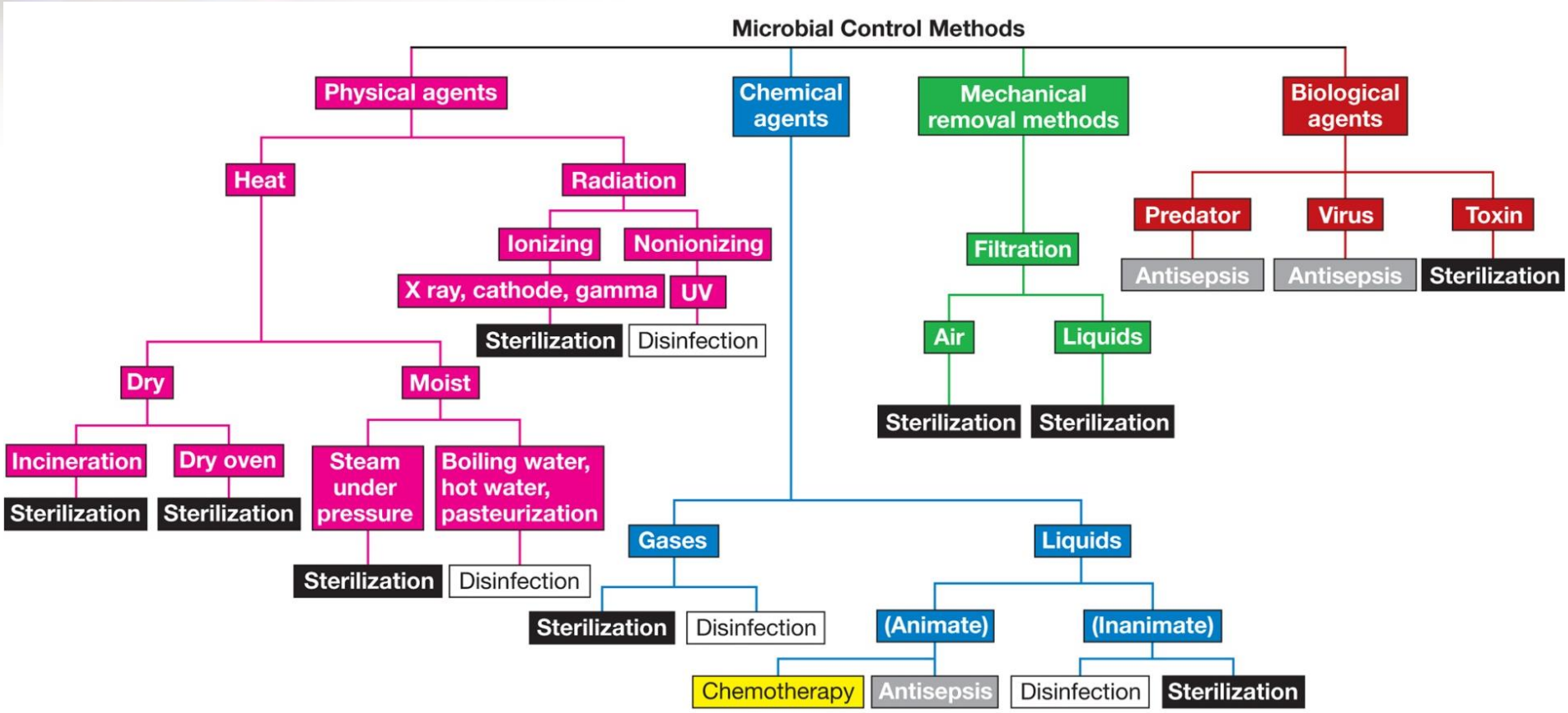
Microbial control methods



Disinfection: The destruction or removal of vegetative pathogens but not bacterial endospores. Usually used only on inanimate objects.
Sterilization: The complete removal or destruction of all viable microorganisms. Used on inanimate objects.
Antisepsis: Chemicals applied to body surfaces to destroy or inhibit vegetative pathogens.
Chemotherapy: Chemicals used internally to kill or inhibit growth of microorganisms within host tissues.

