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Disinfectants

Disinfectants are antimicrobial agents that are applied to non-living objects to destroy microorganisms that are living on the objects. Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores; it is less effective than sterilization, which is an extreme physical and/or chemical process that kills all types of life. Disinfectants are different from other antimicrobial agents such asantibiotics, which destroy microorganisms within the body, and antiseptics, which destroy microorganisms on living tissue. Disinfectants are also different from biocides — the latter are intended to destroy all forms of life, not just microorganisms. Disinfectants work by destroying the cell wall of microbes or interfering with the metabolism.

Sanitizers are substances that simultaneously clean and disinfect. Disinfectants are frequently used in hospitals, dental surgeries, kitchens, and bathrooms to kill infectious organisms.

Bacterial endospores are most resistant to disinfectants, but some viruses and bacteria also possess some tolerance.

In wastewater treatment, a disinfection step with chlorine, ultra-violet (UV) radiation or ozonation can be included as tertiary treatment to remove pathogens from wastewater, for example if it is to be reused to irrigate golf courses. An alternative term used in the sanitation sector for disinfection of waste streams, sewage sludge or fecal sludge is **sanitisation** or **sanitization**.

Properties:-

A perfect disinfectant would also offer complete and full microbiological sterilisation, without harming humans and useful form of life, be inexpensive, and noncorrosive. However, most disinfectants are also, by nature, potentially harmful (even toxic) to humans or animals. Most modern household disinfectants contain Bitrex, an exceptionally bitter substance added to discourage ingestion, as a safety measure. Those that are used indoors should never be mixed with other cleaning products as chemical reactions can occur. The choice of disinfectant to be used depends on the particular situation. Some disinfectants have a wide spectrum (kill many different types of microorganisms), while others kill a smaller range of disease-causing organisms but are preferred for other properties (they may be non-corrosive, non-toxic, or inexpensive.

Types:-dynamics of disinfection

Air disinfectants:-

Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air. Disinfectants are generally assumed to be limited to use on surfaces, but that is not the case. In 1928, a study found that airborne microorganisms could be killed using mists of dilute bleach. An air disinfectant must be dispersed either as an aerosol or vapour at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

In the 1940s and early 1950s, further studies showed inactivation of diverse bacteria, influenza virus, and *Penicillium chrysogenum* (previously *P. notatum*) mold fungus using various glycols, principally propylene glycol and triethylene glycol. In principle, these chemical substances are ideal air disinfectants because they have both high lethality to microorganisms and low mammalian toxicity.

Although glycols are effective air disinfectants in controlled laboratory environments, it is more difficult to use them effectively in real-world environments because the disinfection of air is sensitive to continuous action. Continuous action in real-world environments with outside air exchanges at door, HVAC, and window interfaces, and in the presence of materials that adsorb and remove glycols from the air, poses engineering challenges that are not critical for surface disinfection. The engineering challenge associated with creating a sufficient concentration of the glycol vapours in the air have not to date been sufficiently addressed.

Alcohols:-

Alcohol and alcohol plus Quaternary ammonium cation based compounds comprise a class of proven surface sanitizers and disinfectants approved by the EPA and the Centers for Disease Control for use as a hospital grade disinfectant. Alcohols are most effective when combined with distilled water to facilitate diffusion through the cell membrane; 100% alcohol typically denatures only external membrane proteins. A mixture of 70% ethanol or isopropanol diluted in water is effective against a wide spectrum of bacteria, though higher concentrations are often needed to disinfect wet surfaces. Additionally, high-concentration mixtures (such as 80% ethanol + 5% isopropanol) are required to effectively inactivate lipid-enveloped viruses (such as HIV, hepatitis B, and hepatitis C). The efficacy of alcohol is enhanced when in solution with the wetting agentdodecanoic acid (coconut soap). The synergistic effect of 29.4% ethanol with dodecanoic acid is effective against a broad spectrum of bacteria, fungi, and viruses. Further testing is being performed against Clostridium difficile (C.Diff) spores with higher concentrations of ethanol and dodecanoic acid, which proved effective with a contact time of ten minutes.

Aldehydes:-

Aldehydes, such as formaldehyde and glutaraldehyde, have a wide microbiocidal activity and are sporicidal and fungicidal. They are partly inactivated by organic matter and have slight residual activity.

Some bacteria have developed resistance to glutaraldehyde, and it has been found that glutaraldehyde can cause asthma and other health hazards, hence ortho-phthalaldehydeis replacing glutaraldehyde.

Oxidizing agents:-

Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death. A large number of disinfectants operate in this way. Chlorine and oxygen are strong oxidizers, so their compounds figure heavily here.

