

Chapter 7

Control of Microorganisms by Physical and Chemical Agents

To control the growth of microorganisms is to prevent the reproduction of those microorganisms.

Definition of Frequently Used Terms

- ✓ sterilization
 - destruction or removal of all viable organisms
 - *Sterility is an absolute! Either something is sterile or it is not!!*
- ✓ disinfection
 - killing, inhibition, or removal of 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)
 - Usually 99.9%
 - Used on inanimate objects (hospitals, food service, etc.)
- ✓ antiseptics
 - prevention of infection of living tissue by microorganisms
 - antiseptics
 - chemical agents that kill or inhibit growth of microorganisms when applied to tissue

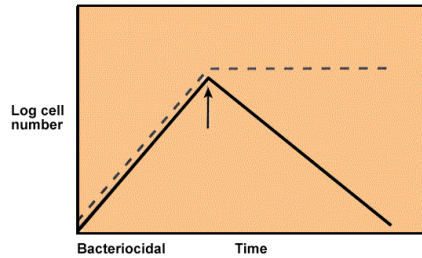
Antimicrobial agents

- ✓ agents that kill microorganisms or inhibit their growth
- ✓ -cidal agents kill
- ✓ -static agents inhibit growth

-cidal agents

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

Bacteriocidal

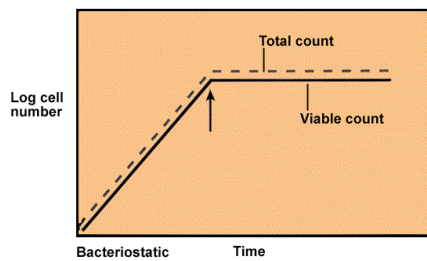


-static agents

-static

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

Bacteriostatic



The Pattern of Microbial Death

- ✓ microorganisms are not killed instantly
- ✓ population death usually occurs exponentially
- ✓ microorganisms are considered to be dead
 - unable to reproduce
 - conditions that normally support their reproduction

Table 7.1 A Theoretical Microbial Heat-Killing Experiment

Minute	Microbial Number at Start of Minute ^a	Microorganisms Killed in 1 Minute (90% of total) ^a	Microorganisms at End of 1 Minute	Log ₁₀ of Survivors
1	10 ⁶	9 × 10 ⁵	10 ⁵	5
2	10 ⁵	9 × 10 ⁴	10 ⁴	4
3	10 ⁴	9 × 10 ³	10 ³	3
4	10 ³	9 × 10 ²	10 ²	2
5	10 ²	9 × 10 ¹	10	1
6	10 ¹	9	1	0
7	1	0.9	0.1	-1

^aAssume that the initial sample contains 10⁶ vegetative microorganisms per ml and that 90% of the organisms are killed during each minute of exposure. The temperature is 121°C.

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
 - Endospores

More conditions...

- ✓ **concentration** or intensity of an antimicrobial agent
 - usually higher concentrations or intensities kill more rapidly
 - relationship is not linear
- ✓ **duration** of exposure
 - longer exposure ⇒ more organisms killed

More conditions...

- ✓ **temperature**
 - higher temperatures usually increase amount of killing
- ✓ **local environment**
 - many factors (e.g., pH, viscosity and concentration of organic matter) can profoundly impact effectiveness

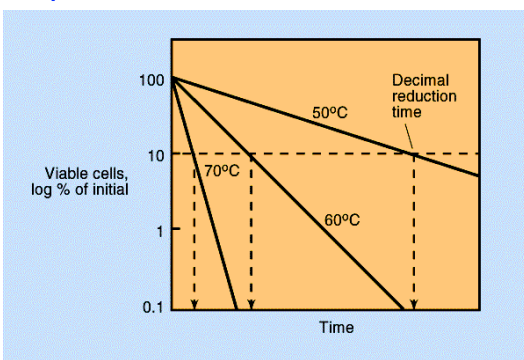
The Use of Physical Methods in Control

- ✓ heat
- ✓ low temperatures
- ✓ filtration
- ✓ radiation

Heat

- ✓ **moist heat**
 - effective against all types of microorganisms
 - degrades nucleic acids, denatures proteins, and disrupts membranes
- ✓ **dry heat sterilization**
 - less effective, requiring higher temperatures and longer exposure times
 - oxidizes cell constituents and denatures proteins

