

PUSHPENDRA PATEL

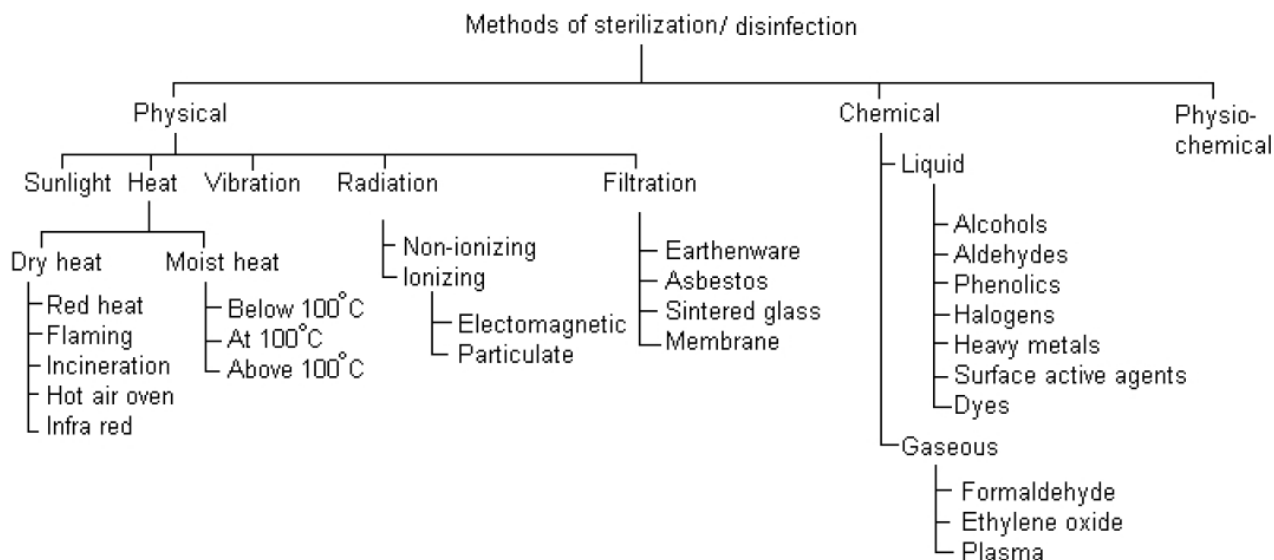
GPAT-MICROBIOLOGY

DISINFECTANT & STERILIZATION

1. Disinfection is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects. Disinfection can be achieved by physical or chemical methods. Chemicals used in disinfection are called disinfectants. Different disinfectants have different target ranges, not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out.

2. Methods of disinfection

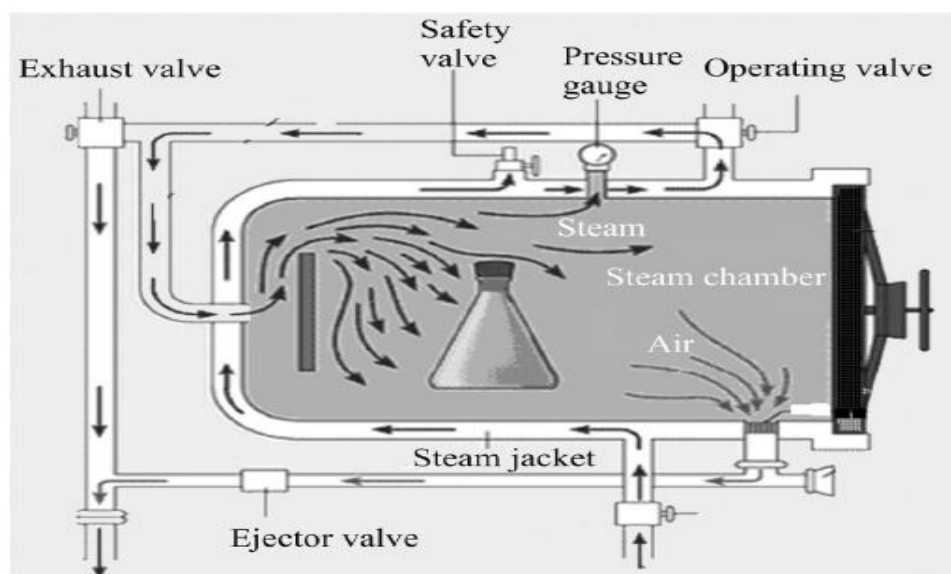
Solution
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Mechanism of action of chemical disinfectant