- **Sodium hypochlorite**:- is very commonly used. Common household bleach is a sodium hypochlorite solution and is used in the home to disinfect drains, toilets, and other surfaces. In more dilute form, it is used in swimming pools, and in still more dilute form, it is used in drinking water. When pools and drinking water are said to be chlorinated, it is actually sodium hypochlorite or a related compound—not pure chlorine—that is being used. Chlorine partly reacts with proteinaceous liquids such as blood to form non-oxidizing N-chloro compounds, and thus higher concentrations must be used if disinfecting surfaces after blood spills. Commercial solutions with higher concentrations contain substantial amounts of sodium hydroxide for stabilization of the concentrated hypochlorite, which would otherwise decompose to chlorine, but the solutions are strongly basic as a result.
- Other hypochlorites such as calcium hypochlorite are also used, especially as a swimming pool additive. Hypochlorites yield an aqueous solution of hypochlorous acid that is the true disinfectant. Hypobromite solutions are also sometimes used.
- **Electrolyzed water** or "Anolyte" is an oxidizing, acidic hypochlorite solution made by electrolysis of sodium chloride into sodium hypochlorite and hypochlorous acid. Anolyte has an oxidation-reduction potential of +600 to +1200 mV and a typical pH range of 3.5—8.5, but the most potent solution is produced at a controlled pH 5.0–6.3 where the predominant oxychlorine species is hypochlorous acid.
- **Chloramine**:- is often used in drinking water treatment.
- **Chloramine-T** is antibacterial even after the chlorine has been spent, since the parent compound is a sulfonamide antibiotic.
- **Chlorine dioxide**:- is used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts. Sodium chlorite, sodium chlorate, and potassium chlorate are used as precursors for generating chlorine dioxide.
- **Hydrogen peroxide**:- is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.
- **Hydrogen peroxide vapor**:- is used as a medical sterilant and as room disinfectant. Hydrogen peroxide has the advantage that it decomposes to form oxygen and water thus leaving no long term residues, but hydrogen peroxide as with most other strong oxidants is hazardous, and solutions are a primary irritant. The vapor is hazardous to the respiratory system and eyes and should be employed where high concentrations of hydrogen peroxide are used in the workplace..
- The antimicrobial action of hydrogen peroxide can be enhanced by surfactants and organic acids. The resulting chemistry is known as Accelerated Hydrogen Peroxide. A 2% solution, stabilized for extended use, achieves high-level disinfection in 5 minutes, and is suitable for disinfecting medical

equipment made from hard plastic, such as inendoscopes. The evidence available suggests that products based on Accelerated Hydrogen Peroxide, apart from being good germicides, are safer for humans and benign to the environment.

- **Iodine**:- is usually dissolved in an organic solvent or as Lugol's iodine solution. It is used in the poultry industry. It is added to the birds' drinking water. In human and veterinary medicine, iodine products are widely used to prepare incision sites prior to surgery. Although it increases both scar tissue formation and healing time, tincture of iodine is used as an antiseptic for skin cuts and scrapes, and remains among the most effective antiseptics known. Also used as an iodophor.
- **Ozone**:- is a gas used for disinfecting water, laundry, foods, air, and surfaces. It is chemically aggressive and destroys many organic compounds, resulting in rapid decolorization and deodorization in addition to disinfection. Ozone decomposes relatively quickly. However, due to this characteristic of ozone, tap water chlorination cannot be entirely replaced by ozonation, as the ozone would decompose already in the water piping. Instead, it is used to remove the bulk of oxidizable matter from the water, which would produce small amounts of organochlorides if treated with chlorine only. Regardless, ozone has a very wide range of applications from municipal to industrial water treatment due to its powerful reactivity.
- **Peracetic acid** is a disinfectant produced by reacting hydrogen peroxide with acetic acid. It is broadly effective against microorganisms and is not deactivated by catalase andperoxidase, the enzymes that break down hydrogen peroxide. It also breaks down to food safe and environmentally friendly residues (acetic acid and hydrogen peroxide), and therefore can be used in non-rinse applications. It can be used over a wide temperature range (0-40 °C), wide pH range (3.0-7.5), in clean-in-place (CIP) processes, inhard water conditions, and is not affected by protein residues.
- **Performic acid** is the simplest and most powerful perorganic acid. Formed from the reaction of hydrogen peroxide and formic acid, it reacts more rapidly and powerfully than peracetic acid before breaking down to water and carbon dioxide.
- Potassium permanganate ($KMnO_4$) is a purplish-black crystalline powder that colours everything it touches, through a strong oxidising action. This includes staining "stainless" steel, which somehow limits its use and makes it necessary to use plastic or glass containers. It is used to disinfect aquariums and is also widely used in community swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of $KMnO_4$/water solution is kept near the pool ladder. Participants are required to step in the basin and then go into the pool. Additionally, it is widely used to disinfect community water ponds and wells in tropical countries, as well as to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution.
- **Potassium peroxymonosulfate**, the principal ingredient in Virkon, is a wide-spectrum disinfectant used in laboratories. Virkon kills bacteria, viruses, and fungi. It is used as a 1% solution in water, and keeps for one week once it is made up. It is expensive, but very effective; its pink colour fades as it is used up so it is possible to see at a glance if it is still fresh.

