaraldehyde is especially effective against tubercle bacilli, fungi, and viruses.

It can be used for cleaning cystoscopes and bronchoscopes, corrugated rubber anesthetic tubes and face masks, plastic endotracheal tubes, metal instruments, and polythene tubing.

**GAS VAPOUR STERILISATION**

**Ethylene oxide**

Ethylene oxide is colourless gas at ordinary room temperature (boiling point, $10^{o}C$) and active against all types of bacteria and spores. Ethylene oxide exerts lethal effect on proteins of bacteria; moreover it can react with DNA and RNA. The use of ethylene oxide as a disinfectant presents a potential hazard to human beings because of its toxicity, mutagenicity and carcigenicitiy. Plastic goods, polythene tube, artery and bone grafts, cystoscopes, vaccines, and culture media can be sterilised by ethylene oxide.

**Formaldehyde gas**

Paraformaldehyde vaporised by heat is used for decontaminating biological safety cabinets, bedding, furniture, books and clothings.

**Hydrogen peroxide vapours**

$H_2O_2$ vapours are effective sterilants because of the oxidising nature of the gas. It is used for sterilising instruments.

**SURFACE ACTIVE AGENTS**

Substances that alter energy relationship at interfaces, producing a reduction in surface or interfacial tension are called surface active agents. Disinfectants, acting as surface agents, are widely used and commercially available as wetting agents detergents and emulsifiers.

They are classified into four groups: anionic, cationic, non-ionic, and amphoteric. As antibacterial agents, the cationic surface active agents are most important. Cationic detergents include **ammonium compounds** and **cetrimide.**

**Ammonium compounds (quats)**

A wide range of quats possess antimicrobial activity; they are positively charged, cationic surface-active detergents that disrupt the cell membrane associating the negatively charged phosphate groups of the phospholipids. Examples of quats include **benzalkonium chloride** and cetylpyridinium chloride; they kill most bacteria but not *Pseudomonas*, *Mycobacterium,* fungus *Trichophyton*, endospores, and viruses. Quats are stable and non-toxic, but they are inactivated by ionic detergents, organic matter, and dilution. Anionic disinfectants include common **soaps** that have mild to moderate disinfectant action. Soaps prepared from saturated fatty acids (e.g., **coconut oil**) have more effective action against Gram-negative bacteria.

**OXIDIZING AGENTS**

This group includes halogens, hydrogen peroxide, potassium permanganate, etc. They are good disinfectants and antiseptics but are less effective in the presence of organic matter. **Hydrogen peroxide** ($H_2O_2$), used as 3% solution, is a weak antiseptic. **Potassium permanganate** ($KMnO_4$) is bactericidal in nature and active against viruses also. Preparing antiseptic solution requires mixing a gramme of $KMnO_4$ in a litre of water; the solution can also be used to wash out the mouth. Preparing a disinfecting solution requires mixing 10 grammes of $KMnO_4$ in a litre of water.

**DYES** (e.g., aniline and acridine groups)

Aniline and acridine groups of dyes are bacteriostatic in high dilution but have low bactericidal action. These agents are more effective against Gram-positive bacteria than against Gram-negative organisms. The aniline dyes include gentian violet, brilliant green, and malachite green. These dyes are used in the laboratory as selective agents in culture media and also in bacterial staining. The acridine dyes include acriflavine, euflavine, proflavine, and aminacrine. They impair the DNA complexes of the organism, and thereby, the organism is either killed or loses its reproductive activity. The two most widely used dyes in clinical practice for skin disinfection are **gentian violet** and **acriflavine**.

**SALTS OF HEAVY METALS**

Soluble salts of some heavy metals have antibacterial activity, both bactericidal and bacteriostatic. They combine with proteins, often with their sulfhydryl groups and inactivate them. They may also precipitate cell proteins. Silver compounds are widely used as antiseptics. **Copper sulphate** is an effective algaecide in lakes and swimming pools. **Mercuric chloride** is used as disinfectant.

**ACIDS AND ALKALIS**

Acids and alkalis are germicidal in nature; they kill microorganisms by hydrolysis and altering the pH of the medium. At high concentrations, sodium hydroxide, potassium hydroxide, and ammonium hydroxides have marked microbicidal properties. Caustic alkaline solutions are effective against many viruses including FMD virus. At concentrations over **5%, sodium hydroxide** has a wide antimicrobial spectrum including bacterial endospores. Prions are inactivated by treatment with 2 mol/litre sodium hydroxide at 121°C for 30 minutes. Both sodium hydroxide and potassium hydroxide are corrosive for metals and hazardous for workers. **Ammonium hydroxide** inactivates coccidial oocysts.