- A. Adsorption on the microbes' surface
 - B. Diffusion through the surface
 - C. Binding to the vulnerable sites (e.g. plasma membrane, cytoplasmic proteins, nucleic acids, and so on)
 - D. Disruption of the vulnerable sites
 - E. Injury and death of the microbes
3. Heat acts by oxidative effects as well as denaturation and coagulation of proteins.
 4. Dry heat acts by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes.
 5. The moist heat acts by coagulation and denaturation of proteins. Moist heat is superior to dry heat in action
 6. **Thermal death time** is the minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.
 7. Articles such as soiled dressings; animal carcasses, pathological material and bedding etc should be subjected to incineration
 8. maintenance of the sterilizing temperature for a defined period (holding time)

9. Different temperature-time relations for holding time are 60 minutes at 160°C, 40 minutes at 170°C and 20 minutes at 180°C.
10. Increasing temperature by 10 degrees shortens the sterilizing time by 50 percent.
11. Biological Validation: 10^6 spores of *Bacillus subtilis* var niger or *Clostridium tetani* on paper strips are placed inside envelopes and then placed inside the hot air oven.
12. Pasteurization: This process was originally employed by Louis Pasteur.
13. There are two methods of pasteurization, the holder method (heated at 63°C for 30 minutes) and flash method (heated at 72°C for 15 seconds) followed by quickly cooling to 13°C.
14. The killing activity of moist heat can be enhanced by addition of 2% sodium bicarbonate. Water boils at 100°C at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases
15. At a pressure of 15 lbs inside the autoclave, the temperature is said to be 121°C.



16. Biological method includes a paper strip containing 10^6 spores of *Geobacillus stearothermophilus*
17. Two types of radiation are used, ionizing and non-ionizing. Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power. Since radiation does not generate heat, it is termed "cold sterilization". In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent
18. Rays of wavelength longer than the visible light are non-ionizing. Microbicidal wavelength of UV rays lie in the range of 200-280 nm, with 260 nm being most effective.
19. Membrane filters with pore sizes between 0.2-0.45 μm are commonly used to remove particles from solutions that can't be autoclaved
20. Earthenware filters: These filters are made up of diatomaceous earth or porcelain.
21. Asbestos filters: These filters are made from chrysotile type of asbestos, chemically composed of magnesium silicate.

22. Membrane filters: These filters are made from a variety of polymeric materials such as cellulose nitrate, cellulose diacetate, polycarbonate and polyester. The older type of membrane, called gradocol.
23. Air can be filtered using HEPA (High Efficiency Particle Air) filters. They are usually used in biological safety cabinets. HEPA filters are at least 99.97% efficient for removing particles $>0.3 \mu\text{m}$ in diameter.
24. Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.
Examples: Ethyl alcohol, isopropyl alcohol and methyl alcohol
25. ALDEHYDES: Mode of action: Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleic acids. It kills all microorganisms, including spores. Examples: Formaldehyde, Gluteraldehyde
26. Phenol act by disruption of membranes, precipitation of proteins and inactivation of enzymes. Examples: 5% phenol, 1-5% Cresol, 5% Lysol (a saponified cresol), hexachlorophene, chlorhexidine, chloroxylonol (Dettol)
27. Halogen are oxidizing agents and cause damage by oxidation of essential sulfhydryl groups of enzymes. Chlorine reacts with water to form hypochlorous acid, which is microbicidal. Examples: Chlorine compounds (chlorine, bleach, hypochlorite) and iodine compounds (tincture iodine, iodophores)
28. Heavy Metals act by precipitation of proteins and oxidation of sulfhydryl groups. They are bacteriostatic. Examples: Mercuric chloride, silver nitrate, copper sulfate, organic mercury salts (e.g., mercurochrome, merthiolate)
29. **Rideal Walker method:** This method relies on the estimation of phenol coefficient. bactericidal activity is determined against *Salmonella typhi* suspension.
30. Chick Martin test: This test also determines the phenol coefficient of the test disinfectant.

	Rideal -Walker	Chick-Martin
Volume medium	5.0 ml	10.0 ml
Diluent for test disinfectant	Water	Yeast suspension
Reaction temperature	$17.5 \pm 0.5^\circ\text{C}$	30°C
Organism	<i>Salmonella typhi</i>	<i>Salmonella typhi</i> , <i>Staphylococcus aureus</i>
Sampling times	2.5, 5.0, 7.5, 10.0 min.	30.0 min.
Calculation of coefficient	Dilution test killing in 7.5 min divided by same for phenol	Mean concentration of phenol showing no growth after 30 min. divided by same for test

The classical tests such as Rideal - Walker or Chick - Martin are not practicable.