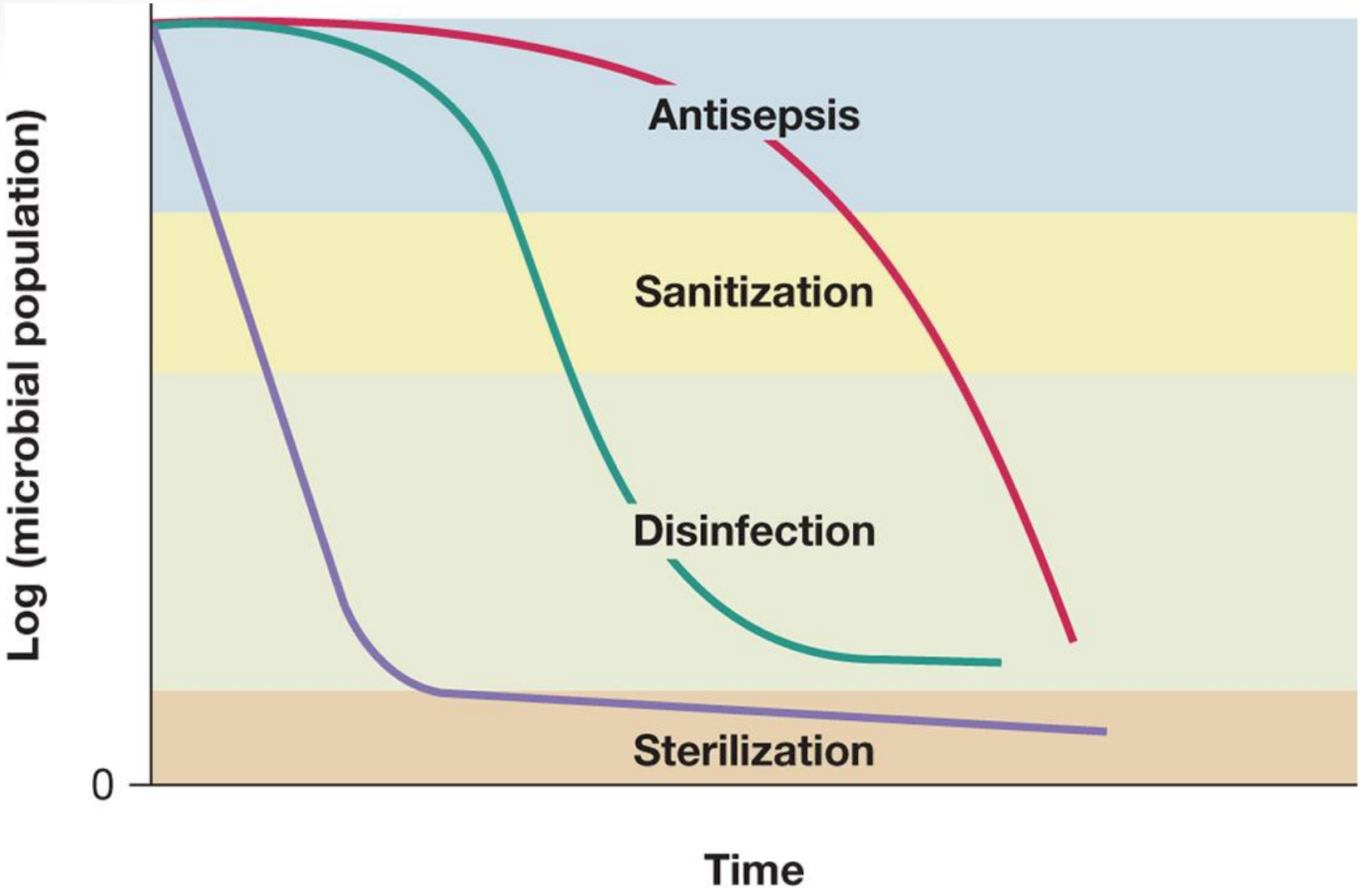
Definition of Frequently Used Terms

- **Sterilization**
  - destruction or removal of all viable organisms
- **Disinfection**
  - killing, inhibition, or removal of disease causing (pathogenic) organisms
  - disinfectants
    - agents, usually chemical, used for disinfection
    - usually used on inanimate objects

More Definitions…

- **Sanitization**
  - reduction of microbial population to levels deemed safe (based on public health standards)
- **Antisepsis**
  - prevention of infection of living tissue by microorganisms
  - antiseptics
    - chemical agents that kill or inhibit growth of microorganisms when applied to tissue

Impact of biocide exposure



Antimicrobial Agents

- **Chemotherapy**
  - use of chemicals to kill or inhibit growth of microorganisms within host tissue
- Agents that kill microorganisms or inhibit their growth
  - **cidal** agents kill
  - **static** agents inhibit growth

-cidal vs. –static Agents

-cide

- suffix indicating that agent kills
- germicide
  - kills pathogens and many nonpathogens but not necessarily endospores
- include bactericides, fungicides, algicides, and viricides

-static

- suffix indicating that agent inhibits growth
- include bacteriostatic and fungistatic

Conditions Influencing the Effectiveness of Antimicrobial Agent Activity

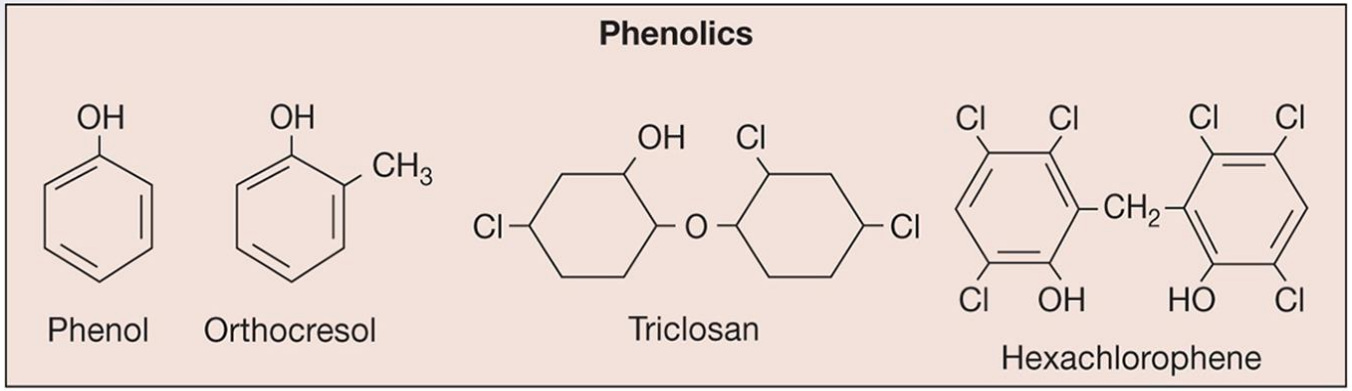
- **Population size** larger populations take longer to kill than smaller populations
- **Population composition**
  - microorganisms differ markedly in their sensitivity to antimicrobial agents

More Conditions…

- **Concentration or intensity of an antimicrobial agent**
  - usually higher concentrations kill more rapidly
  - relationship is not linear
- **Duration of exposure**