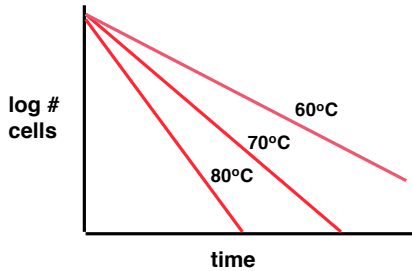
Exponential death



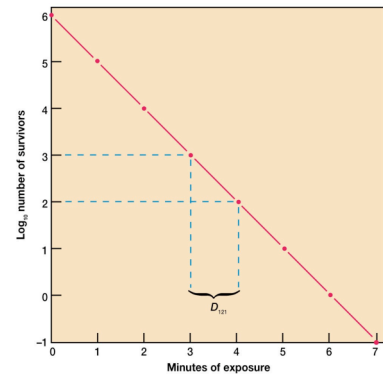
Measuring heat-killing efficiency

- ✓ **thermal death time (TDT)**
 - time needed to kill all microorganisms at a specific temperature
 - under defined conditions
- ✓ **decimal reduction time (*D* or *D value*)**
 - time required to kill 90% of microorganisms or spores at a specific temperature
 - decrease by one log

Thermal Death Time (TDT)



D value is equal to 1 minute



Other measures...

✓ Z value

- increase in temperature required to reduce D by 1/10

✓ F value

- time in minutes at a specific temperature needed to kill a population of cells or spores (usually 121°C)

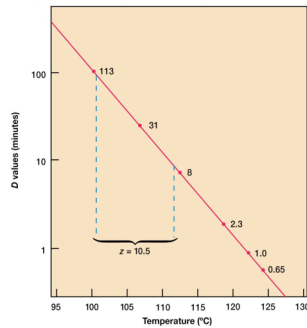


Table 7.3 D Values and z Values for Some Food-Borne Pathogens

Organism	Substrate	D Value (°C) in Minutes	z Value (°C)
<i>Clostridium botulinum</i>	Phosphate buffer	$D_{121} = 0.204$	10
<i>Clostridium perfringens</i> (heat-resistant strain)	Culture media	$D_{90} = 3-5$	6-8
<i>Salmonella</i>	Chicken à la king	$D_{60} = 0.39-0.40$	4.9-5.1
<i>Staphylococcus aureus</i>	Chicken à la king	$D_{60} = 5.17-5.37$	5.2-5.8
	Turkey stuffing	$D_{60} = 15.4$	6.8
	0.5% NaCl	$D_{60} = 2.0-2.5$	5.6

Values taken from F.L. Bryan, 1979, "Processes That Affect Survival and Growth of Microorganisms," *Time-Temperature Control of Foodborne Pathogens*, 1979. Atlanta: Centers for Disease Control and Prevention, Atlanta, GA.

Moist heat

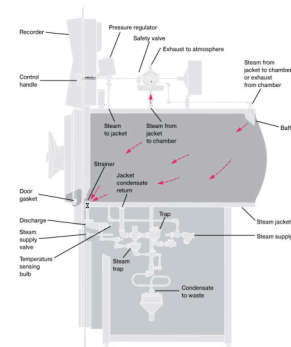
Table 7.2 Approximate Conditions for Moist Heat Killing

Organism	Vegetative Cells	Spores
Yeasts	5 minutes at 50-60°C	5 minutes at 70-80°C
Molds	30 minutes at 62°C	30 minutes at 80°C
Bacteria ^a	10 minutes at 60-70°C	2 to over 800 minutes at 100°C
Viruses	30 minutes at 60°C	0.5-12 minutes at 121°C

^aConditions for mesophilic bacteria.

autoclaves

- used to kill endospores efficiently
- use saturated steam under pressure to reach temperatures above boiling



Moist heat

✓ pasteurization

- controlled heating at temperatures well below boiling
- reduces total microbial population and thereby increases shelf life of treated material
- Denatures proteins, membranes, DNA

