UNIT – IV CONTROL OF MICROORGANISMS

SATHYABAMA UNIVERSITY **FACULTY OF BIO AND CHEMICAL ENGINEERING**

SBT1103	MICROBIOLOGY	L	T	P	Credits	Total Marks
		3	0	0	3	100

COURSE OBJECTIVES

- To enable students to learn about the principles of Microbiology to emphasize structure and biochemical aspects of various micro organisms.
- To know the control and preventive measures of microbial infections and environmental pollutions.

UNIT 1 INTRODUCTION TO MICROBIOLOGY **11 Hrs.**

Introduction, History and scope of microbiology, Contributions of Leewenhoek, Pasteur, Koch, Jenner and Fleming, Microbial classification: Classical and Current systems, Methods of identifying microbes.

Basics of Microscopy, Staining: simple, differential (Gram staining, Acid fast staining), special staining (flagella,capsule,endospore)

UNIT 2 MICROBIAL STRUCTURE AND REPRODUCTION **9 Hrs.**

Morphology and Reproduction: Bacteria - General structure and forms, Reproduction methods - Fission, budding and sporulation, Virus - TMV, HIV & T4 bacteriophage - lytic, lysogenic cycle, Fungi - Fungal morphology - Mycelial and yeast forms - sexual and asexual Reproduction, Actinomycete

UNIT 3 MICROBIAL GROWTH AND PHYSIOLOGY **7 Hrs.**

Microbial Growth and Nutrition, Types of media - Based on Consistency, Nutritional components, Funtional uses and application, Microbial types based on nutrition, Growth of microbes in culture - Pure culture techniques, Batch & Continous - Growth curve - Enumeration methods , Types of fungal growth media. Aerobic and Anaerobic metabolism of sugars, mixed acid fermentation.

UNIT 4 CONTROL OF MICROORGANISMS **9 Hrs**

Definitions of frequently used terms - Pattern or Rate of Microbial Death, Physical methods of Microbial Control: Heat (Moist & Dry), Low temperature, Filtration, High pressure, Desiccation, Osmotic pressure, Radiation. Chemical methods of Microbial Control: Liquids - Alcholos, Aldehydes, Phenolics, Halogens - Heavy metals, Surface active agents & Dyes, Gases - Formaldehyde, Ethylene Oxide, Plasma - Physico-chemical methods - Chemotherapeutic agents - Evaluation of effectiveness of antimicrobial agents. Difference between cleaning - sanitizing - sterilizing agents. Moist heat sterilization: D, Z and F Values and significance.

UNIT 5 APPLICATIONS OF MICROBIOLOGY **9 Hrs.**

Microbial ecology: Microbe-Microbe interaction - Mutualism, Commensalism, Altruism, Microbe - host interactions - Colonization and Infection- Causes and Transmission of Infectious Diseases, Emerging and re-emerging infectious diseases - Mechanism and examples, Multidrug resistance - MRSA, Diagnostic Microbiology, Childhood and adult vaccinations - MMR, Polio, Rabies etc, bioterrorism agents, Biofilm - Quorum sensing,

Max. 45 Hours.

TEXT / REFERENCE BOOKS

1. Pelczar, Jr E.C.S Chan and noel R.Krieg, Microbiology, 5th edition Tata McGrawHill -2006
2. Joanne M. Willey, Linda Sherwood, Christopher J. Woolverton, Prescott's Microbiology, 8th Edition, McGraw-Hill Higher Education, 2008
3. Jawetz, Melnick and Adelberg's Medical Microbiology . McGraw-Hill Medical, 2007
4. University of South Carolina School of Medicine (http://pathmicro.med.sc.edu/book/bact-sta.htm)

END SEMESTER EXAMINATION QUESTION PAPER PATTERN

Max Marks : 80 **Exam Duration : 3 Hrs.**

PART A : 10 questions of 2 marks each - No choice **20 Marks**

PART B : 2 questions from each unit of internal choice; each carrying 12 marks **60 Marks**

Sterilization is the process of killing all forms of microbial life in or on the given object or preparation. Microbiologically, sterile material is one that contains no living organisms at all and the term sterile is therefore an absolute one. Accordingly, an object is sterile or non sterile but it can never be semisterile or almost sterile.

DEFINITIONS OF TERMS RELATED TO STERILIZATION:

1. Antiseptic: A substance that arrests sepsis i.e prevents the growth or action of microorganisms by inhibiting their activity without necessarily destroying them. These can be applied on human body.
2. Bactericide: An agent that kills bacteria.
3. Bacteriostat: An agent that arrests or retards the growth of bacteria.
4. Disinfection: A process that removes infection potential by destroying microorganisms but not ordinarily bacterial spores.
5. Disinfectants: These are generally meant for application on inanimate objects.
6. Germicides: A substance that kills disease microorganisms (i.e. pathogens / germs) but not necessarily bacterial spores.
7. Sterility: The absence of viable organisms.
8. Viable: Live and growing bacteria (or microorganisms) + spores
9. Vegetative microorganisms: Growing organisms.

Sterilization Parameters:

Now let us have a simple overview regarding the sterilization parameters which help us in better understanding of the sterilization processes.

The exponential relationship between number of organisms living and the extent of treatment is studied from the inactivation kinetics of pure culture of organisms by exposing them to physical or chemical sterilization procedures. Survivor curves have been used to develop inactivation data for a specific sterilization procedure using suitable biological indicator. This data is helpful in establishing a sterilizing regimen for a specific preparation.

D value: It is the parameter calculated as the time taken for one log (90%) reduction in the number of microorganisms.

Z value: It calculates the temperature or dose of radiation sterilization required to produce a one log (90%) reduction in D value for a particular organism.

F value: This value is used to compare the lethality of different heat sterilization procedures.

METHODS OF STERILISATION:

Sterilization process can be basically separated as terminal and non terminal process based on the stage at which the preparation is subjected to the process pf sterilization.

Terminal Sterilization: Different types of terminal sterilization techniques employed are Physical sterilization and Chemical sterilization.