Phenolics:-

Phenolics are active ingredients in some household disinfectants. They are also found in some mouthwashes and in disinfectant soap and handwashes. Phenols are toxic to cats and newborn humans

- Phenol is probably the oldest known disinfectant as it was first used by Lister, when it was called carbolic acid. It is rather corrosive to the skin and sometimes toxic to sensitive people. Impure preparations of phenol were originally made from coal tar, and these contained low concentrations of other aromatic hydrocarbons including benzene, which is an IARC Group 1 carcinogen.
- *o*-Phenylphenol is often used instead of phenol, since it is somewhat less corrosive.
- Chloroxylenol is the principal ingredient in Dettol, a household disinfectant and antiseptic.
- Hexachlorophene is a phenolic that was once used as a germicidal additive to some household products but was banned due to suspected harmful effects.
- Thymol, derived from the herb thyme, is the active ingredient in some "broad spectrum" disinfectants that bears ecological claims.
- Amylmetacresol is found in Strepsils, a throat disinfectant.
- Although not a phenol, 2, 4-dichlorobenzyl alcohol has similar effects as phenols, but it cannot inactivate viruses.

Quaternary ammonium compounds:-

Quaternary ammonium compounds ("quats"), such as benzalkonium chloride, are a large group of related compounds. Some concentrated formulations have been shown to be effective low-level disinfectants. Quaternary Ammonia at or above 200ppm plus Alcohol solutions exhibit efficacy against difficult to kill non-enveloped viruses such as norovirus,rotavirus, or polio virus, Newer synergous, low-alcohol formulations are highly effective broad-spectrum disinfectants with quick contact times (3–5 minutes) against bacteria, enveloped viruses, pathogenic fungi, and mycobacteria. Quats are biocides that also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth.

Silver:-

Silver has antimicrobial properties, but compounds suitable for disinfection are usually unstable and have a limited shelf-life. Silver dihydrogen citrate (SDC) is a chelated form of silver that maintains its stability. SDC kills microorganisms by two modes of action: 1) the silver ion deactivates structural and metabolic membrane proteins, leading to microbial death; 2) the microbes view SDC as a food source, allowing the silver ion to enter the microbe. Once inside the organism, the silver ion denatures the DNA, which halts the microbe's ability to replicate, leading to its death. This dual action makes SDC highly and quickly effective against a broad spectrum of microbes. SDC is non-toxic, non-caustic, colorless, odorless, and tasteless, and does not produce toxic fumes. SDC is non-toxic to humans and animals: the United States Environmental Protection Agency classifies it into the lowest toxicity category for disinfectants, category IV.

Copper alloy surfaces:-

Copper-alloy surfaces have natural intrinsic properties to destroy a wide range of microorganisms (e.g., *E. coli* O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA),*Staphylococcus, Clostridium difficile*, influenza A virus, adenovirus, and fungi). In addition, extensive tests on E. coli O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA),*Staphylococcus, Enterobacter aerogenes*, and *Pseudomonas aeruginosa* sanctioned by the United States Environmental Protection Agency (EPA) using Good Laboratory Practices found that when cleaned regularly, some 355 different copper alloy surfaces:

- Continuously reduce bacterial contamination, achieving 99.9% reduction within two hours of exposure;
- Kill greater than 99.9% of Gram-negative and Gram-positive bacteria within two hours of exposure;
- Deliver continuous and ongoing antibacterial action, remaining effective in killing greater than 99.9% of bacteria within two hours;
- Kill greater than 99.9% of bacteria within two hours, and continue to kill 99% of bacteria even after repeated contamination;
- Help inhibit the buildup and growth of bacteria within two hours of exposure between routine cleaning and sanitizing steps.

Thymol-based disinfectant:-

Thymol, a phenolic chemical found in thyme, can be as effective as bleach in terms of disinfecting as both are considered an intermediate level disinfectant Thyme essential oils have bacteriostatic activity against a variety of microorganisms, including *E. coli* and *S. aureus*.

Other:-

The biguanide polymer polyaminopropyl biguanide is specifically bactericidal at very low concentrations (10 mg/l). It has a unique method of action: The polymer strands are incorporated into the bacterial cell wall, which disrupts the membrane and reduces its permeability, which has a lethal effect to bacteria. It is also known to bind to bacterial DNA, alter its transcription, and cause lethal DNA damage. It has very low toxicity to higher organisms such as human cells, which have more complex and protective membranes.

Ultraviolet germicidal irradiation is the use of high-intensity shortwave ultraviolet light for disinfecting smooth surfaces such as dental tools, but not porous materials that are opaque to the light such as wood or foam. Ultraviolet light is also used for municipal water treatment. Ultraviolet light fixtures are often present in microbiology labs, and are activated only when there are no occupants in a room (e.g., at night).

Common sodium bicarbonate ($NaHCO_3$) has antifungal properties, and some antiviral and antibacterial properties, though those are too weak to be effective at a home environment.

Lactic acid is a registered disinfectant. Due to its natural and environmental profile, it has gained importance in the market.

Measurements of effectiveness:-

One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly. Phenol is the standard, and the corresponding rating system is called the "Phenol coefficient". The disinfectant to be tested is compared with phenol on a standard microbe (usually *Salmonella typhi* or *Staphylococcus aureus*). Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective have a coefficient < 1.

The standard European approach for disinfectant validation consists of a basic suspension test, a quantitative suspension test (with low and high levels of organic material added to act as 'interfering substances') and a two part simulated-use surface test.