## MONITORING EFFICACY OF DISINFETANTS

The efficiency of disinfectants can be determined with the help of several tests. These are:

1. Phenol coefficient (Rideal-Walker) test
2. Chick Martin test
3. Capacity (Kelsey–Sykes) test
4. In-use (Kelsey–Maurer) test

**Phenol coefficient test (aka Rideal-Walker test)**

A series of dilutions of phenol and the disinfectant being tested are prepared. A standard amount of *Salmonella typhi* and *Staphylococcus aureus* are added to each dilution; the dilutions are then placed in a 20 or 37°C water bath. At 5-minute intervals, samples are withdrawn from each dilution and used to inoculate a growth medium, which is incubated for two or more days and then examined for growth. If there is no growth in the growth medium, the dilution at that particular time of sampling killed the bacteria. The highest dilution (i.e., the lowest concentration) that kills the bacteria after a 10-minute exposure, but not after 5 minutes, is used to calculate the phenol coefficient. The reciprocal of the appropriate test disinfectant dilution is divided by that for phenol to obtain the coefficient. Suppose that the phenol dilution was 1/90 and maximum effective dilution for disinfectant X tested was 1/450, then the phenol coefficient of X would be 5. The higher the phenol coefficient value, the more effective the disinfectant under these test conditions.

A value greater than 1 means that the disinfectant is more effective than the phenol. The test, however, does not show the action of disinfectant in natural conditions, i.e., in the presence of organic contaminants. The phenol coefficient test is a useful initial screening procedure, but the phenol coefficient can be misleading if taken as a direct indication of disinfectant potency during normal use. This is because the phenol coefficient is determined under carefully controlled conditions with pure bacterial strains, whereas disinfectants are normally used on complex populations in the presence of organic matter and with significant variations in environmental factors like pH, temperature, and presence of salts.

**Chick Martin test**

It is a modification of Rideal-Walker test, in which the disinfectant acts in the presence of organic contaminants (e.g., dried yeast, faeces, etc.) to simulate the natural conditions.

Phenol coefficient of the disinfectant is calculated as in Phenol coefficient test.

**Kelsey-Sykes test (capacity test)**

Kelsey–Sykes test determines the appropriate use of dilutions of the disinfectants. It measures the capacity of a disinfectant to retain its activity when repeatedly used microbiologically. The disinfectant is assessed by its ability to kill bacteria by demonstrating growth or no growth on recovery culture media. Three successive lots of test disinfectants are taken. Now, suspension of standard organism (Staphylococ*cus aureus, Escherichia coli, Pseudomonas aeruginosa*) is added to the three lots of disinfectants at 0, 10 and 20 minutes. The three lots of disinfectants are allowed to remain in contact with organism for 8 minutes and samples are transferred at 8, 18 and 28 minutes, respectively, to a recovery medium. The recovery medium is incubated overnight and noted for bacterial growth or no growth. Thus the disinfectant is judged by its capacity or ability to kill bacteria. The tests are performed both in clean and dirty conditions and thus simulate natural conditions.

**In-use test (Kelsey–Maurer test)**

The "in-use" test is a test that determines whether the chosen disinfectant is effective, in actual use, in hospital practice and also for the period of its use. The effectiveness of the disinfectant is determined by its ability to inactivate a known number of standard strains of pathogenic staphylococci on a given surface within a certain given time. In-use test allows a more accurate determination of effectiveness of a disinfectant compared to phenol coefficient test. In case of a new disinfectant, it is challenged with a bacterium suspension and samples are withdrawn and cultured in recovery media. The result is compared with previously standardised disinfectant.

**RESOURCES**

- **Books**

Antimicrobial therapy in veterinary medicine (2013): edited by Steeve Giguère, John F. Prescott, Patricia M. Dowling), fifth edition, John Wiley & Sons, Inc

Boyce P. Wanamaker and Kathy Lockett Massey (2009): Applied Pharmacology for veterinary technicians, fourth edition, Elsevier (USA), ISBN: 978-1-4160-5633-1

Hany M. Elsheikha and Naveed Ahmed Khan (2011): Essentials of Veterinary Parasitology, Caister Academic Press, Norfolk, UK. Horizon Scientific Press.

Joanne M. Willey, Linda M. Sherwood and Christopher Woolverton J. 2008. Prescott, Harley, and Klein's Microbiology, Seventh Edition, the McGraw-Hill Companies, New York, USA

Judith A. Owen, Jenni Punt, Sharon A. Stranford, Patricia P. Jones 2013. Immunology, 7th edition, W.H. Freeman and Company, New York.

K.W. Clarke, CM Trim, L.W. Hall (2013): Veterinary Anaesthesia, 11th edition, ISBN: 978-0-7020-2793-2.

Quinn PJ., Markey B K., Leonard F C., FitzPatrick E S., Fanning S. and Hartigan P. 2011. Veterinary Microbiology and Microbial Diseases. Second edition, Wiley-Blackwell, Oxford, UK

Subhash Chandra Parija 2012. A textbook of microbiology and immunology, Reed Elsevier India Private Limited, New Delhi, India

Susan M. Ford and Sally S. Roach (2010): Roach's Introductory Clinical Pharmacology, Lippincott Williams & Wilkins, ISBN 1605476331, 9781605476339.

Walter H. Hsu. (2008). Handbook of Veterinary Pharmacology. 1st Edition. Wiley-Blackwell

**Journals**

- International Journal of Pharmacy and Pharmaceutical Sciences
- Veterinary Medicine and Science

**Key websites and on-line resources**

- AJOL
- AGORA
- HINARI