Pasteurization of milk

- ✓ Low Temp- Long time. 63°C for 30 min
- ✓ flash pasteurization (high temperature short-term – HTST)
 - 72°C for 15 seconds then rapid cooling
- ✓ ultrahigh-temperature (UHT) sterilization
 - 140 to 150°C for 1 to 3 seconds

Dry heat

✓ Dry heat Sterilization

- 160 to 170°C for 2 to 3 hours
 - Oxidation of cells molecules and protein denaturation
- ☞ Clostridium botulinum spores
- 5 min at 121°C using an autoclave
 - 2 hours at 160°C using dry heat

Incineration

- ✓ Hundreds of degrees.
- ✓ Sterilizes transfer loops, needles.
- ✓ Disposal of animal carcasses and contaminated objects.
- ✓ Some materials are destroyed by high temperatures.

Low Temperatures

✓ freezing

- stops microbial reproduction due to lack of liquid water
- some microorganisms killed by ice crystal disruption of cell membranes

✓ refrigeration

- slows microbial growth and reproduction

Freezers

- ✓ Less than 0°C - usually -20°C
 - ✓ Preserves foods and other materials.
 - ✓ Microbiostatic, rather than microbiocidal.
 - ✓ Thaw and refreeze can allow growth of microbes
- -60 to -80°C is used for preserving specimens
 - 4°C - psychrotrophic organisms grow

Liquid Nitrogen Refrigerators

- ✓ minus 180°C.
- ✓ Very effective preservation of microorganisms.
- ✓ Liquid N is expensive.

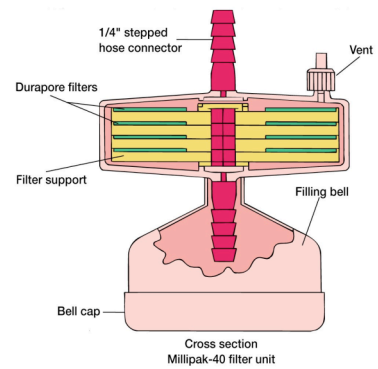
Filtration

- ✓ reduces microbial population or sterilizes solutions of heat-sensitive materials by removing microorganisms
- ✓ also used to reduce microbial populations in air
 - HEPA

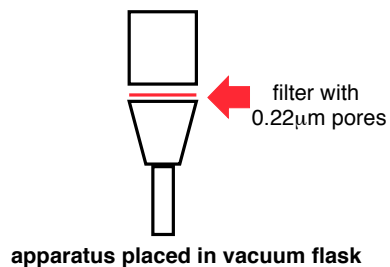
Filtering liquids

- ✓ depth filters
 - thick fibrous or granular filters that remove microorganisms by physical screening, entrapment, and/or adsorption
 - Glass, asbestos, diatomaceous earth
- ✓ membrane filters
 - porous membranes with defined pore sizes that remove microorganisms primarily by physical screening
 - Nylon, cellulose acetate, Teflon,

Membrane filter design

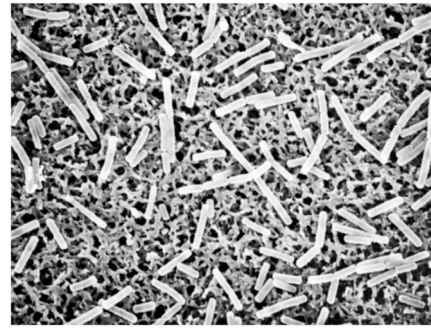


Membrane filters

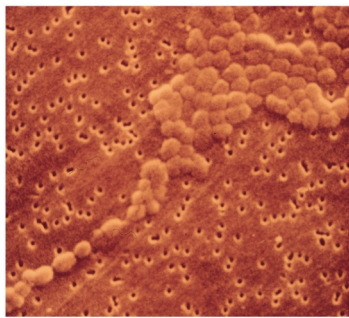


Filter sizes vs particles

PORE SIZE (in μm)	PARTICLES THAT PASS THROUGH THEM
10	Erythrocytes, yeast cells, bacteria, viruses, molecules
5	Yeast cells, bacteria, viruses, molecules
3	Some yeast cells, bacteria, viruses, molecules
1.2	Most bacteria, viruses, molecules
0.45	A few bacteria, viruses, molecules
0.22	Viruses, molecules
0.10	Medium-sized to small viruses, molecules
0.05	Small viruses, molecules
0.025	Only the very smallest viruses, molecules
Ultrafilter	Small molecules