A less specific measurement of effectiveness is the United States Environmental Protection Agency (EPA) classification into either *high*, *intermediate* or *low* levels of disinfection. "High-level disinfection kills all organisms, except high levels of bacterial spores" and is done with a chemical germicide marketed as a sterilant by the U.S. Food and Drug Administration (FDA). "Intermediate-level disinfection kills mycobacteria, most viruses, and bacteria with a chemical germicide registered as a 'tuberculocide' by the Environmental Protection Agency. Low-level disinfection kills some viruses and bacteria with a chemical germicide registered as a hospital disinfectant by the EPA.

An alternative assessment is to measure the Minimum inhibitory concentrations (MICs) of disinfectants against selected (and representative) microbial species, such as through the use of microbroth dilution testing.

Home disinfectants:-

By far the most cost-effective home disinfectant is the commonly used chlorine bleach (a 5% solution of sodium hypochlorite), which is effective against most common pathogens, including difficult organisms such as tuberculosis (mycobacterium tuberculosis), hepatitis B and C, fungi, and antibiotic-resistant strains of staphylococcus and enterococcus. It even has some disinfectant action against parasitic organisms.[36]

The use of some antimicrobials such as triclosan, in particular in the uncontrolled home environment, is controversial because it may lead to the germs becoming resistant. Chlorine bleach and alcohol do not cause resistance because they are so completely lethal, in a very direct physical way.

Factor influencing disinfectants

Concentration and Potency of Disinfectants:-

With other variables constant, and with one exception (iodophors), the more concentrated the disinfectant, the greater its efficacy and the shorter the time necessary to achieve microbial kill. Generally not recognized, however, is that all disinfectants are not similarly affected by concentration adjustments. For

example, quaternary ammonium compounds and phenol have a concentration exponent of 1 and 6, respectively; thus, halving the concentration of a quaternary ammonium compound requires doubling its disinfecting time, but halving the concentration of a phenol solution requires a 64-fold (i.e., 2^6) increase in its disinfecting time.

Considering the length of the disinfection time, which depends on the potency of the germicide, also is important. This was illustrated by Spaulding who demonstrated using the mucin-loop test that 70% isopropyl alcohol destroyed 10^4 *M. tuberculosis* in 5 minutes, whereas a simultaneous test with 3% phenolic required 2–3 hours to achieve the same level of microbial kill.

Physical and Chemical Factors:-

Several physical and chemical factors also influence disinfectant procedures: temperature, pH, relative humidity, and water hardness. For example, the activity of most disinfectants increases as the temperature increases, but some exceptions exist. Furthermore, too great an increase in temperature causes the disinfectant to degrade and weakens its germicidal activity and thus might produce a potential health hazard.

Organic and Inorganic Matter:-

Organic matter in the form of serum, blood, pus, or fecal or lubricant material can interfere with the antimicrobial activity of disinfectants in at least two ways. Most commonly, interference occurs by a chemical reaction between the germicide and the organic matter resulting in a complex that is less germicidal or nongermicidal, leaving less of the active germicide available for attacking microorganisms. Chlorine and iodine disinfectants, in particular, are prone to such interaction.

Duration of Exposure:-

Items must be exposed to the germicide for the appropriate minimum contact time. Multiple investigators have demonstrated the effectiveness of low-level disinfectants against vegetative bacteria (e.g., *Listeria, E. coli, Salmonella,* VRE, MRSA), yeasts (e.g., *Candida*), mycobacteria (e.g., *M. tuberculosis*), and viruses (e.g., poliovirus) at exposure times of 30–60 seconds.

Biofilms:-

Microorganisms may be protected from disinfectants by production of thick masses of cells [428] and extracellular materials, or biofilms . Biofilms are microbial communities that are tightly attached to surfaces and cannot be easly removed. Once these masses form, microbes within them can be resistant to disinfectants by multiple mechanisms, including physical characteristics of older biofilms, genotypic variation of the bacteria, microbial production of neutralizing enzymes, and physiologic gradients within the biofilm (e.g., pH). Bacteria within biofilms are up to 1,000 times more resistant to antimicrobials than are the same bacteria in suspension . Although new decontamination methods are being investigated for removing biofilms, chlorine and monochloramines can effectively inactivate biofilm bacteria.

Number and Location of Microorganisms:-

All other conditions remaining constant, the larger the number of microbes, the more time a germicide needs to destroy all of them. Spaulding illustrated this relation when he employed identical test conditions and demonstrated that it took 30 minutes to kill 10 *B. atrophaeus* (formerly *Bacillus subtilis*) spores but 3 hours to kill 100,000 *Bacillus atrophaeus* spores. This reinforces the need for scrupulous cleaning of medical instruments before disinfection and sterilization.

Evaluation of disinfectants:-

The **Phenol coefficient**, is now largely of historical interest, although the principles upon which it is based are still used.[1] It is a measure of the bactericidal activity of a chemical compound in relation to phenol. When listed numerically, the figure expressing the disinfecting power of a substance by relating it to the disinfecting power of phenol may be a function of the standardized test performed. For example, the Rideal–Walker method, introduced in 1903 gives a Rideal–Walker coefficient and the U.S. Department of Agriculture method gives a U.S. Department of Agriculture coefficient.