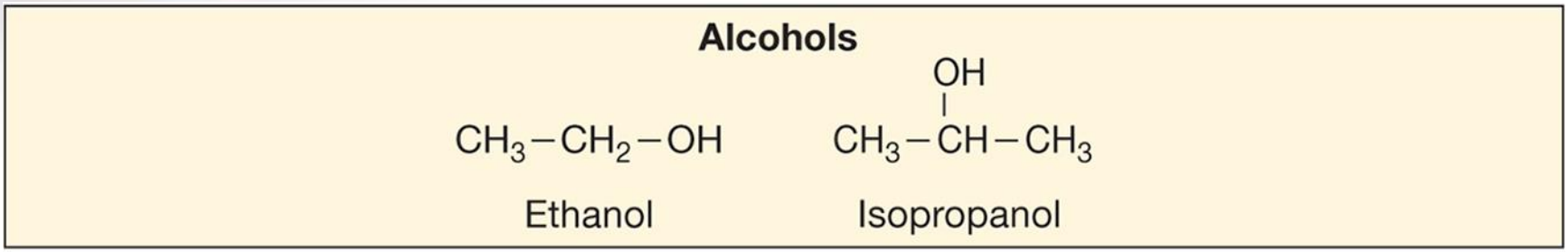
  longer exposure ⇒ more organisms killed
- **Temperature**
  - higher temperatures usually increase killing
- **Local environment**
  - pH, viscosity, concentration of organic matter, etc. can profoundly impact effectiveness
  - organisms in biofilms are less susceptible to many antimicrobial agents

Phenolics



- Commonly used as laboratory and hospital disinfectants
- **Act by denaturing proteins and disrupting cell membranes**
- Tuberculocidal and remain active on surfaces long after application
- Disagreeable odor and can cause skin irritation

Alcohols



- Among the most widely used disinfectants and antiseptics
- Two most common are ethanol and isopropanol
- **Bactericidal, fungicidal, but not sporicidal**
- Inactivate some viruses
- Denature proteins and possibly dissolve membrane lipids

Halogens - Iodine

- Important antimicrobial agent
- Skin antiseptic
- **Oxidizes cell constituents and iodinates proteins**
- At high concentrations may kill spores
- Skin damage, staining, and allergies can be a problem
- **Iodophore**
  - iodine complexed with organic carrier
  - released slowly to minimize skin burns

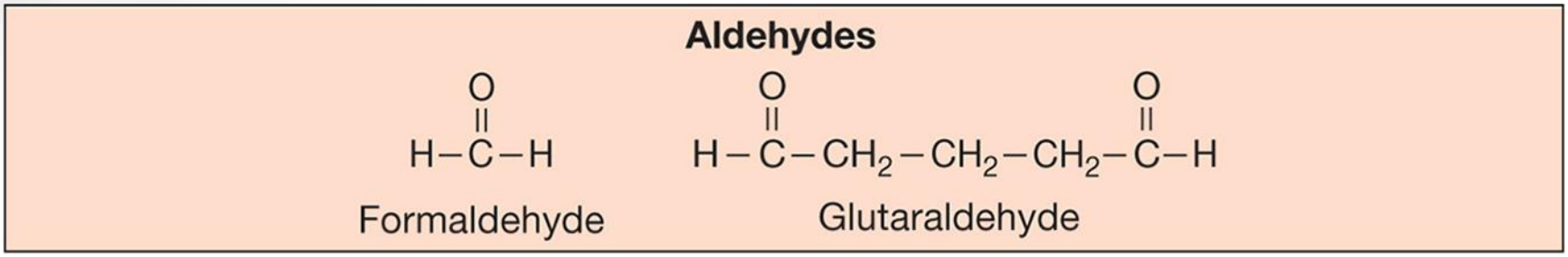
Halogens - Chlorine

- Oxidizes cell constituents
- Important in disinfection of water supplies and swimming pools, used in dairy and food industries, effective household disinfectant
- Destroys vegetative bacteria and fungi,
- Chlorine gas is **sporicidal**
- Can react with organic matter to form carcinogenic compounds

Heavy Metals

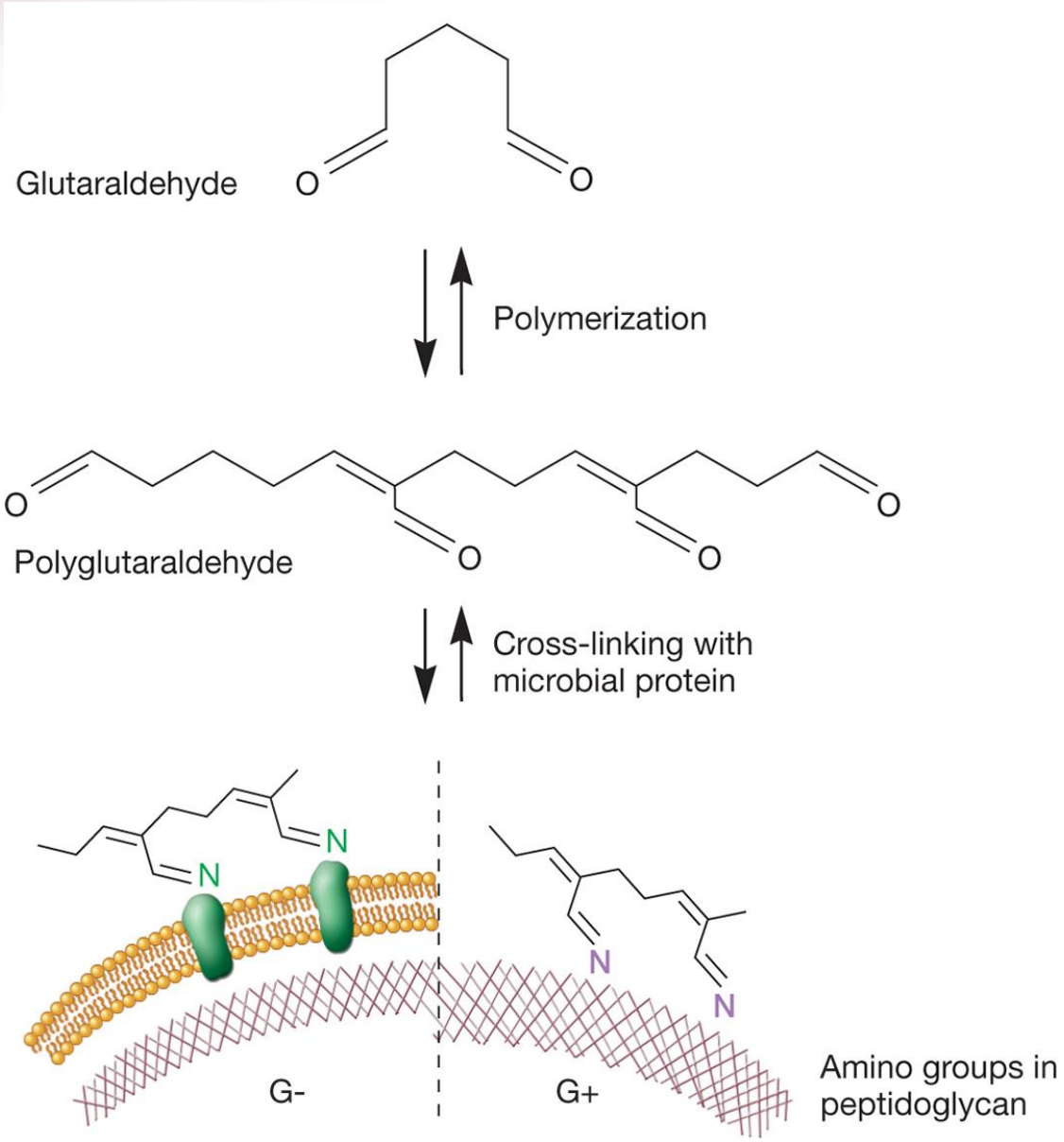
- e.g., ions of mercury, silver, arsenic, zinc, and copper
- ($CuSo_4$) potent against algae in swimming pools, fish tanks.
- $ZnCl_2$ is common ingredients in mouth washes
- **Combine with and inactivate proteins; may also precipitate proteins**