nylon membrane with bacterial removal rating of 0.2 μm



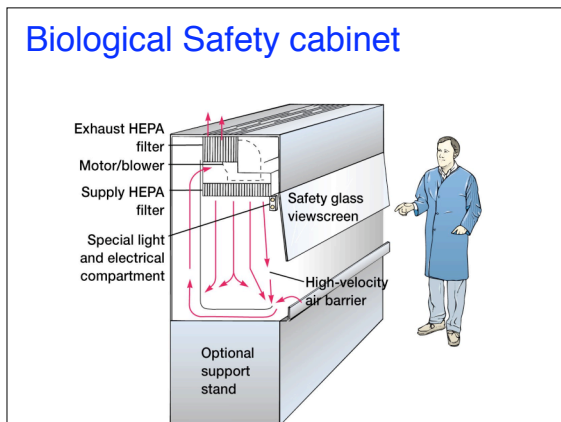
polycarbonate membrane with 0.4 μm pores

Filtering air

- ✓ surgical masks
- ✓ cotton plugs on culture vessels
- ✓ high-efficiency particulate air (HEPA) filters
 - used in laminar flow biological safety cabinets
 - 99.97% of 0.3 μm particles



Biological Safety cabinet



Radiation

- ✓ ultraviolet (UV) radiation
 - limited to surface sterilization
 - ▷ UV radiation does not penetrate glass, dirt films, water, and other substances
- ✓ ionizing radiation
 - penetrates deep into objects
 - destroys bacterial endospores; not always effective against viruses
 - used for sterilization and pasteurization of antibiotics, hormones, sutures, plastic disposable supplies, and food

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Table 7.4
Activity Levels of Selected Germicides

Class	Use Concentration of Active Ingredient	Activity Level ^a
Gas		
Ethylene oxide	450-500 mg/liter ^b	High
Liquid		
Glutaraldehyde, aqueous	2%	High to intermediate
Formaldehyde + alcohol	8 + 70%	High
Stabilized hydrogen peroxide	6-30%	High to intermediate
Formaldehyde, aqueous	6-8%	High to intermediate
Iodophors	750-5,000 mg/liter ^c	High to intermediate
Iodine + alcohol	75-150 mg/liter ^c	Intermediate to low
Chlorine compounds	0.1-0.5% ^d	Intermediate
Phenolic compounds, aqueous	0.5-3%	Intermediate to low
Iodine, aqueous	1%	Intermediate
Alcohols (ethyl, isopropyl)	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

Source: From Seymour S. Block, *Disinfection, Sterilization and Preservation*, Copyright © 1983 Lea & Febiger, Malvern, Pa, 1983. Reprinted by permission.
^aHigh-level disinfectants destroy vegetative bacterial cells including *M. tuberculosis*, bacterial endospores, fungi, and viruses. Intermediate-level disinfectants destroy all of the above except endospores. Low-level agents kill bacterial vegetative cells except for *M. tuberculosis*, fungi, and certain sized lipid-containing viruses that are bacterial endospores or small, nonlipid viruses.
^bUse in a well-ventilated area.
^cAvailable iodine.
^dFree chlorine.