To calculate phenol coefficient, the concentration of phenol at which the compound kills the test organism in 10 minutes, but not in 5 minutes, is divided by the concentration of the test compound that kills the organism under the same conditions (or, probably more common, dividing the dilution factor at which the tested substance shows activity by the dilution factor at which phenol shows comparable activity). The Rideal–Walker test was widely used, but the test conditions chosen were unrealistic, and impossibly high values for the coefficient were claimed by disinfectant manufacturers. Distinguished bacteriologist Sir Ashley Miles, reviewing the subject, described the test as "...at best a grossly over-simplified answer to a difficult problem and, at worst little short of bacteriological prostitution". Modifications were made by Dame Harriette Chick and Sir Charles James Martinin 1908. They used more realistic conditions, including 3% sterile faeces to mimic the conditions in which many disinfectants were used. The Chick–Martin test was then widely used until replaced by more suitable tests not reliant on phenol and reflecting the conditions in which modern disinfectants are used.

One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly using the Phenol coefficient. The disinfectant to be tested is compared with phenol on a standard microbe (usually Salmonella typhi or Staphylococcus aureus). Disinfectants that are more effective than phenol have a coefficient greater than 1; those that are less effective have a coefficient less than 1

Various dilutions of phenol are done on follows to determine Phenol coefficient.

Dilution	Phenol 1: 20	Distilled H_2O	Total volume	Mix discard
1 : 50	2ml	3ml	5ml	0
1 : 60	2ml	4ml	6ml	1ml
1 : 70	2ml	5ml	7ml	2ml
1 : 80	2ml	6ml	8ml	3ml
1 : 90	2ml	7ml	9ml	4ml

Disinfectant dilutions:

	1 : 25 disinfectant	H_2o Dist.	Total	Mix discard
1 : 150	1ml	5ml	6ml	1ml
1 : 175	1ml	6ml	7ml	2ml
1 : 200	1ml	7ml	8ml	3ml
1 : 225	1ml	8ml	10ml	4ml
1 : 250	1ml	9ml	10ml	5ml

Calculation of phenol coefficient method:-

Determination of Phenol Coefficient of a Given Disinfectant

Phenol	Conc.	5min	10min	15min
	1:50	+	-	-
	1:60	+	-	-
	1:70	+	-	-
	1:80	+	+	+
	1:90	+	+	+
Disinfectant Conc.				
	1:150	+	-	-
	1:175	+	-	-
	1:200	+	-	-
	1:222	+	+	+
	1:250	+	+	+

$$\text{Phenol coefficient Of Disinfectant.} = \frac{\text{Highest dilution of disinfectant killing microorganism in 10min but not in 5 mins}}{\text{Highest dil}^{n} \text{ of phenol killing micro organism in 10 min But not is 5 min.}}$$

$$= \frac{200}{70} = 2.8571$$

Therefore Phenol co-efficient of given disinfectant is = 2.8571

Antiseptic

Antiseptic(s) (from Greek ἀντί *anti*, "against and *sēptikos*, "putrefactive") are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. Antiseptics are generally distinguished from *antibiotics* by the latter's ability to be transported through the lymphatic system to destroy bacteriawithin the body, and from *disinfectants*, which destroy microorganisms found on non-living objects.

Disinfectants do not kill bacterial spores e.g., on surgical instruments; a sterilization process is required for that. Even sterilization may not destroy prions.

Some antibiotics– are true *germicides*, capable of destroying microbes (bacteriocidal), while others are bacteriostatic and only prevent or inhibit their growth.

Antibacterials are antiseptics that have the proven ability to act against bacteria. Microbicides which destroy virus particles are called viricides or antivirals.

Function:-

Bacterial growth requires a food supply, moisture, oxygen (if the bacteria are obligate aerobes), and a certain minimum temperature (see bacteriology). These conditions have been studied and dealt with in food preservation and the ancient practice of embalming the dead, which is the earliest known systematic use of antiseptics.

Evaluation of Alcohol Antisepsis

Objectives 1. To evaluate alcohol as an antiseptic.

2. Compare antiseptic effectiveness of alcohol wipes, soap and liquid hand sanitizers.

3. Quantify the effectiveness of an antiseptic and compare to other antiseptic methods.

Introduction :-The body parts of healthy humans and animals are hosts to a variety of microbes known as resident microbes. But through contact with other objects, the body also picks up other microbes known as transient microbes. For example, a typical person's hand can carry 10,000 to 10 million bacteria, some resident and some transient. When humans or animals are sick or infected with specific microbes the number of microbes may increase. Antiseptics are chemicals that are used to remove bacteria and potential pathogens from the skin. This is common in medical procedures, such as before an injection or surgery. Alcohol is a very common antiseptic. It is used in wipes ubiquitously before injections. Hand sanitizers are typically alcohol based. Many believe they are more effective than soap and water, while others do not believe this is so. The majority of alcohol-based sanitizers in the United States contain ethanol or isopropanol or a combination of these two products. Most brands also contain a moisturizer to minimize irritation to the skin. Alcohol works immediately and effectively to kill bacteria and most viruses. The antimicrobial activity of alcohol is its ability to change proteins in microorganisms. Proteins and fats on soiled hands will decrease the effectiveness of alcohol as a sanitizer. Alcohol