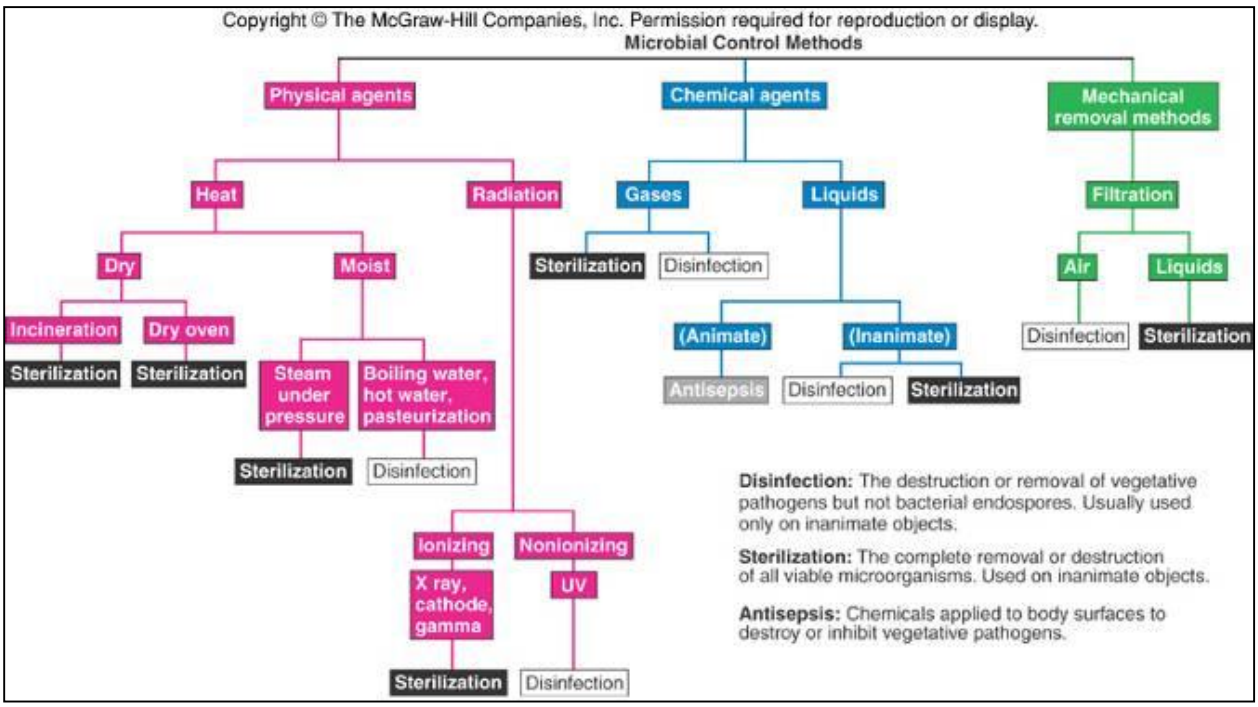
Non terminal Sterilization: It includes the filtration procedure.

Based on the principle of mechanism involved in the process they are further classified as follows:

Physical Sterilization: This class includes heat sterilization and radiation sterilization as well. Heat sterilization procedure is the one in which the destruction of microorganisms mainly occurs due to the high temperatures employed. This process includes
(a). Moist heat sterilization.
(b). Dry heat sterilization.

Radiation Sterilization: This process is accomplished by exposure to ultraviolet (UV) light or high-energy ionizing radiation such as gamma rays and accelerated electrons i.e particulate radiation as well..

Chemical sterilization: The procedures which involve treatment of the preparations to be sterilized with certain chemicals in either gaseous form or in liquid form are categorized under this class.



General Consideration for Microbial Growth

- Decontamination Methods
  - Relative Resistance of Microbial Forms
    - Highest Resistance
      - Endospores of bacteria
    - Moderate Resistance
      - Mycobacteria
      - Cysts of protozoa
      - Vegetative protozoa
      - Gram- bacteria
    - Fungi, including most fungal spores.
    - Least Resistance
      - Viruses without envelopes.
      - Gram+ bacteria.
      - Viruses with lipid envelopes. (least resistance)
- General Terms
  - Sterilization: a process that kills all life, including viruses & endospores.
  - Disinfection: a process of possibly killing or reducing growth of microbes on a non-living surface.
  - Sanitization: any cleaning technique that removes dirt and usually microbes.
  - Antisepsis: a process of killing or reducing growth of microbes on living tissue.
  - Chemotherapy: process of treating a disease with chemicals inside the host.
  - Bacteriocidal: a substance that kills bacteria.
  - Bacteriostatic: a substance that kills the growth of bacteria. If the substance is removed, bacteria resumes growth.
  - Antibiotic: a natural substance produced by one organism (usually a microbe) in tiny quantities to kill or inhibit another.
  - Aseptic: microbe-free, asepsis prevents entry of pathogens into host.
  - Pasteurized: heat-treated to reduce numbers of microbes and destroy pathogens (eg liquids).
  - Sanitary: safe and clean, pathogen-free non-living objects (eg water).
  - Fungicidal/static, Virucidal/static, aglicidal/static, pesticidal/static.
- Microbial Death
  - The permanent loss of reproductive capability, even under optimum growth conditions, has become the accepted microbiological definition of death.

        - Most practical way to detect damage/cell death for microorganisms is by exposing it to a suitable environment and determine if it can reproduce.
    - Most susceptible: cells to microbicidal agent are younger, actively growing cells.
    - Least susceptible: older, inactive cells.
    - Factors of Microbial Death
        - Temperature: if temp goes up or down , so does metabolism. Diffusion rates!
            - Cold & slow growing cells are going to be hard to kill.
        - Environment:
            - pH , Chemical interference.
            - Organic matter: chemical interference and may protect microbes.
            - Presence of solvents and other molecules.
        - Number of Microbes
        - Age & Stage in Life Cycle of Microbe: actively growing/dormant.
        - Cell Structure & function: bacerial cell wall type.
            - Gram+ or Gram- or wall-less: Mycobacteria (mycolic acid wall), endospores, cysts, spores/eucaryotes.

How Microbial Agents Work: Their Modes of Action

- Mode (or mechanism) of action: antimicrobial agent's adverse effect on cells.
- Agents on the Cell Wall
    - Chemical agents used to damage cells wall by blocking its synthesis, digesting it, or breaking down its surface.
        - Result in fragile cells which later lyses very easily.
    - Anitibial drugs (penicillin): interfere with synthesis of cell wall in some bacteria.
    - Detergents and alcohols can disrupt cell walls especially gram-negative bacteria.
- Agents on the Cell Membrane
    - Surfactant: detergents that work by lowering the surface tension of cell membranes.
        - Surfactants are polar molecules with hydrophilic regions that can bind to membrane and penetrate internal hydrophobic regions.
        - Similar structure to lipids in membrane, but they act as replacements with lower tension.
- Agents on Protein and Nucleic Acid Synthesis