The Use of Chemical Agents in Control

Table 7.5 Relative Efficacy of Commonly Used Disinfectants and Antiseptics

Class	Disinfectant	Antiseptic	Comment
Gas			
Ethylene oxide	3-4 ^b	0 ^a	Sporicidal; toxic; good penetration; requires relative humidity of 30% or more; microbicidal activity varies with apparatus used; absorbed by porous material; dry spores highly resistant; moisture must be present, and presoaking is most desirable
Liquid			
Glutaraldehyde, aqueous	3	0	Sporicidal; active solution unstable; toxic
Stabilized hydrogen peroxide	3	0	Sporicidal; use solution stable up to 6 weeks; toxic orally and to eyes; mildly skin toxic; little inactivated by organic matter
Formaldehyde + alcohol	3	0	Sporicidal; noxious fumes; toxic; volatile
Formaldehyde, aqueous	1-2	0	Sporicidal; noxious fumes; toxic
Phenolic compounds	3	0	Stable; corrosive; little inactivation by organic matter; irritates skin
Chlorine compounds	1-2	0	Fast action; inactivation by organic matter; corrosive; irritates skin
Alcohol	1	3	Rapidly microbicidal except for bacterial spores and some viruses; volatile; flammable; dries and irritates skin
Iodine + alcohol	0	4	Corrosive; very rapidly microbicidal; causes staining; irritates skin; flammable
Iodophors	1-2	3	Somewhat unstable; relatively bland; staining temporary; corrosive
Iodine, aqueous	0	2	Rapidly microbicidal; corrosive; stains fabrics; stains and irritates skin
Quaternary ammonium compounds	1	0	Bland; inactivated by soap and anionics; compounds absorbed by fabrics; old or dilute solution can support growth of gram-negative bacteria
Hexachlorophene	0	2	Bland; insoluble in water; soluble in alcohol; not inactivated by soap; weakly bactericidal
Chlorhexidine	0	3	Bland; soluble in water and alcohol; weakly bactericidal
Mercurial compounds	0	±	Bland; much inactivated by organic matter; weakly bactericidal

Source: From Seymour S. Block, *Disinfection, Sterilization and Preservation*, Copyright © 1983 Lea & Febiger, Malvern, Pa, 1983. Reprinted by permission.
^aSubjective ratings of practical usefulness in a hospital environment—4 is maximal usefulness; 0 is little or no usefulness; ± signifies that the substance is sometimes useful but not always.

Mechanisms of action of Chemical agents

✓ Protein denaturation

- Hydrogen and disulfide bonds are disrupted
- Heat, acids, & alkalis denature
- Permanent denaturation
 - Bacteriocidal
- Temporarily denaturation
 - Bacteriostatic

Temporarily denatured protein

Active protein → Inactive protein

Reconfiguration; temporarily denatured

Example: warmed milk

Permanently denatured protein

Active protein → Inactive protein

No reconfiguration; permanently denatured

Example: fried egg

Phenol and derivatives

Phenol: Oc1ccccc1

Orthocresol: Oc1ccccc1C

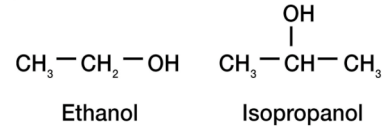
Orthophenylphenol: Oc1ccc(cc1)-c2ccccc2

Hexachlorophene: ClC1=C(Cl)C(Cl)=C(Cl)C(Cl)=C1Cl

Phenolics

- ✓ commonly used as laboratory and hospital disinfectants
 - act by denaturing proteins and disrupting cell membranes
 - tuberculocidal
 - effective in presence of organic material,
 - long lasting
- ✓ disagreeable odor and can cause skin irritation

Alcohols



- ✓ bactericidal, fungicidal, but not sporicidal
- ✓ inactivate some viruses
- ✓ denature proteins and possibly dissolve membrane lipids
- ✓ 70% concentration is best

Halogens

Halogens include:

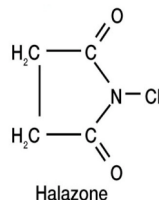
- ✓ iodine
- ✓ chlorine
- ✓ bromine

Halogens

- ✓ Iodine
 - Tincture iodine (2% KI in 70% EtOH)
 - skin antiseptic
 - oxidizes cell constituents and iodates proteins
 - at high concentrations may kill spores
 - skin damage, staining, and allergies can be a problem
 - Iodophore (Betadine or Wescodyne)
 - ☐ iodine complexed with organic carrier
 - ☐ Reduces irritation