Aldehydes

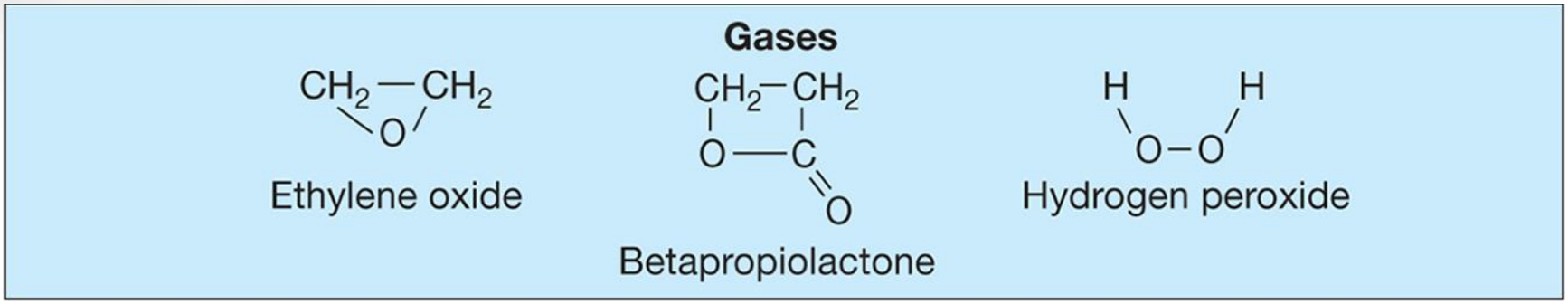


- Commonly used agents are formaldehyde and glutaraldehyde
- Highly reactive molecules
- **Combine with and inactivate nucleic acids and proteins**
- Sporicidal and can be used as chemical sterilants

Glutaraldehydes

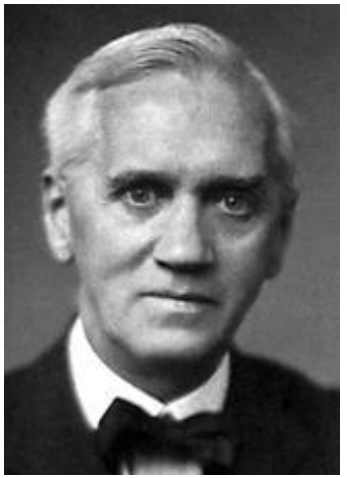


Sterilizing Gases

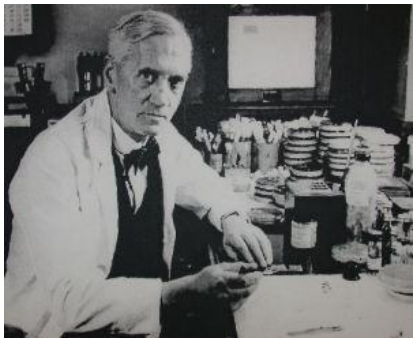


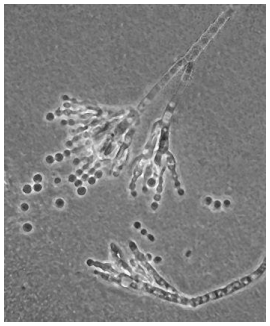
- Used to sterilize heat-sensitive materials
- Microbicidal and sporicidal
- Ethylene oxide sterilization is carried out in equipment resembling an autoclave
- Betapropiolactone and vaporized hydrogen peroxide
- Combine with and inactivate DNA and proteins

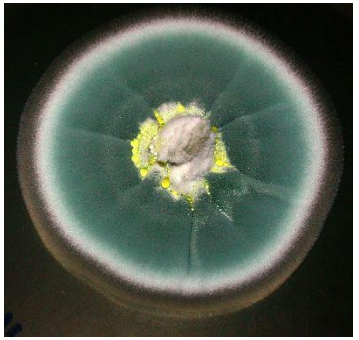
The story of penicillin
P. chrysogenum (*P. notatum*)