    - Some agents used to inhibit ability to reproduce and metabolize by inhibiting protein synthesis.
        - Chlororamphenicol: a microbiotic that binds to the ribosomes of bacteria in a way that stops peptide bonds from forming.
    - Some agents impede transcription or translation.
        - An agent can bind to DNA and prevent both transcription and and translation.
        - Others are mutagenic.
            - X-radiation causes mutation that result in permanent inactivation of DNA.
        - Formaldehyde and ethylene oxide interfere with DNA and RNA function.
- Agents That Alter Protein Function
    - Denature: disrupt proteins by bonds of secondary or tertiary structure are broken.
        - Example: coagulation by moist heat.
        - Chemicals: organic solvents (alcohol, acids) or phenolics.
        - Metalic Ions
    - Methods of inhibiting metabolism.
- Practical Concerns
    - 1. Must spores be destroyed or only vegetative pathogens?
    - 2. Re-usability?
        - If reusable, it must be a quick and least expensive method.
            - Can it withstand head, pressure, radiation, or chemicals?
    - 3. Practical application?
    - 4. Penetrate to the necessary extent?
    - 5. Safe

With the advent of the germ theory of disease, it became obvious that disease could be spread by organisms too small for the eye to see. Pioneers such as Ignaz Semmelweis and Joseph Lister utilized techniques such as the washing of hands and disinfecting of surfaces to decrease the likelihood of infection. In time, hospitals, clinics, and laboratories began to adopt these methods and improve upon them.

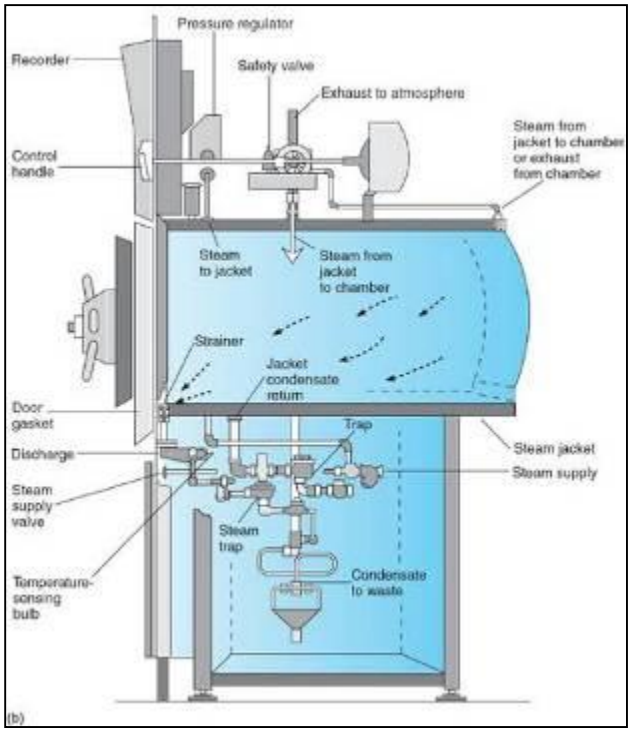
Methods used to control the growth of microbial growth can be placed into two broad categories, physical and chemical. **Physical methods** either exclude microbes, or reduce their numbers in a solution, or on the surface of a **fomite** (any nonliving material

which might come into contact with the individual) . **Chemical methods** involve the application of specific chemical agents which inhibit growth or kill microbes on fomites or the surface of skin. The selection of an appropriate technique is important, since many physical and chemical agents can cause damage to the cells and tissues of the individual as well as the microbe.

Agents of microbial control either sterilize or disinfect. **Sterilizing agents** kill all living things, thus removing the living source of contagion. **Disinfecting agents** kill some microbes, but inhibit the growth of others. Most techniques only provide disinfection. Also, several factors influence the effectiveness of any method of microbial control. These include population size, susceptability of the microorganism to the agent, concentration of the dose used, and the duration of treatment.

Physical Methods

- Physical agents: physical means to destroy or remove contaminant.
- Heat
    - Elevated temperatures are microbicidal where lowered temperatures are microbistatic.
    - Moist Heat (hot water, boiling water, steam)
        - Range: 60-135 degrees Celsius
        - Most heat works faster than dry heat; at the same length of time, moist heat kills cells at lower temp.
        - Moist heat denatures cell proteins.
        - Membranes, ribosomes, RNA, and DNA are also damaged by moist heat.



- Dry Heat (flame or electric coil)
  - Range: 160-several thousand degrees Celsius.
  - Oxidizes cells.
  - Dehydrates cell components.
  - Can denatures protein and DNA, but requires more heat than moist heat due to proteins more stable in dry heat.

- Heat Resistance
  - Endospore
    - Endospores have greatest resistance to heat.
    - Spores of B. antrhacis requires boiling water at 100 degrees Celsius for minutes.
    - Thermophilic spores can take hours.
  - Vegetative Cells (including yeasts, molds, and other spores)
    - Moist heat: 50-60 degrees Celsius for 60 minutes.
    - All non-heat resistant forms of bacteria, yeast, molds, protozoa, worms, and viruses destroyed by exposure to 80 degrees Celsius for 20 minutes.
  - Viruses
    - Relatively resistant to heat.
  - Thermal Death Time (TDT): shortest length of time required to kill all microbes at a specified temperature.
- Common Methods of Moist Heat
  - Autoclave: pressure-temperature method that can subject pure steam to pressures greater than 1atm.
    - Most efficient pressure-temperature is 15psi which results in 121 degree Celsius.
    - Great for glassware, cloth (surgical dressings), rubber (gloves), metallic instruments, liquids, paper, some media, and some heat-resistant plastics.
    - Ineffective for sterilizing substances that repel moisture (oils, waxes, powders).
  - Intermittent Sterilization (Tyndallization)

        - Items in chamber are exposed to free-flowing steam for 30 to 60 minutes, incubated for 23-24 hours, then repeat the process 3 days in a row.
        - Used for culture media with sera, egg, or carbohydrates and some canned foods.
    - Pasteurization
        - Technique in which heat is applied to liquids to kill potential agents of infection and spoilage.
        - Flash Method: 71.6 degrees Celsius for 15 seconds.
        - Batch Method: 63- 66 degrees Celsius for 30 minutes
        - Ultrahigh Temperature (UHT) Method: 134 degrees Celsius for 1 to 2 seconds.
            - Used to produce sterile milk which has shelf-life of 3 months.
        - Do not kill endospores, Coxiella, Mycobacterium, or thermoduric species (lactobacilli, micrococci, and yeasts).
    - Boiling Water
        - Exposing items to boiling water (100 degrees Celsius) for 30 minutes can kill most non-spore forming pathogens.
        - Can quickly decontaminate items.
        - Items can easily be recontaminated when removed from water.
- Common Methods of Dry Heat
    - Incineration: heat treatment from flame or heating coil.
        - Flame of Bunsen burner is 1,870 degrees Celsius.
        - Furnaces/incinerators burn from 800-6,500 degrees Celsius.
    - Dry oven: heat is circulated in an enclosed compartment.
        - Sterilization requires 150-180 degrees Celsius for 2 to 4 hours (destroys spores).
        - Used for glass-ware, metallic instruments, powders, and oils.
        - Method not suitable for plastics, cotton, and paper, which may burn at high temp.