Chlorine

- ✓ oxidizes cell constituents
 - Hypochlorous acid (HClO) → O + HCl
- ✓ Uses
 - important in disinfection of water supplies and swimming pools
 - used in dairy and food industries
 - effective household disinfectant
- ✓ destroys vegetative bacteria and fungi, but not spores
- ✓ can react with organic matter to form carcinogenic compounds (trihalomethanes)



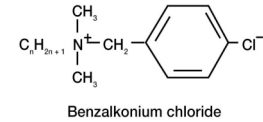
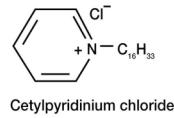
Heavy Metals

- ✓ Ions of mercury, silver, arsenic, zinc, and copper
- ✓ effective but usually toxic
- ✓ combine with and inactivate proteins; may also precipitate proteins
 - Silver nitrate - infant's eyes,
 - Silver sulfadiazine - burns

Antimicrobial uses of heavy metals

- ✓ Mercurochrome, methiolate and metaphen are used to treat minor cuts, wounds and skin infections.
- ✓ Silver nitrate - treat eyes of infants at birth to prevent gonococcal infections.
- ✓ Copper sulfate is an algicide in water.
- ✓ Zinc compounds are fungicidal
- ✓ Silver sulfadiazine - burn dressings

Quaternary Ammonium Compounds



Quaternary Ammonium Compounds

- ✓ Detergents
 - organic molecules with hydrophilic and hydrophobic ends
 - act as wetting agents and emulsifiers
- ✓ cationic detergents are effective disinfectants
 - Benzalkonium chloride & cetylpyridinium chloride
 - kill most bacteria, but not *Mycobacterium tuberculosis* or endospores
 - safe and easy to use, but inactivated by hard water and soap

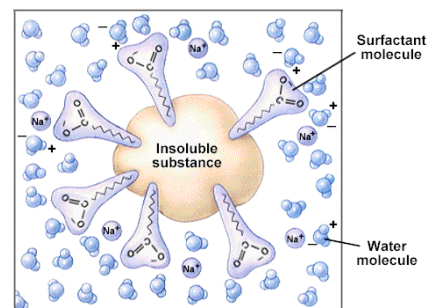
Soaps & Detergents

- ✓ Soaps remove dirt and microbes
 - They may kill some
- ✓ Quaternary Ammonium Compounds (quats)
 - bacteriocidal for both G+ and G- bacteria
 - low toxicity
 - detergent action
 - high stability
 - noncorrosive

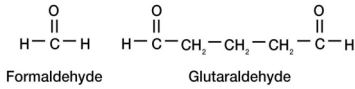
Mechanisms of action of Chemical agents

- ✓ Reactions on membranes
 - **Surfactants** disrupt lipid membranes
 - Alcohols, detergents, & quaternary ammonium compounds (benalkonium chloride)
 - Dissolve lipids
 - Phenols
 - Dissolve lipids
 - Denature proteins

Surfactants reduce surface tension

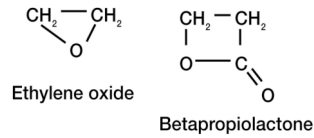


Aldehydes



- ✓ highly reactive molecules
- ✓ sporicidal and can be used as chemical sterilants
- ✓ combine with and inactivate nucleic acids and proteins

Sterilizing Gases



- ✓ used to sterilize heat-sensitive materials
- ✓ microbicidal and sporicidal
- ✓ Alkylating agents
 - Replace chemical groups on proteins and nucleic acids
 - Hydrogen, amino, or alcohol groups

Oxidizing agents

- ✓ Disrupt disulfide bonds
 - Disrupt membranes and proteins
- ✓ H₂O₂
- ✓ O₃
 - fruits

Dyes

- ✓ DNA mutations - acridine
- ✓ cell wall synthesis - crystal violet

Phenol coefficient test

- ✓ potency of a disinfectant is compared to that of phenol

Table 7.6 Phenol Coefficients for Some Disinfectants

Disinfectant	Phenol Coefficients ^a	
	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>
Phenol	1	1
Cetylpyridinium chloride	228	337
<i>o</i> -phenylphenol	5.6 (20°C)	4.0
<i>p</i> -cresol	2.0-2.3	2.3
Hexachlorophene	5-15	15-40
Merthiolate	600	62.5
Mercurochrome	2.7	5.3
Lysol	1.9	3.5
Isopropyl alcohol	0.6	0.5
Ethanol	0.04	0.04
2% I ₂ solution in EtOH	4.1-5.2 (20°C)	4.1-5.2 (20°C)

^aAll values were determined at 37°C except where indicated.