solutions containing 60-95% alcohol are the most effective. Higher concentrations are less potent, because proteins are not denatured easily without water. Alcohol gels work by stripping away the outer layer of oil on the skin, thereby destroying any "transient" microorganisms present on the surface of the hands. After use, re-growth of bacteria on the skin tends to occur slowly, thereby effectively keeping "residual" micro-flora that reside in deeper layers of skin from coming to the surface. To be most effective, a dimesize dollop of alcohol gel should be rubbed into the hands for 30 seconds. Chemically speaking, “soaps” are surfactants. In the cleaning process, soaps or detergents help reduce surface tension. They make water mix better with dirt and soil on surfaces and skin. Through their ability to loosen and remove soil from a surface or from skin, they contribute to good personal hygiene by reducing the presence of germs that cause infectious diseases. Plain soap is used primarily in the mechanical removal of transient microorganisms whereas antimicrobial products are used for the mechanical removal and killing or inhibition of both resident and transient microbes. Antimicrobial soaps contain an antiseptic agent to help lower the number of microbes, in addition to mechanical removal. Triclosan is the most commonly used chemical ingredient in antimicrobial soaps. In this lab we will evaluate the effectiveness of alcohol wipes, alcohol gel sanitizers and soap and water.

Lab Exercises Supplies per group of 3 3 trypticase soy agar (TSA) plates, 2 sterile cotton swabs, 1 broth of TSB

Protocol Day 1 1. Prepare lab bench by removing extraneous items and cleaning surface with table disinfectant. 2. Label one plate. Divide your plate in half, one side labeled pre-treatment, the other half, post-treatment. 3. Dip one swab into the TSB broth. Press the swab against the inside of the tube to remove excess broth; you just need it moist, not dripping. 4. Rub sterile cotton swab over your palm and between your fingers of your left hand. 5. Roll the swab over the half of plate labeled pre-treatment. 6. Select an antiseptic treatment (each group will have one wiper, one washer and one hand sanitizer). Treat your hands and let dry for 3 minutes. 7. Use the second swab, as above, dip, squeeze and then swab your right hand as you did with your left hand before. 8. Roll the swab over the half of the plate labeled post-treatment. 9. Incubate all media at 37° C for 2 days or 25° C for 5 days.

Day 2 1. Obtain your plate. 2. Count all the colonies on the half labeled pre-treatment. Record in the table below. 3. Count all the colonies on the half labeled post-treatment. Record in the table below. 4. Calculate the percent reduction from pre to post treatment. You can determine this by taking the difference in colony numbers and dividing by the number in the pre-treatment sample. Then multiply by 100 to get the percent reduction. Example if you had 10 colonies pre-treatment, and 6 colonies post-treatment, then you would have the difference of 4 colonies. Take 4 divide by 10. Multiply by 100. This would be 40% reduction. 5. Compare with the other treatments in your group.

Sterilization method & their equipment

Sterilization (or **sterilization**) is a term referring to any process that eliminates (removes) or kills (deactivates) all forms of life and other biological agents (such as prions, as well as viruses which some do not consider to be alive but are biological pathogens nonetheless), including transmissible agents (such as fungi, bacteria, viruses, prions, spore forms, unicellular eukaryotic organisms such as Plasmodium, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media. Sterilization can be achieved with one or more of the

following: heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization in that sterilization kills, deactivates, or eliminates all forms of life and other biological agents.

Method:-sterilization by heat.

Moist heat: - A widely used method for heat sterilization is the autoclave, sometimes called a converter or steam sterilizer. Autoclaves use steam heated to 121-134 °C under pressure. To achieve sterility, the article is heated in a chamber by injected steam until the article reaches a time and temperature setpoint. The article is then held at that set point for a period of time which varies depending on the disburden present on the article being sterilized and its resistance (D-value) to steam sterilization. A general cycle would be anywhere between 3 and 15 minutes, (depending on the generated heat)[10] at 121 °C at 100 kPa, which is sufficient to provide a sterility assurance level of 10^{-4} for a product with a bioburden of 10^{6} and a D-value of 2.0 minutes, Following sterilization, liquids in a pressurized autoclave must be cooled slowly to avoid boiling over when the pressure is released. This may be achieved by gradually depressurizing the sterilization chamber and allowing liquids to evaporate under a negative pressure, while cooling the contents.

For autoclaving, cleaning is critical. Extraneous biological matter or grime may shield organisms from steam penetration. Proper cleaning can be achieved through physical scrubbing, sonication, ultrasound or pulsed air, Pressure cooking and canning are analogous to autoclaving, and when performed correctly renders food sterile.

Moist heat causes destruction of micro-organisms by denaturation of macromolecules, primarily proteins. This method is a faster process than dry heat sterilization. Proper autoclave treatment will inactivate all resistant bacterial spores in addition to fungi, bacteria, and viruses, but is not expected to eliminate all prions, which vary in their resistance. For prion elimination, various recommendations state 121-132 °C for 60 minutes or 134 °C for at least 18 minutes.

Dry heat sterilization:- Dry heat was the first method of sterilization, and is a longer process than moist heat sterilization. The destruction of microorganisms through the use of dry heat is a gradual phenomenon. With longer exposure to lethal temperatures, the number of killed microorganisms increases. Forced ventilation of hot air can be used to increase the rate at which heat is transferred to an organism and reduce the temperature and amount of time needed to achieve sterility. At higher

temperatures, shorter exposure times are required to kill organisms. This can reduce heat-induced damage to food products.