Hot air oven

Hot air ovens are electrical devices which use dry heat to sterilize. They were originally developed by Pasteur.[1] Generally, they can be operated from 50 to 300 °C, using a thermostat to control the temperature. Their double walled insulation keeps the heat in and conserves energy, the inner layer being a poor conductor and outer layer being metallic. There is also an air filled space in between to aid insulation. An air

circulating fan helps in uniform distribution of the heat. These are fitted with the adjustable wire mesh plated trays or aluminium trays and may have an on/off rocker switch, as well as indicators and controls for temperature and holding time. The capacities of these ovens vary. Power supply needs vary from country to country, depending on the voltage and frequency (hertz) used. Temperature sensitive tapes or biological indicators using bacterial spores can be used as controls, to test for the efficacy of the device during use.

Filtration

Filtration is the passing of either a solution or gasses through a device which traps microbes on one side of a container or space, preventing them from passing to the other. Filters are materials which have pores (openings) of varying sizes. Particulate matter larger than the pore size in a filter is excluded from passage and is this physically excluded. The earliest form of filter used in microbiological was cotton, a fiberous material derived from plants. Cotton fibers form a densely packed matrix which offers a torturous path for particulate matter containing microbes to pass, while still allowing air to do so. This is only true however, as long as a cotton plug, filter, or bandage remains dry, since water clings to each fiber allowing microbes unrestricted access.

In most cases, cotton has been replaced as a filter by ceramic filters and synthetic plastics such as nitrocellulose which offer very small pore sizes (0.2 m to 0.45 m) without taking up as much space. Since these materials are not fiberous, all but the very smallest microbes can be removed from a solution passing through them. This solution,

called a **filtrate**, is generally free from contaminants so long as the original pre-filtered solution did not contain organisms such as mycoplasma bacteria or viruses, both of which are smaller than most filters. As a consequence, filtration should be considered an agent of disinfection rather than sterilization.

Dessication

Dessication (drying) is the removal of moisture from the body of an organism. Many bacteria are very sensitive to water loss and can be killed simply by removal of water. For example, *Treponema pallidum*, the agent of syphillis, is so intolerant to water loss that it will die within twenty seconds on the surface of a dry fomite. The physical preservation of foodstuffs by drying has been practiced by humans for thousands of years and in most cases does reduce the number of potentially pathogenic microbes. One process, called **lyophilization** or freeze-drying, is used to rapidly remove water from the body of an organism under very cold temperatures in a partial vacuum. This process does not kill organisms such as bacteria, but does inactivate their metabolic processes. Lyophilization is used to preserve living bacterial cultures for storage and transport. To restore the freeze-dried cells, an individual has only to rehydrate them in a nutrient broth solution and incubate the culture at the optimum temperature for growth of the microbe.

It is important to note, however, that not all microbes are killed or inactivated by dessication. Bacteria which form spores such as members of the genera *Bacillus* and *Clostridium*, cyst-forming protists, and viruses can withstand drying, simply becoming inactive until moisture becomes available. For this reason, dessication can only be considered a form of disinfection.

Radiation

Radiation describes a physical phenomenon which occurs when matter releases either energy, atomic particules, or both. Radiation can affect the chemical makeup of the cell by altering or disrupting the structure of biological molecules. **Ionizing radiation** strips electrons away from biological molecules. Both **gamma** and **X-radiation** are ionizing forms. **Ultraviolet radiation** is absorbed by the pyrimidine

bases cytosine and thymine in DNA. When two thymine or cytosine molecules lie adjacent to one another on a nucleoside, ultraviolet radiation with wavelengths between 250 nm and 280 nm causes them to have a greater affinity for one another than for their complementary adenines on the opposite nucleoside. The two bond together, forming a **dimer**, which disrupts the normal sequence of nucleotide bases. This kind of mutation prevents the cell from producing proteins which may be necessary for normal metabolism to occur. Some cells can repair this damage if exposed to visibile light through a process called **photoreactivation** (light repair), wherein the dimer is nicked by a restriction endonuclease, then cut away and replaced by DNA polymerase. The new thymine or cytosine bases are then bonded to their complementary adenines or guanines by DNA ligase. Since light repair can occur, the use of ultraviolet radiation has only disinfecting activity and cannot be considered a sterilizing agent.

Temperature

Excess heat energy can cause proteins to become **denatured**, meaning that they lose their normal three-dimensional shape. Effective temperature for the reduction of microbes is measured as the **thermal death point** (**TDP**) of each organism, which is the temperature at which all growth stops. **Thermal death time** (**TDT**) is the amount of time it takes to kill all of the microbes in a sample, and the **decimal reduction factor** (**DRF**) is the amount of time at a specific heat necessary to reduce the population of microbes in a sample tenfold.

The most common methods of applying excess heat energy are **flaming** and **incineration**, which completely destroy all life. Flaming of inoculating loops and needles, as well as the tops of glass culture tubes and flasks insures that no contaminating microbes can infect sterile media. Applying **dry heat** by forcing hot air onto the surface of an object can be used in a similar fashion, though many spore formers are capable of withstanding this.

The application of **moist heat**, such as **boiling**, **steaming**, and **pasteurization** (application of high heat to a solution for a short period of time), is also commonly used. These methods work well for most microbes, but are incapable of killing organisms which are **thermoduric** (capable of withstanding elevated temperatures), or are spore formers. For example, the spores of *Clostridium botulinum*, the bacterium which causes botulism, can be boiled for up to five hours and still remain viable. The

most effective application of moist heat is through the use of a device called an **autoclave**. The autoclave works on the principle of **saturated steam**. The inner chamber is raised to an air pressure of 15 lb/inch2, then steam at a temperature of 121º C is injected. The steam strikes the surface of the object to be sterilized and condenses into water as its excess heat energy is released. This condensation creates a partial vacuum which draws more steam to the object. Saturated steam is extremely effective as a sterilizing agent, at least 1500 times more effective than the application of dry heat. Autoclaves are usually operated in cycles between 15 and 90 minutes, and can be used to sterilize glassware, surgical implements, soil, water, and microbiological media such as broths and agars. They cannot, however, be used to sterilize hydrophyllic powders which would clump, or hydrophobic oils since microbes suspended in oils would only be subjected to dry heat. Also, while contaminated bandages can be placed in an autoclave, the toxins or exoenzymes left behind by killed microorganisms such as *Clostridium perfringens* (the agent of gas gangrene) may still be capable of causing host cell damage, so these should be rinsed thoroughly with sterile water prior to reuse.