Other evaluation methods

- ✓ use dilution test
 - determines rate at which selected bacteria are destroyed by various chemical agents
 - ⇨ Polished stainless steel cylinder
 - Bacteria is dried on the surface
 - Immersed into the disinfectant
 - Transferred to culture media for 48 hr
- ✓ in-use testing
 - testing done using conditions that approximate normal use of disinfectant
 - ⇨ Contaminate a surface
 - ⇨ Spray the surface with disinfectant
 - ⇨ Determine killing

Activity levels

Table 7.4 Activity Levels of Selected Germicides

Class	Use Concentration of Active Ingredient	Activity Level ^a
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Liquid		
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Formaldehyde + alcohol	8 + 70%	High
Stabilized hydrogen peroxide	6-30%	High to intermediate
Formaldehyde, aqueous	6-8%	High to intermediate
Iodophors	750-5,000 mg/liter ^c	High to intermediate
Iodophors	75-150 mg/liter ^c	Intermediate to low
Iodine + alcohol	0.5 + 70%	Intermediate
Chlorine compounds	0.1-0.5% ^d	Intermediate
Phenolic compounds, aqueous	0.5-3%	Intermediate to low
Iodine, aqueous	1%	Intermediate
Alcohols (ethyl, isopropyl)	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

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^bThe active stage requires at 55 to 60°C.
^cAvailable iodine.
^dFree chlorine.

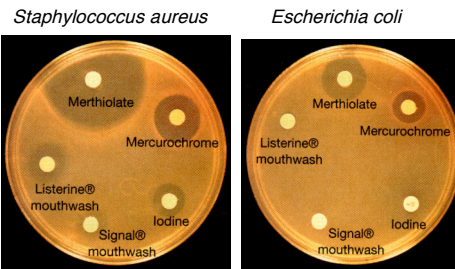
Antibiotics

- ✓ Substances produced by microorganisms that has antimicrobial activity.
- ✓ Active in small quantities.
- ✓ Typically act on the metabolism of cells whose growth they inhibit.

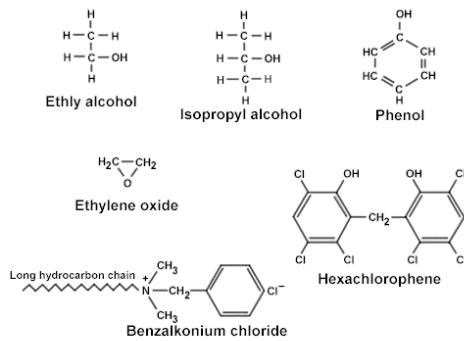
Measuring Antimicrobial Activity

- ✓ Tube dilution method
- ✓ Disk-plate method

Disk-plate method



Structures of some important disinfectants (fig. 12.4)

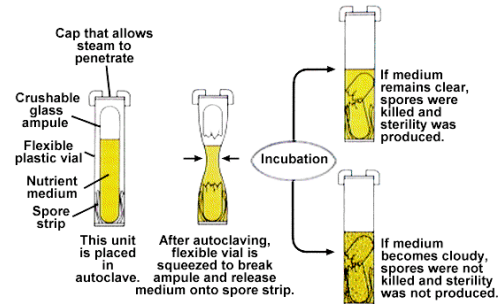


Other information

Boiling Water

- ✓ 100°C for 10 minutes
- ✓ Kills vegetative cells on instruments and containers.
- ✓ Not reliable for sterilization; spores are not killed

Checking for sterility



Drying (Desiccation)

- ✓ Absence of water
 - Preserves food
 - Will destroy some pathogens
 - No effect on most spores
- ✓ Lyophilization
 - Freeze drying
 - Long term preservation.
 - Does not normally kill microbes