The standard setting for a hot air oven is at least two hours at 160 °C. A rapid method heats air to 190 °C for 6 minutes for unwrapped objects and 12 minutes for wrapped object. Dry heat has the advantage that it can be used on powders and other heat-stable items that are adversely affected by steam (e.g. it does not cause rusting of steel objects). The standard setting for a hot air oven is at least two hours at 160 °C. A rapid method heats air to 190 °C for 6 minutes for unwrapped objects and 12 minutes for wrapped objects.[14][15] Dry heat has the advantage that it can be used on powders and other heat-stable items that are adversely affected by steam (e.g. it does not cause rusting of steel objects).

Flaming:-

Flaming is done to loops and straight-wires in microbiology labs. Leaving the loop in the flame of a Bunsen burner or alcohol lamp until it glows red ensures that any infectious agent gets inactivated. This is commonly used for small metal or glass objects, but not for large objects). However, during the initial heating infectious material may be "sprayed" from the wire surface before it is killed, contaminating nearby surfaces and objects. Therefore, special heaters have been developed that surround the inoculating loop with a heated cage, ensuring that such sprayed material does not further contaminate the area. Another problem is that gas flames may leave carbon or other residues on the object if the object is not heated enough. A variation on flaming is to dip the object in 70% or higher ethanol, then briefly touch the object to a Bunsen burner flame. The ethanol will ignite and burn off rapidly, leaving less residue than a gas flame.

Incineration:- Incineration is a waste treatment process that involves the combustion of organic substances contained in waste materials. This method also burns any organism to ash. It is used to sterilize medical and other biohazardous waste before it is discarded with non-hazardous waste. Bacteria incinerators are mini furnaces used to incinerate and kill off any micro organisms that may be on an inoculating loop or wire.

Tyndallization:- Named after John Tyndall, Tyndallization is an obsolete and lengthy process designed to reduce the level of activity of sporulating bacteria that are left by a simple boiling water method. The process involves boiling for a period (typically 20 minutes) at atmospheric pressure, cooling, incubating for a day, then repeating the process a total of three to four times. The incubation periods are to allow heat-resistant spores surviving the previous boiling period to germinate to form the heat-sensitive vegetative (growing) stage, which can be killed by the next boiling step. This is effective because many spores are stimulated to grow by the heat shock. The procedure only works for media that can support bacterial growth, and will not sterilize non-nutritive substrates like water. Tyndallization is also ineffective against prion.

Pasteurization: - Unlike sterilization, pasteurization is not intended to kill all microorganisms in the food. Instead, it aims to reduce the number of viable pathogens so they are unlikely to cause disease (assuming the pasteurized product is stored as indicated and is consumed before its expiration date). Commercial-scale sterilization of food is not common because it adversely affects the taste and quality of the product. Certain foods, such as dairy products, may be superheated to ensure pathogenic microbes are destroyed.

Chemical sterilization:-

Chemicals are also used for sterilization. Heating provides a reliable way to rid objects of all transmissible agents, but it is not always appropriate if it will damage heat-sensitive materials such as biological materials, fiber optics, electronics, and many plastics. In these situations chemicals, either as gases or in liquid form, can be used as sterilants.

Ethylene oxide

Ethylene oxide (EO,EtO) gas treatment is one of the common methods used to sterilize, pasteurize, or disinfect items because of its wide range of material compatibility. It is also used to process items that are sensitive to processing with other methods, such as radiation (gamma, electron beam, X-ray), heat (moist or dry), or other chemicals. Ethylene oxide treatment is the most common sterilization method, used for approximately 70% of total sterilizations, and for over 50% of all disposable medical devices.

Ethylene oxide treatment is generally carried out between 30 °C and 60 °C with relative humidity above 30% and a gas concentration between 200 and 800 mg/l. typically; the process lasts for several hours. Ethylene oxide is highly effective, as it penetrates all porous materials, and it can penetrate through some plastic materials and films. Ethylene oxide kills all known microorganisms such as bacteria (including spores), viruses, and fungi (including yeasts and molds), and is compatible with almost all materials even

when repeatedly applied. It is flammable, toxic and carcinogenic, however, with a reported potential for some adverse health effects when not used in compliance with published requirements. Ethylene oxide sterilizers and processes require biological validation after sterilizer installation, significant repairs or process changes.

Nitrogen dioxide

Nitrogen dioxide (NO_2) gas is a rapid and effective sterilant for use against a wide range of microorganisms, including common bacteria, viruses, and spores. The unique physical properties of NO_2 gas allow for sterilant dispersion in an enclosed environment at room temperature and ambient pressure. The mechanism for lethality is the degradation of DNA in the spore core through nitration of the phosphate backbone, which kills the exposed organism as it absorbs NO_2. This degradation occurs at even very low concentrations of the gas NO_2 has a boiling point of 21 °C at sea level, which results in a relatively high saturated vapor pressure at ambient temperature.

Ozone

Ozone is used in industrial settings to sterilize water and air, as well as a disinfectant for surfaces. It has the benefit of being able to oxidize most organic matter. On the other hand, it is a toxic and unstable gas that must be produced on-site, so it is not practical to use in many settings. The high reactivity of ozone means that waste ozone can be destroyed by passing over a simple catalyst that reverts it to oxygen and ensures that the cycle time is relatively short.