All of the above physical means of control can be checked for effectiveness utilizing various bacteria as quality control agents. Devices which emit ionizing radiation can be tested with *Micrococcus radiouridans*, U.V. devices with *Bacillus pumilis*, and heat disinfecting and sterilizing units such as hot-air ovens, pressure cookers, and autoclaves with *Bacillus stearothermophilus*. These organisms are generally supplied to laboratories live or in ampules or tape strips, which can be placed in the control device. After a normal operating cycle, the organisms are incubated in microbiological media. If growth occurs, the device is not operating properly and should be repaired. Quality control checks and maintenance are vital to the effective microbiological laboratory or health-care facility, and should be performed on a regular basis to prevent contamination and the spread of disease.

- Cold and Desiccation
  - Cold merely retards the activities of most microbes.
  - Vegetative cells exposed to normal room air gradually become dehydrate (desiccated).
  - Many viruses and fungal spores can also withstand long periods of desiccation.
  - Lyophilization: freezing and drying is a common method to preserve microorganisms and other cells in a viable state for many years.
- Radiation: energy emitted from atomic activities and dispensed at high velocity

through matter or space.

- Electromagnetic Radiation
  - Microbial controlled done with gamma ray, X-ray, and ultraviolet ray levels.
- Particle Radiation
  - High-speed electron (beta-particle or cathode ray)
- Ionizing Radiation Versus Non-Ionizing Radiation
  - Ionizing radiation: radiation ejects orbital electrons from an atom and causes ions to form.
    - Causes chemical changes in organelles and the production of toxic substances.
    - Gamma rays, X-rays, and high-speed electrons are all ionizing in their effects.
    - Method of cold sterilization (absence of heat).
    - Dosage measured in rads (radiation absorbed dose).
      - Gamma ray most penetrating, then X-rays, then finally cathode rays.
  - Non-ionizing: radiation that excites atoms by raising them to a higher energy state.
    - Results in abnormal linkages within molecules such as DNA and results in mutations.
    - UV rays (100nm-400nm)
      - Most lethal dose from 240nm to 280nm, but peaks at 260nm.
      - UV rays are absorbed by DNA and damages pyrimidine bases (thymine and cytosine) to form pyrimidine dimers.
      - UV rays also disrupt cells by generating toxic photochemical products (free radicals).
      - Destroys fungal cells and spores, bacterial vegetative cells, protozoa, and viruses.
        - Vegetative spores are 10Xs more resistant to radiation, but longer exposure time can kill them.
      - Disinfectant technique but can also be used to treat water.
      - Poor penetrating power through solid material.

- Sound Waves
  - Frequencies from 15,000 to 200,000 cycles per seconds (supersonic to ultrasonic) disrupt cells.
  - Gram negative rods most sensitive to ultrasonic vibrations.

        - Gram positive cocci, fungal spores, and bacterial spores are most resistant to them.
    - Heat is also generated from sonic waves (up to 80 degrees Celsuis).
    - Used in dental and some medical centers to clear debris and saliva from instruments before sterilization and to clean dental restorations.
        - Also used for removing plaque and calculus from teeth.
- Sterilization by Filtration: Techniques for Removing Microbes
    - Effective for removing microbes from air and liquids.
    - Most made from thin membranes of cellulose acetate, polycarbonate, and a variety of plastic materials (Teflon, nylon).

Chemical Agents in Microbial Control

- Chemical agents: chemical means to destroy or remove contaminants.
- Three Levels of Chemical Decontamination
    - High-level germicides kill endospores, and if properly used, are sterilants.
    - Intermediate germicides kill fungal (not bacterial) spores, resistant pathogens such as tubercle bacillus, and viruses (used for non-invasive equipment).
    - Low-level germicides kill vegetative bacteria and fungal cells, and some viruses (used for materials that may touch the skin, not mucous membrane).
- Dilutions
    - Chemical (solute) is added to water (solution), aqueous.
        - Noted at (solute):(solution) --> For 1 part Lysol there are 100 parts water in 1:100.
- Penetration
    - Smooth, solid objects are easier to disinfect due to rough objects can collect dirt or contaminants in crevices that the disinfectant can't penetrate.
- Germicidal Categories According to Chemical Group
    - Halogens: non-metallic elements that commonly occur in minerals, seawater, and salts
        - Fluorine, bromine, chlorine, and iodine.
            - Fluorine and bromine difficult and dangerous to handle.
        - Microbicidal (sporicidal with longer exposure time)
        - Chlorine (CL2), hypochlorite (Clorox), chloramines.
            - Denaturation of proteins.
            - (disrupts disulfide bonds)
            - Can be sporicidal.
            - Formula: 8 drops of solid bleach per gallon, if looks cloudy,

double. Boil for 10 minutes after.

- Iodine (I2), iodophors (betaiodine)
  - Denatures proteins.
  - Can be sporicidal.
  - Milder medical & dental degerming agents, disinfectants, ointments.
  - Kills protozoans.

- Phenol (Phenolics)
  - Damage cell membrane & precipitate proteins; bactericidal, fungicidal, virucidal, not sporicidal.
  - Active in organic matter.
  - Stable & Persistent
  - Lysol
  - Triclosan: soap antibacterial additive.
- Chlorhexidine
  - Hibiclens , Hibitane.
  - Halogen & phenol compound.
  - A surfactant & protein denatured with broad microbicidal properties.
  - Not sporicidal.
  - Low toxicity.
  - Used as skin degerming agents for scrubs.
- Alcohol
  - Ethyl alcohol, isopropyl.
  - Solutions of 70-95%
  - 70% better than 100% due to more dilutive more effective.
  - Dissolve membrane lipids and coagulating proteins of vegetative bacterial cells and fungi.
  - Swabs
  - Not sporicidal.
  - Action depends on concentration, but generally microbistatic.
- Hydrogen Peroxide
  - Weak (3%) to strong (35%)
  - Produce highly reactive hydroxyls: (free radicals) that damage protein & DNA while also decomposing to O2 gas, toxic to anaerobes.
  - Strong solutions are sporicidal.
- Detergents & soaps <--QUATS
  - Quaternary ammonium compounds are surfactants that alter membrane proteins of some bacteria and fungi. Hospital cleaners.
  - Not sporicidal.