Sir Alexander Fleming







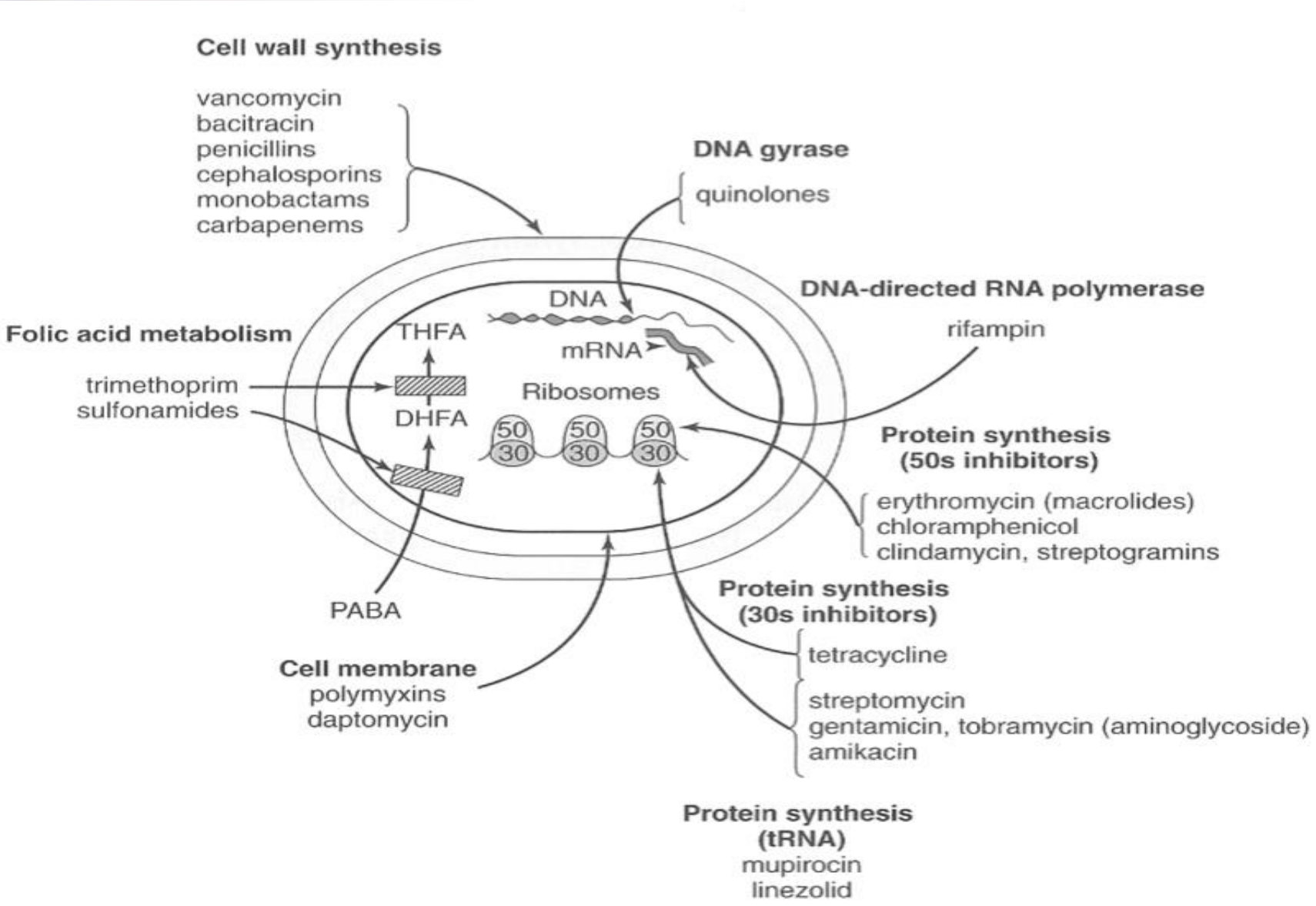
The Nobel Prize in Physiology or Medicine 1945

"for the discovery of penicillin and its curative effect in various infectious diseases"

Antimicrobial Agents

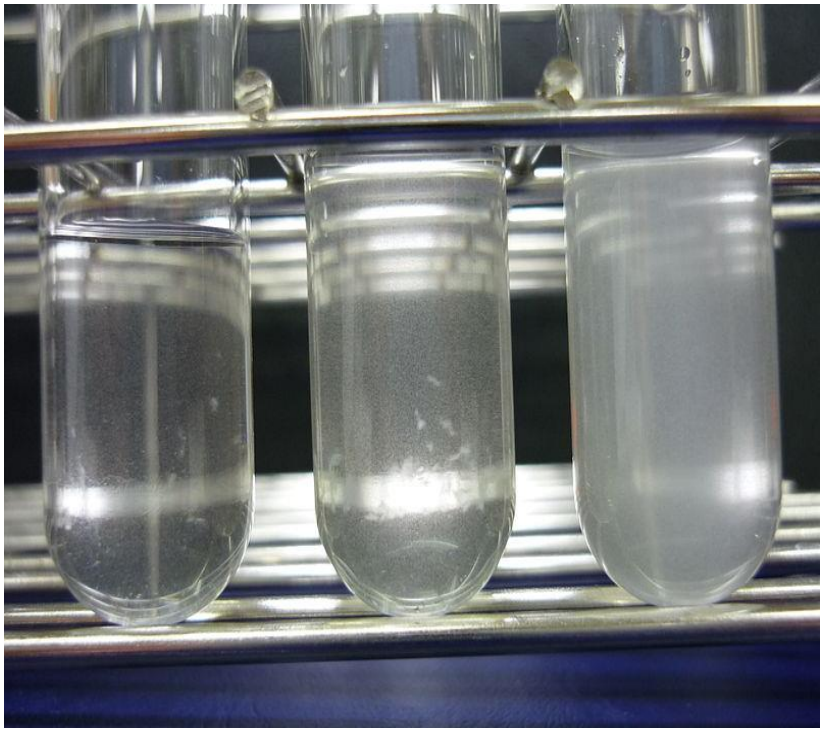
- **Antibiotics are either:**
  - **Natural, semi-synthestic or synthetic**
  - Natural antibiotics are synthesized by molds and bacteria
  - Antibiotics made by **streptomyces** such as:
    - Streptomycin, neomycin, tetracycline, chloramphenicol, erythromycin,
  - Antibiotics produced by **Bacillus sp**. Such as
    - Bacitracin, gramicidin, tyrocidin.