31. Assay of antibiotics- For their potency and efficacy
32. Site of action of antibiotics

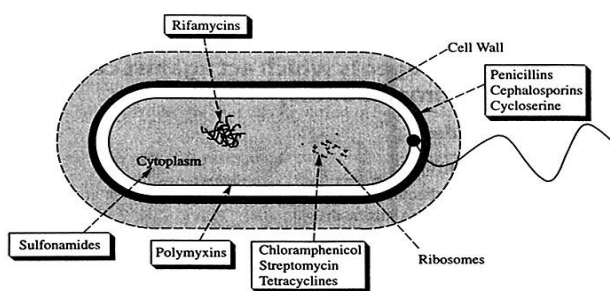
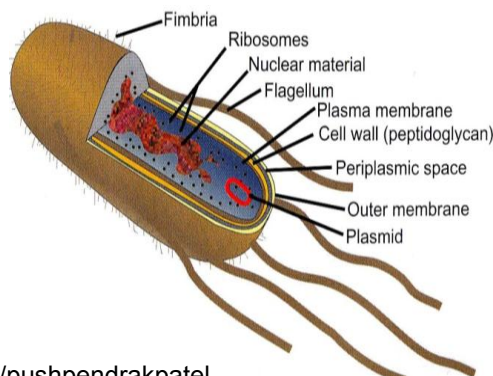
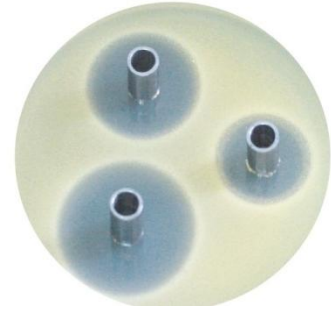


Fig. 14.5 Sites of antibacterial action.



33. The potency of an antibiotic is estimated by comparing the inhibition of growth of sensitive micro-organisms produced by known concentrations of the antibiotic to be examined and a reference substance.
34. Two types of assay- diffusion and turbidity methods.
35. **Kirby-Bauer antibiotic testing (KB testing or disc diffusion antibiotic sensitivity testing)** is a test which uses antibiotic-impregnated wafers to test whether bacteria are affected by antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition.



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MICROBIOLOGY

- Identify the correct definition of an antiseptic:
 A-Chemicals that kill or prevent infection and damage living tissues
B Chemicals that kill or prevent infection without damaging living tissues
 C Chemicals that can only kill or prevent infection on non-living tissues
 D Chemicals that can only kill or prevent infection on animal housing
- Identify the term that describes a disinfectant that can kill bacteria:
 A. **Bactericidal** B. Bacteriostatic C. Pathogen D. Bacteriosis
- Identify the term that is used to ensure surgical instruments are free from micro-organisms:
 A. Disinfected B. Cleaned C. Debrided **D. Sterilization**
- Sterilization involves the use of a physical or chemical procedure to:
 A. Removes visible soil from surgical instruments
 B. Destroy non-pathogenic organisms
C. Destroy all forms of microbial life including highly resistant bacteria spores
 D. Destroy all forms of microbial life except highly resistant bacteria spores
- The most widely used, effective, economical and reliable method of sterilization used in the health care setting is:
 A. Gas plasma B. Ethylene oxide C. Peracetic acid **D. Steam**
- The essential parameters of steam sterilization are:
 A. Securely wrapped packages
B. Time, temperature, saturated steam and pressure
 C. Chemical and biological indicators D. Both A and C
- Ethylene Oxide is a highly toxic agent, which destroys microorganisms by a process called-
 A. Cavitation B. Oxidation C. Osmosis **D. Alkylation**
- Halogen is an example of –
 A. Physical **B. Chemical** C. Biological D. None of them
- What is holding time

A. Maintenance of the sterilizing temperature for a defined period

- B. Hold the substance as per their properties
- C. Technique how to sterilize the object
- D. How to kill the microorganism

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10. Bacillus subtilis var niger or Clostridium tetani on paper strips are used for
A. Validation B. Sterilization C. Disinfection D. Separation
11. Killing activity of the water in autoclave can be increased by adding-
A. Sodium carbonate B. **Sodium bicarbonate** C. Sodium salicylate D. Ammonia
12. "Cold sterilization". Is another name for-
A. Gas **B. Radiation** C. Ice Killing D. Filtration
13. Cup-Plate is used for-
A. Sterilization **B. Assay** C. Validation D. Agar plate preparation
14. Assay of Ampicillin is done by
A. **Micrococcus luteus** B. Pseudomonas aeruginosa C. None D. Bacillus subtilis
15. Staphylococcus aureus is used for
A. Tetracycline **B. Bitadine** **C. Diabetic** **D. Antianxiety**
16. Culture media can be sterilized by
A. Hot Air oven B. Filtration **C. Autoclave** D. Ionization
17. RW test is performed for-
A. Phenols **B. Disinfectants** C. Antibiotics D. Remand Factor
18. Which of below factor affect the efficacy of disinfectants-
A. Temperature B. Time C. Organic Matter **D. All**
19. Dyes affect which part of bacteria-
A. **Nucleic acid** B. Cell C. Mitochondria D. None
20. Term "Absolute" is used for-
A. Autoclave **B. Sterilization** C Disinfection D. Both B and C