        - Smells nice.
        - Soaps: mechanically remove dirt/soil and grease containing microbes.
    - Heavy metals
        - Solutions of silver & mercury kill vegetative (wont kill spores) cells in low concentrations by inactivating proteins.
        - Oligodynamic action.
        - Not sporicidal.
        - Eg. silver nitrate, thimerosol.
    - Aldehyde
        - Glutaraldehyde & Formaldehyde kill everything by crosslinking/alkylating proteins & DNA.
        - Glutaraldehyde in 2% solution (T.B.) used as sterilant for heat sensitive instruments.
        - Formaldehyde: disinfectant, preservative, toxicity limits use.
        - Formaldehyde sources: common in household.
    - Gases and Aerosols
        - Ethylene oxide, propylene oxide, betapropiolactone, and chlorine dioxide.
        - Strong alkylating agents, sporicidal.
        - Equipment, bedding, disposables, sterilization.
        - Very toxic (eyes, skin, mucus, membranes). Carcinogenic?
    - Organic Acids & Food Preservatives
        - Used in foods to inhibit microbial growth.
        - Sulfur dioxide
        - Sodium benzoate/benzoic acid
        - Sorbic acid
        - Propionic acid/Ca propionate
        - Nitrates and Nitrites
- Germicidal Categories Summary

Agent	Category	Level of Activity	Target Microbes
Chlorine		Intermediate	Sporicidal (slowly)
Iodine		Intermediate	Sporicidal (slowly)
Phenolics		Intermediate to Low	Some bacteria, viruses, fungi

Alcohols		Intermediate	Most bacteria, viruses, fungi
Hydrogen peroxide, stabalized		High	Sporicidal
Quaternary Ammonia Compounds		Low	Some bactericidal, virucidal, fungicidal activity.
Soaps		Very Low	Certain very sensitive species.
Mercurials		Low	Weakly microbistatic.
Silver Nitrate		Low	Bactericide
Glutaraldehyde		High	Sporicidal
Formaldehyde		Low to Intermediate	Sporicidal
Ethylene Oxide Gas		High	Sporicidal
Dyes		Low	Weakly bactericidal, fungicidal.
Chlorhexidine		Low to Intermediate	Most bacteria, some viruses, fungi

Chemical Methods

Chemical agents for the control of microbial growth are either **microbiocidal** or **microbiostatic**. Microbiocidal agents are **sterilants** which kill all living cells. Microbiostatic agents kill some cells and inhibit the growth of others. The spectrum of activity exhibited by any microbiocidal or microbiostatic agent is an important factor in choice, and should be considered, along with potential harmful effects on the user. An agent which kills staphylococci may be totally ineffective against mycobacteria, and would be useless in a tuberculosis ward. Also, a broadly killing sterilant may release gasses which are toxic to patients and staff. Most often, chemical agents which disinfect are utilized by clinics, hospitals, and laboratories. While these agents do not sterilize, their toxicity is usually much less than that of a sterilant, and prevention of infection is stressed.

There are four large categories for agents of chemical control. **Antibiotics** are produced by microorganisms to kill or inhibit the growth of other microbes. These agents are generally selectively toxic, and can be naturally produced, synthesized, or or semisynthetic. **Antiseptics** are synthetic compounds which kill or inhibt the growth of microbes on the surface of the skin. **Disinfectants** are chemical compounds which kill or inhibit microbes on the surface of fomites. **Preservatives**, such as sugars, salt, nitrates, nitrites, sulfate, and sulfites inhibit microbial growth in food, usually by producing osmotic environments which are unfavorable to microbial growth. These can be further subdivided as high-, intermediate, or low level agents. **High-level germicides** sterilize fomites, but are toxic to skin and mucus membranes. **Intermediate-level disinfectants and antiseptics** kill and inhibit on fomites and skin, but can be toxic to the user at medium to high concentrations. Examples include phenolics and halogens. **Low-level disinfectants**, such as alcohols, hydrogen peroxide, heavy metals, and soaps kill some microbes but inhibit the growth of most.

High-Level Germicides

These are called **agents of cold sterilization**, since no heat needs to be applied to increase their activity. These are generally alkylating agents, which kill by adding ethyl or methyl groups to nucleic acids or proteins. While the agents are capable of killing vegetative cells, spores, and inactivating viruses, some take up to several hours to complete their germicidal activity.

Aldehydes, such as **formaldehyde** and **gluteraldehyde**, fix tissues by alkylating and forming cross-links between adjacent proteins. They are commonly used as fixitive compounds for electron microscopy, preservatives of specimens and cadavers, in some synthetic plastic compounds, and can be used to sterilize anesthesia tubing and surgical implements. Aldehydes can fix living tissues such as mucus membranes and have the ability to vaporize or **outgas** from compounds containing them, so they should be handled with caution. **b-propiolactone** is a liquid alkylating sterilant with a high boiling point (155^{o}C). It is generally used to sterilize bone used in grafts. It quickly breaks down into nontoxic compounds when it comes into contact with organic matter, but can burn skin.

Ethylene oxide (carboxide) kills vegetative cells and spores. It is a liquid at temperatures below 10.8^{o} C, but rapidly sublimates into a highly inflammable gaseous state above this temperature. It is generally used in a chamber similar to an autoclave at 60^{o} C for 1-10 hours, where it is mixed in a 9:1 ratio with carbon dioxide (90% CO_2, 10% ethylene oxide), which reduces its toxicity, but also its inflammability. Carboxide can be used to sterilize surgical implements and glassware, but these fomites must be allowed to degass before use, since residues can stimulate mutations in bacteria.

Ozone (O_3) occurs naturally in the upper atmosphere, where it serves to shield the surface of the earth from solar radiation, and is produced as an exhaust gas by vehicles and industry, acting as a pollutant in the lower atmosphere. Applied properly in a chamber, ozone is a powerful oxidizing agent which kills cells and spores on the surface of glassware, surgical implements, and bandages. An advantage of sterilization with this compound is that it outgasses quickly, leaving no toxic residues as can ethylene oxide and b-propiolactone.