Antimicrobial Agents



McFarland Standard

McFarland Standard No.	0.5	1	2	3	4
1.0% Barium chloride (ml)	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**
1.0% Sulfuric acid (ml)	**9.95**	**9.9**	**9.8**	**9.7**	**9.6**
Approx. cell density (1-2X10^8 CFU/mL)	1.5	3.0	6.0	9.0	12.0
% Transmittance*	74.3	55.6	35.6	26.4	21.5
Absorbance*	**0.08 to 0.1**	**0.257**	**0.451**	**0.582**	**0.669**



To do

1. Prepare a standard turbidity inoculum of the test bacterium so that a certain density of bacteria will be put on the plate.

2. Inoculate a 150mm Mueller-Hinton agar plate with the standardized inoculum so as to cover the entire agar surface with bacteria.

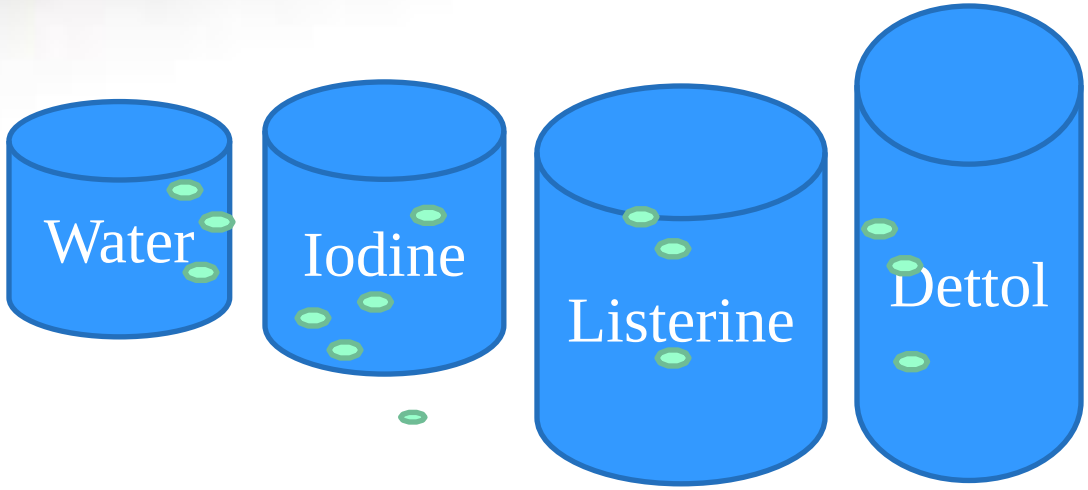
3. Place standardized antibiotic or chemicals discs on the plate.

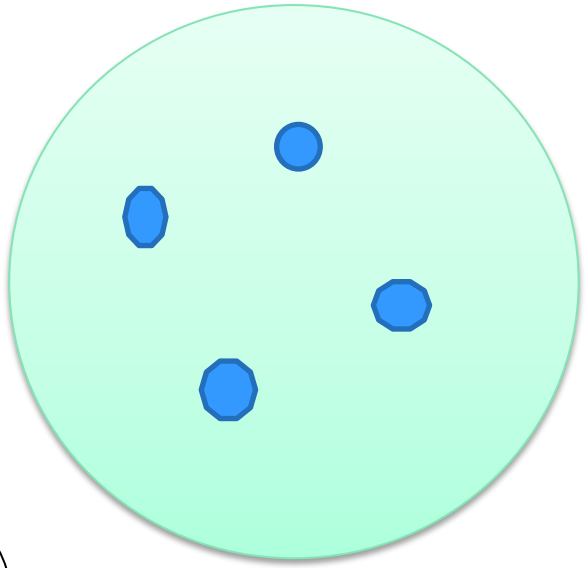
4. Incubate the plate at 37°C for 24 hours.

5. Measure the diameter of any resulting zones of inhibition in millimetres (mm).

6. Determine if the bacterium is susceptible, moderately susceptible, intermediate, or resistant to each antimicrobial agent.