Glutaraldehyde and formaldehyde

Glutaraldehyde and formaldehyde solutions (also used as fixatives) are accepted liquid sterilizing agents, provided that the immersion time is sufficiently long. To kill all spores in a clear liquid can take up to 22 hours with glutaraldehyde and even longer with formaldehyde. The presence of solid particles may lengthen the required period or render the treatment ineffective. Sterilization of blocks of tissue can take much longer, due to the time required for the fixative to penetrate Glutaraldehyde and formaldehyde are volatile, and toxic by both skin contact and inhalation. Glutaraldehyde has a short shelf life (<2 weeks), and is expensive.

Radiation sterilization:-

Sterilization can be achieved using electromagnetic radiation such as electron beams, X-rays, gamma rays, or irradiation by subatomic particles. Electromagnetic or particulate radiation can be energetic enough to ionize atoms or molecules (ionizing radiation), or less energetic (non-ionizing radiation).

Non-ionizing radiation sterilization

Ultraviolet light irradiation (UV, from a germicidal lamp) is useful for sterilization of surfaces and some transparent objects. Many objects that are transparent to visible light absorb UV. UV irradiation is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas, including areas under dirt (which may become polymerized after prolonged irradiation.

Ionizing radiation sterilization

Gamma radiation is very penetrating, and is commonly used for sterilization of disposable medical equipment, such as syringes, needles,cannulas and IV sets, and food. It is emitted by a radioisotope, usually Cobalt-60(^{60}Co) or caesium-137 (^{137}Cs)

X-rays: high-energy X-rays allow irradiation of large packages and pallet loads of medical devices. They are sufficiently penetrating to treat multiple pallet loads of low-density packages with very good dose uniformity ratios. X-ray sterilization does not require chemical or radioactive material: high-energy.

Sterile filtration

Fluids that would be damaged by heat, irradiation or chemical sterilization, such as drug products, can be sterilized by microfiltration using membrane filters. This method is commonly used for heat labile pharmaceuticals and protein solutions in medicinal drug processing. A microfilter with pore size 0.2 µm will usually effectively remove microorganisms.

Validation of sterilization

Sterilization processes require periodic **validation** to demonstrate that they are working correctly and functioning within established norms.

Such validation entails detailed **measuring** of various physical parameters throughout the **sterilization process** and assessing and comparing these results to relevant international **standards**.

To ensure end-users, which include hospitals, clinics and care institutions, the seal of approval must be issued by a qualified entity with worldwide recognition.

Bureau Veritas is a leading partner of choice with a 15-year track record of performing global **sterilization process validation**.

Solution for sterilization process:-

Bureau Veritas validates the following **sterilization processes**:

- **Steam sterilizers**
- **Hot air sterilizers, including infrared sterilisation**
- **Ethylene oxide sterilizers**

Dry heat sterilization validation:-

Dry heat is one of the most commonly used methods to sterilize and/or depyrogenate pharmaceutical components and products. Dry heat sterilization is often used for heat-stable oils, ointments and powders. Most often, depyrogenation of parenteral containers is performed utilizing a dry heat oven. The depyrogenation process is also utilized on certain heat-stabile components, glass containers, metal

equipment, etc. to render the item and final parenteral product free of pyrogens. The equipment utilized to provide the dry heat medium must be validated to ensure that the system is able to provide sterile and/or depyrogenated components, on a reproducible basis. The validation of a dry heat sterilization and depyrogenation process involves approaches and procedures which parallel those utilized for steam sterilization. The efficiency of any heat treatment is determined by the design and source of the heat. Hot air is substantially less efficient in a thermal transfer medium as compared to steam. The validation effort must include heat distribution, heat penetration, bioburden and pyroburden determination, filter integrity, and microbial/end otoxin challenges.

General Considerations

Two types of dry-heat sterilization systems are utilized in the pharmaceutical industry today. They are the conventional hot air oven and the tunnel system. The major difference between the two systems, as far as validation is concerned, is the belt or line speed variable with the tunnel system. The key to validating a dry-heat sterilizer is to prove its repeatability. This means that the unit can consistently perform under a given set of conditions to generate materials that are sterile, pyrogen-free, and particulate-free. Repeatability in dry-heat sterilization obviously involves consistency and reliability in attaining and maintaining a desired temperature. The desired temperature must be reached in all areas of the heating chamber. There will always be an area in the chamber that represents a cold spot; that is, an area that is most difficult to heat up to the desired temperature. This cold spot must be identified so that validation studies involving thermocouple monitoring and microbial challenges can be done at this location.

As with any sterilization process, the first step in dry-heat sterilizer validation involves qualification of all the equipment and instrumentation used. This step includes examination and documentation of all utilities, ductwork, filters, and control valves or switches for the oven or tunnel unit, and the calibration of the instrumentation used in validating and monitoring the process. The instruments used are as follows:

1. Temperature recorders and thermocouples
2. Constant-temperature baths
3. Amp meters
4. Monometers
5. Dioctylphthalate generators
6. Particle counters
7. Velometers
8. Tachometers