Intermediate-Level Disinfectants and Antiseptics

Phenol (carbolic acid) is one of the earliest disinfectant compounds to be used in health care facilities and laboratories. Joseph Lister used atomized phenolic compounds in the 1860's to disinfect his surgery during invasive procedures as a means of cutting

down on postoperative septic infections. Phenol kills microorganisms by denaturing proteins and destabilizing cell membranes, is bacteriocidal, fungicidal, and virucidal at high concentrations, but is not effective against bacterial endospores, and is effective against many potential pathogens, including mycobacteria, staphylocci, streptococci, and gram-negative coliforms, such as *E. coli*. It can be used to disinfect garbage cans, surgical operating facilities, laboratory equipment, feces, urine, and sputum, but it is very corrosive at higher concentrations and its fumes can be lethal. Because of its toxicity, this compound is generally used as a solution between 2% to 5% in concentration, and many less toxic derivitives have been produced. The most common of th phenolic derivatives are called **cresols** and **bisphenols**. Cresols are formed by adding methyl groups to phenol, and are used for the preservation of wood products, as compounds such as creosote (para-cresol). Bisphenols are produced by combining two phenol molecules. Lysol™ is a combination of cresol and soap, which has about the same spectrum of activity as phenol, but is much less toxic to skin. Other cresols include resorcinol, hexylresorcinol, and hexachlorophene. Hexachlorophene soaps such as pHizoHex were once widely utilized as antiseptic soaps by health-care personnel for hand washing and the bathing of newborn infants, but have been discarded, since it was found that this phenolic could be absorbed through the skin and potentially cause birth defects.

Since phenol has such a broad-spectrum of activity, it is used as a standard by which to judge how well other disinfecting compounds work. The **phenol coefficient** (**P.C.**) is a mathematical value used to compare the effectiveness of a test disinfectant to that of phenol, and is derived from the following formula:

$$\text{P.C.} = \frac{\text{dilution of a test disinfectant necessary to kill a standard population of bacteria}}{\text{dilution of phenol which has the same effect}}$$

For example, a 1:250 dilution of a test reagent kills a standard population of *S. aureus*. A 1:60 dilution of phenol kills the same size population. To derive the P.C., we divide 250/60:

P.C. = 250/60 =4.2 Therefore the test disinfectant is 4.2 times as effective as phenol.

Halogens are a family of elements with a high affinity for electrons. This affinity makes them very reactive with biological molecules, and they can serve to disrupt

enzyme activity, break down lipid structure, and produce oxidizing agents such as singlet oxygen (O). The halogens most commonly used as disinfectants are chlorine and iodine. **Chlorine** is used as a disinfectant only, either as a gas or in liquid form which is effective against many vegetative forms of microbes as well as some viruses such as HIV and hepatitis. Commonly, chlorine is supplied in **sodium hypochlorite** (NaOCl) bleach, which is a combination of 94.75% water and 5.25% NaOCl. It is used as a bleaching compound as well as a disinfectant for used hypodermic needles, in swimming pools, toilets, water supplies, and in sewage treatment plants. It is inactivated by organic matter, and produces toxic fumes (the "mustard gas" used in World War I because of its yellow color) which can cause considerable damage to skin and mucus membranes with direct contact. **Iodine** is lethal to all vegetative forms of microorganisms, can inactivate viruses, and is fairly effective in higher concentrations against endospores. Pure iodine is caustic to tissues, so it is diluted with other compounds. Tincture of iodine is produced by dissolving crystaline iodine in alcohol. This solution is a good antiseptic for most minor cuts and scrapes, but it excites pain receptors at the site of an injury, and as the alcohol component dries, the iodine may concentrate and damage exposed tissues. **Iodophore compounds**, such as Betadine™ and Wescodine™ are composed of iodine dissolved in mild detergent and alcohol. These do not excite pain receptors as readily and can be used to clean and disinfect large areas of skin prior to invasive surgical procedures.

Low-Level Disinfectants

Hydrogen peroxide is a good low-level disinfectant agent when used in concentrations of 3% or lower. Higher concentrations are caustic to human skin, and cannot be used. This compound is used as an antiseptic for the treatment of minor cuts and scrapes and as a bleaching agent. When placed on the surface of an injury, hydrogen peroxide bubbles due to the release of the enzyme catalase from tissues, which break it down into water and oxygen. This breakdown also releases the peroxide ion (O_2^{-2}), a strong oxidizing agent, and that the water released provides hydroxide ions which strip hydrogen from biological molecules. Obligate anaerobic microbes are especially sensitive to hydrogen peroxide, since they do not produce catalase and the rapid release of oxygen gas inhibits their growth.

Alcohols such as ethanol and isopropanol are effective antiseptics and disinfectants when used in concentrations between 70% and 80%. Alcohols kill microbes by denaturing proteins, dehydrating (100% concentration), and as nonpolar solvents which disrupt the phospholipid structure of the cell membrane, but are relatively ineffective against spores and viruses. Also, dehydration may actually be beneficial to some microbes, enhancing their survival by extracting extracellular water, so alcohols such as ethanol are normally used in lower concentrations. Isopropanol is used as an antiseptic and to clean the epidermis prior to syringe and I.V. needle use.

Heavy metals such as mercury, silver, and copper tend to combine with sulfur groups in the proteins of microbes, causing them to denature. This **oligodynamic activity** makes the heavy metals useful in small concentrations. These are some of the earliest used agents for the control of microorganisms. Mercury, in the form of mercuric chloride, was used by the Greeks and Romans to disinfect skin. This element is toxic in high concentrations, so it is commonly blended with other compounds such as iodine and alcohol in mercurochrome. Silver, applied as silver nitrate ($AgNO_3$) in a 1% solution, is commonly used to inhibit the growth of *Neisseria gonorrhoeae* in the eyes of newborn infants, a condition called **neonatorum opthamalia** (though many hospitals now use antibiotic ointments such as erythromycin or tetracycline), which can lead to blindness and is acquired as the organisms are transmitted to the infant as it passes through the birth canal. Copper, in the form of copper sulfate, is used to limit the growth of algaes in ponds and lakes, and as an antifungal compound for use on plants.

Detergents and soaps are composed of lipids and compounds having basic pH, such as sodium hydroxide. These break up surface tension, act as wetting agents which release particles attached to the surface of objects, and destabilize the phosphate portions of the plasma membrane of microorganisms. Detergents are either anionic or cationic, releasing negatively or positively- charged ions into solution. Anionic forms are weakly active against gram-positive bacteria but tend to repel negatively-charged cells, thus they are generally used in the production of iodophore compounds. Cationic forms are attracted to bacterial cells and are bacteriostatic, while remaining relatively mild to the surface of skin. **Quartenary ammonium compounds** (**QUATS**) are cationic detergents which contain one or more long-chain akyl groups. Quats such as benzalkonium chloride (Zephiran™) have broad-spectrum inhibitory activity against bacteria, fungi, and protozoa, are mildly antiseptic and disinfecting when used as cleaning agents for laboratory fomites and on the surface of skin, and remain active after drying, but lose much of their activity when mixed with soaps. Cetylpyridium chloride (ceepryn) is the quat in Cepacol™ which is mildly antiseptic and safe for use on the mucus membranes of the oral cavity.