Effect of chemical agents



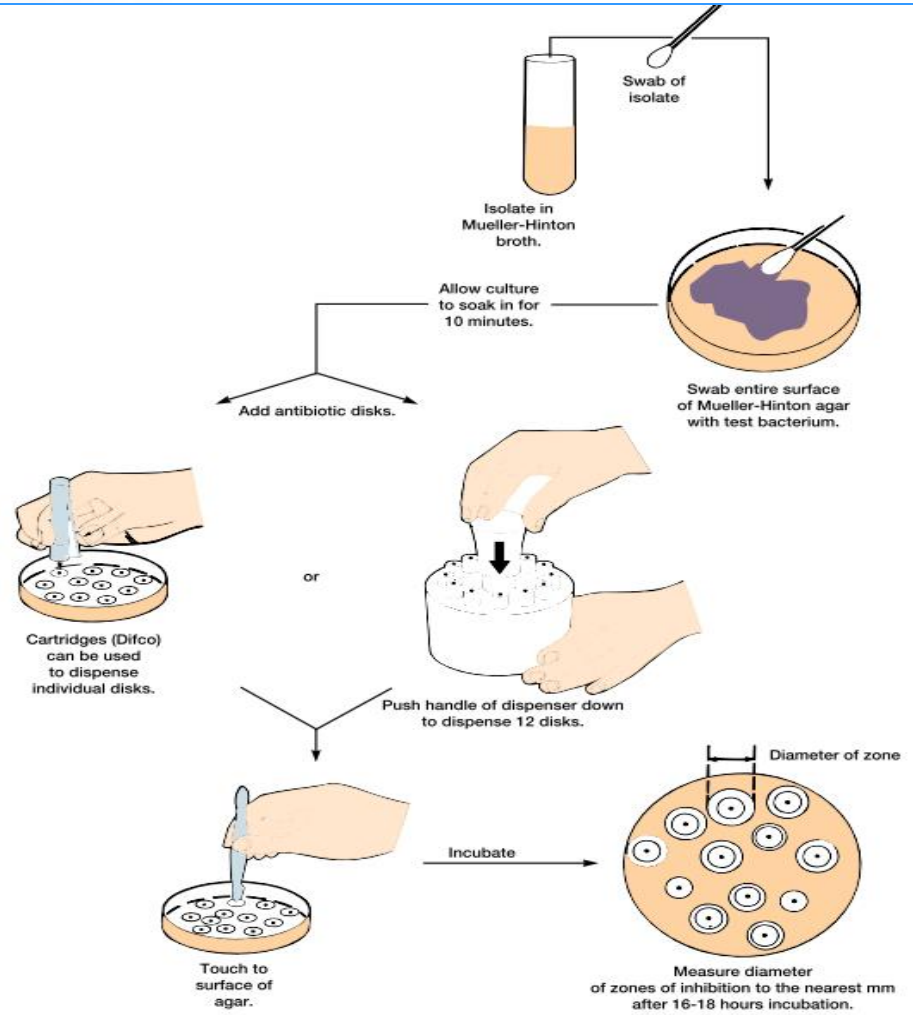


Label the plate with the chemicals used and bacteria species

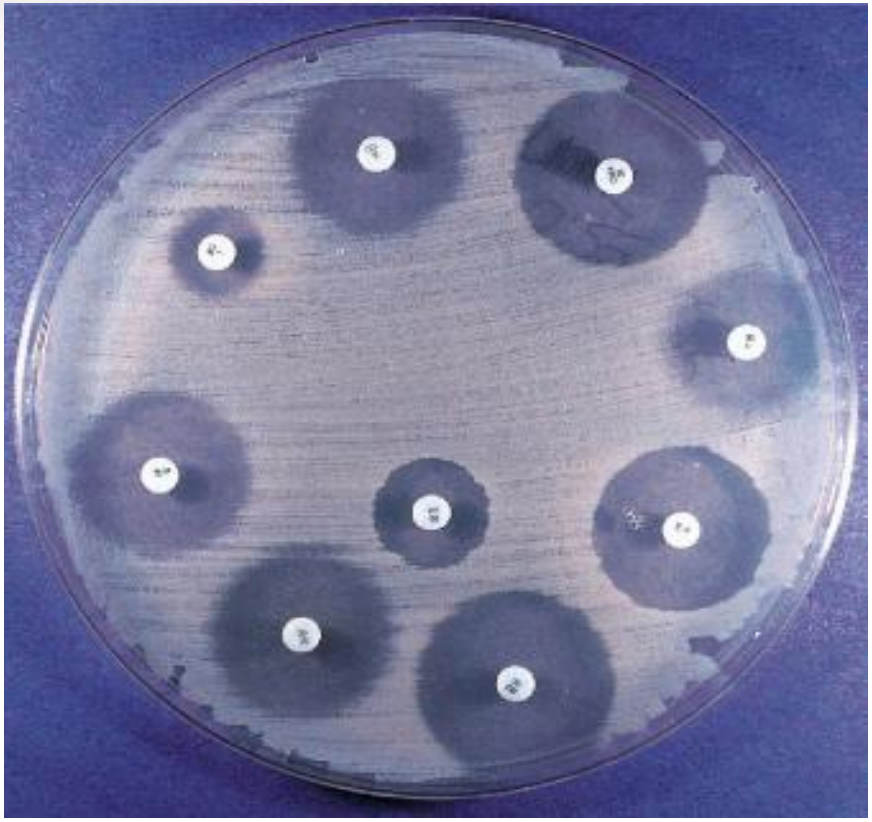
Inoculate the plate with your bacteria

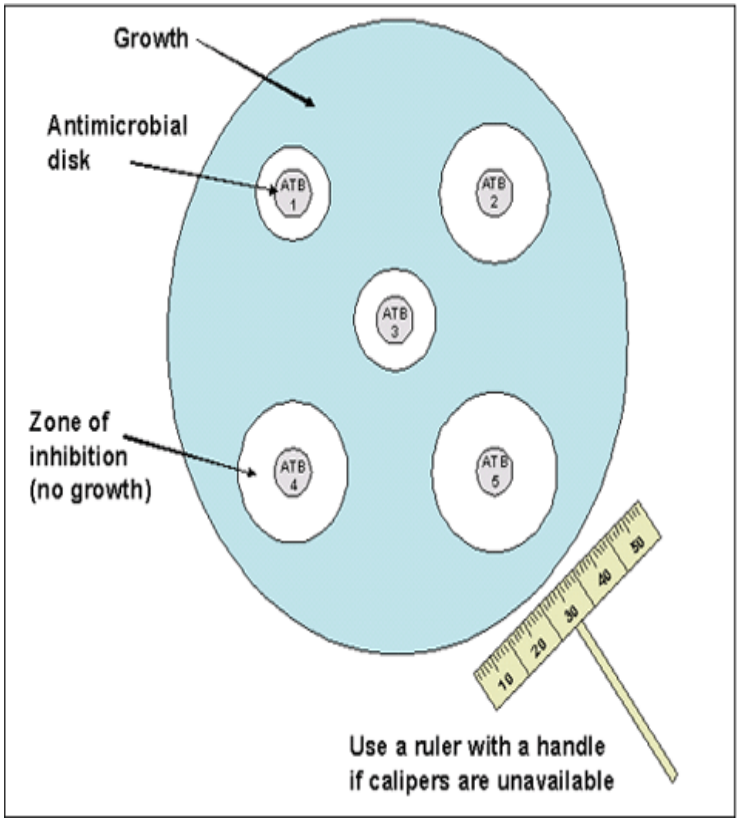
Use sterile forceps to blot the disk into the inoculated plate