Some **dyes** can not only be used to stain microorganisms, but also have antimicrobial activity. Crystal violet (used in very low concentrations as gentian violet) can be used to treat oral infections by bacteria such as *Rochlaemia quintana*, the agent of trenchmouth, and fungal infections such as *Candida albicans*, which causes oral thrush.

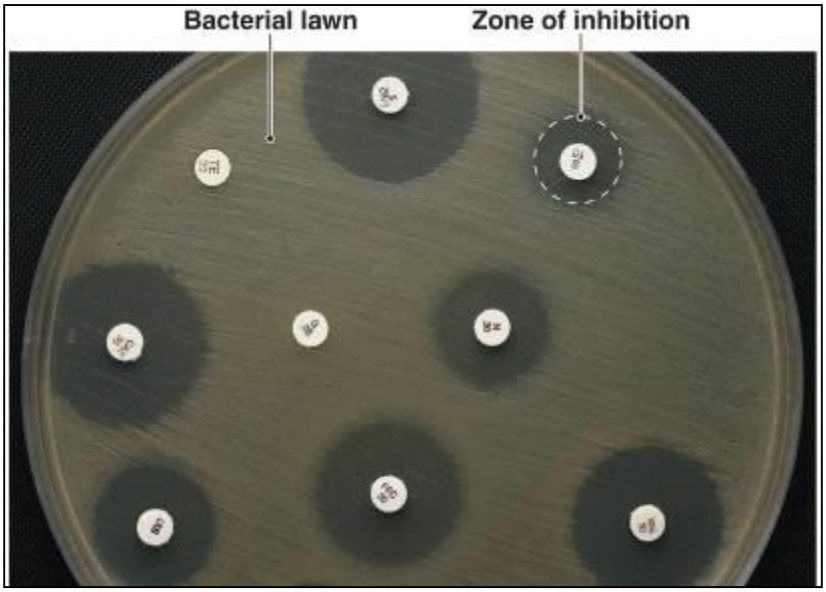
Physico – chemical methods – chemotherapeutic agents

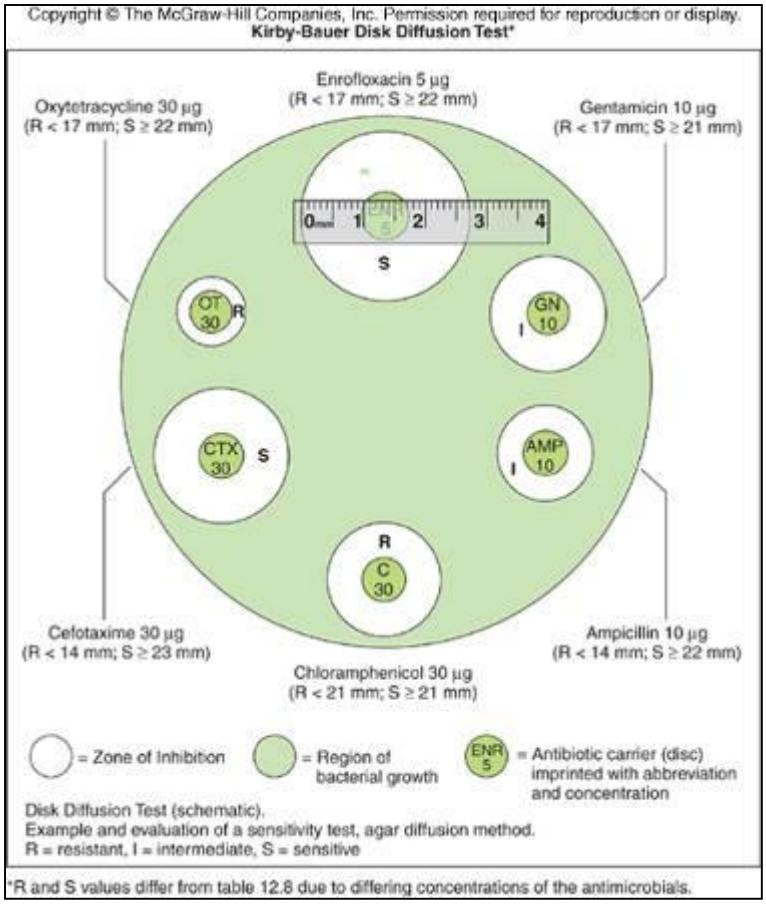
Evaluation of effectiveness of antimicrobial agents

1. Identify the microorganism causing the infection
   - Specimens should be taken before antimicrobials initiated
2. Test the microorganism's susceptibility (sensitivity) to various drugs ***in vitro*** when indicated
   - **(Next slide)**
3. Overall medical condition of the patient

Testing for Drug Susceptibility

- ***Essential*** for groups of bacteria commonly showing resistance
- **Kirby-Bauer disk diffusion test Agar well diffusion method**


Bacterial lawn
Zone of inhibition


Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.
Kirby-Bauer Disk Diffusion Test*
Enrofloxacin 5 µg
(R < 17 mm; S ≥ 22 mm)
Oxytetracycline 30 µg
(R < 17 mm; S ≥ 22 mm)
Gentamicin 10 µg
(R < 17 mm; S ≥ 21 mm)
0 1 2 3 4
S
OT 30
R
GN 10
I
CTX 30
S
AMP 10
I
R
C 30
Cefotaxime 30 µg
(R < 14 mm; S ≥ 23 mm)
Ampicillin 10 µg
(R < 14 mm; S ≥ 22 mm)
Chloramphenicol 30 µg
(R < 21 mm; S ≥ 21 mm)
= Zone of Inhibition
= Region of bacterial growth
ENR 5
= Antibiotic carrier (disc) imprinted with abbreviation and concentration
Disk Diffusion Test (schematic).
Example and evaluation of a sensitivity test, agar diffusion method.
R = resistant, I = intermediate, S = sensitive
*R and S values differ from table 12.8 due to differing concentrations of the antimicrobials.

Tube Dilution tests

- Minimum inhibitory concentration (MIC)
  - smallest concentration of drug that visibly inhibits growth
- *In vitro* activity of a drug is not always correlated with *in vivo* effect
  - If therapy fails, a different drug, combination of drugs, or different administration must be considered
- Best to chose a drug with highest level of selectivity but lowest level toxicity