The antimicrobial susceptibility test



The antimicrobial susceptibility test Disc method



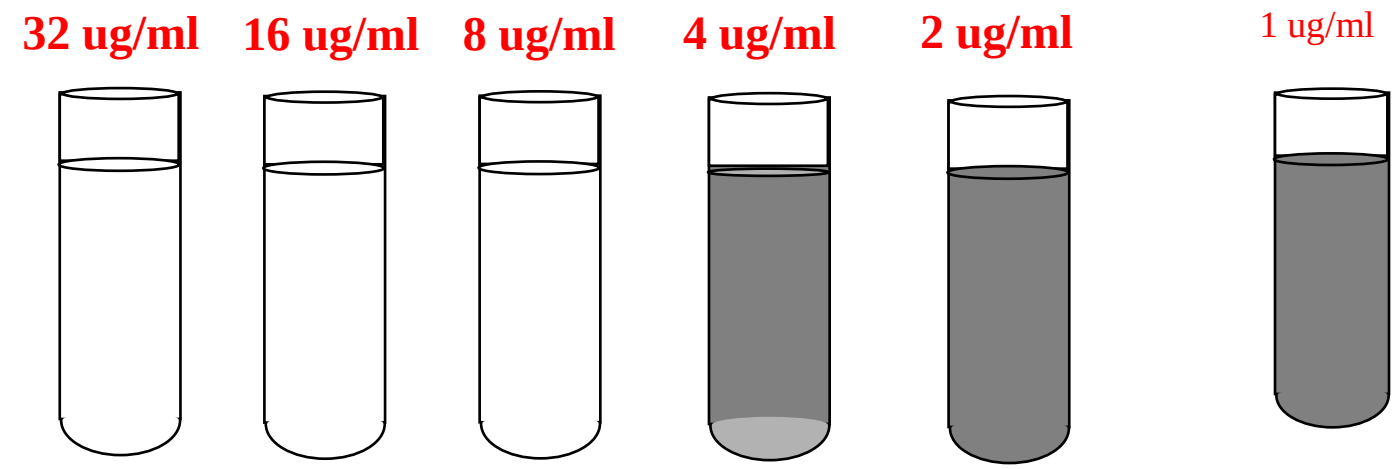


A Kirby-Bauer Plate. A Mueller-Hinton agar plate inoculated with *S. aureus* and various antibiotics. Notice the diameter of the various zones of inhibition.

Approved interpretive criteria for antimicrobials used in food animals…

	Zone Diameter (mm)			Concentrations (µg/ml)		
Antimicrobial	S	I	R	S	I	R
Clindamycin[2] (used for lincomycin testing)	≥21	15-20	≤14	≤0.5	1-2	≥4
Erythromycin[3]	≥23	14-22	≤13	≤0.5	1-4	≥8
Gentamicin	≥15	13-14	≤12	≤4	8	≥16
Oxacillin	≥13	11-12	≤10	≤2	---	≥4
Oxytetracycline	≥19	15-18	≤14	≤4	8	≥16
Penicillin[4]	≥28	20-27	≤19	≤0.12	0.25-2	≥4
Sulfathiazole	≥17	13-16	≤12	≤256	---	≥512
Tetracycline[5]	≥19	15-18	≤14	≤4	8	≥16
Trimethoprim/Sulphamethoxazole[6]	≥16	11-15	≤10	≤0.5/9.5	--	≥4/76

The antimicrobial susceptibility test
Tube dilution method



MIC = 8 ug/ml

MBC = 16 ug/ml

Table 8.3 Activity Levels of Selected Biocides

Class	Use Concentration of Active Ingredient	Activity Level[1]
Gas		
Ethylene oxide	450–500 mg/L[2]	High
Liquid		
Glutaraldehyde, aqueous	2–8%	High to intermediate
Formaldehyde + alcohol	8 + 70%	High to intermediate
Stabilized hydrogen peroxide	6–30%	High to intermediate
Formaldehyde, aqueous	6–8%	High to intermediate
Iodophors, high concentration	5,000–10,000 mg/L[3]	High to intermediate
Iodophors, low concentration	75–150 mg/L[3]	Intermediate to low
Iodine + alcohol	0.5 + 70%	Intermediate
Chlorine compounds	500–5,000 mg/L[4]	Intermediate
Phenolic compounds, aqueous	0.5–3%	Intermediate to low
Iodine, aqueous	1%	Intermediate
Alcohols (ethyl, isopropyl)	62–70%	Intermediate
Quaternary ammonium compounds	0.1–0.2% aqueous	Low
Chlorhexidine	0.75–4%	Low
Hexachlorophene	1–3%	Low
Mercurial compounds	0.1–0.2%	Low

1 High-level disinfectants destroy vegetative bacterial cells including *M. tuberculosis,* bacterial endospores, fungi, and viruses. Intermediate-level disinfectants destroy all of the above except spores. Low-level agents kill bacterial vegetative cells except for *M. tuberculosis,* fungi, and medium-sized lipid-containing viruses (but not bacterial endospores or small, nonlipid viruses).
2 In autoclave-type equipment at 55 to 60°C.
3 Available iodine.
4 Free chlorine.