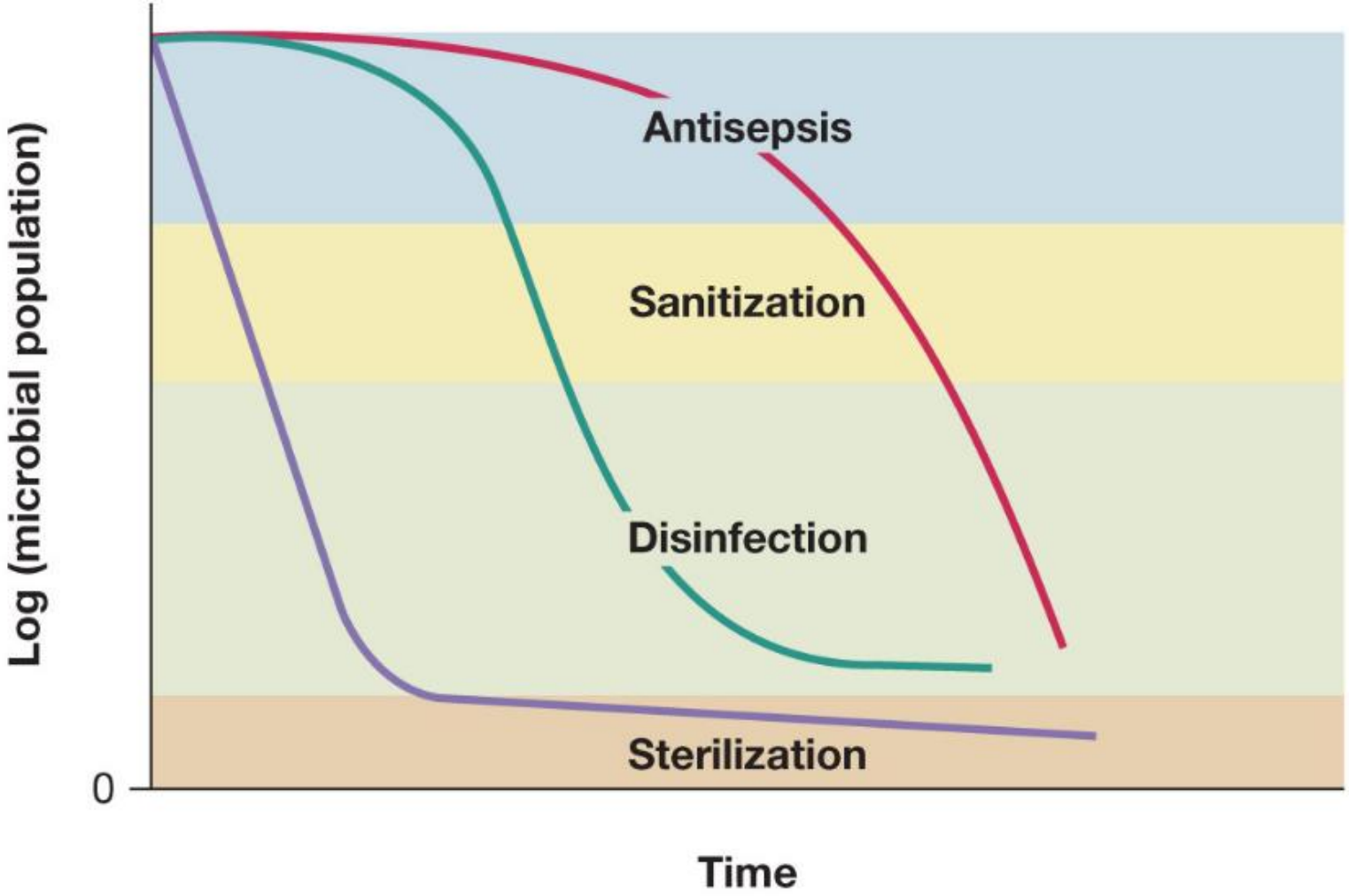
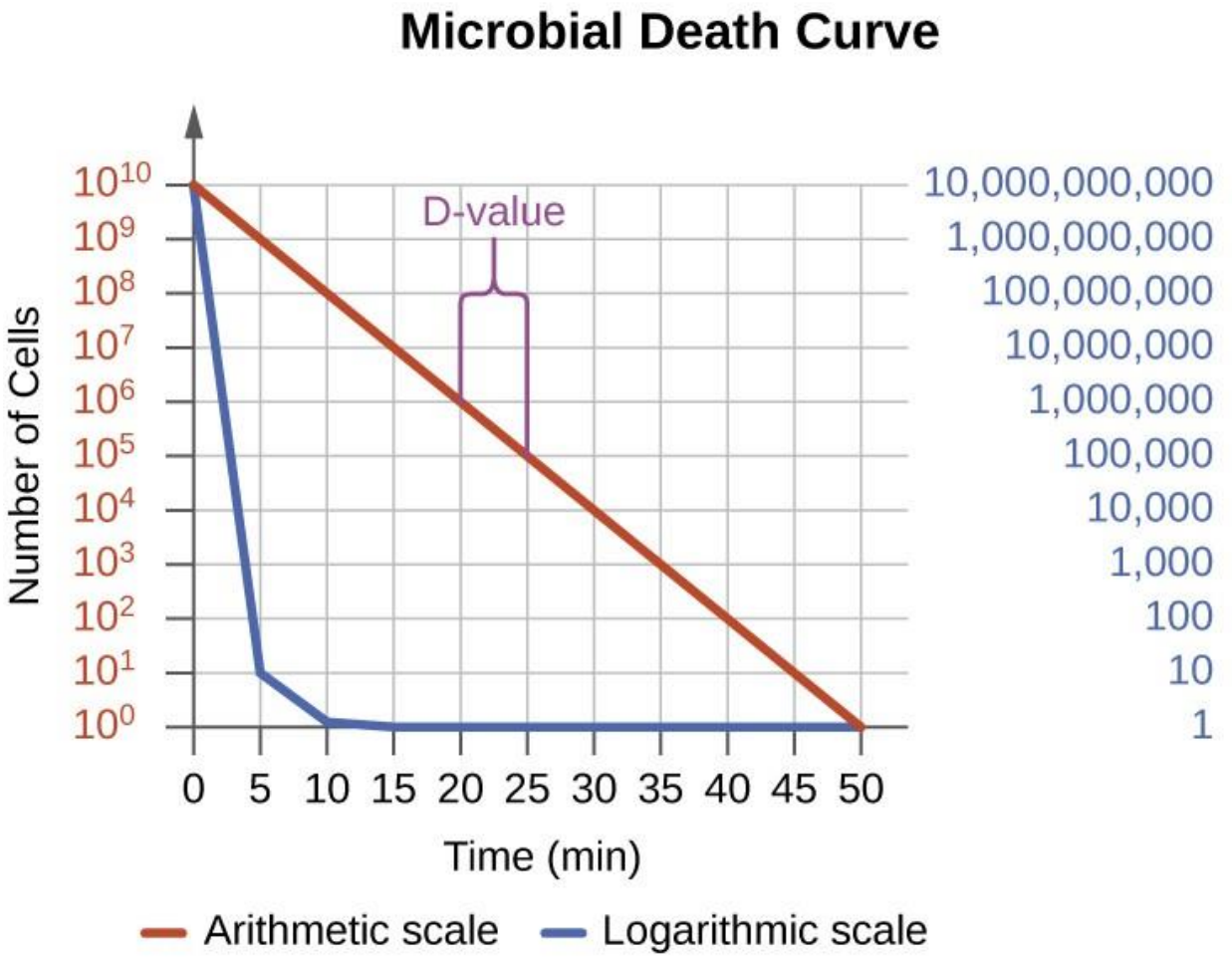
Control of Microbial Growth

Benilda Ramos-Butron

Terminologies Related to Control of Microbial Growth

Common Protocols for Control of Microbial Growth			
Protocol	**Definition**	**Common Application**	**Common Agents**
For Use on Fomites			
Disinfection	Reduces or destroys microbial load of an inanimate item through application of heat or antimicrobial chemicals	Cleaning surfaces like laboratory benches, clinical surfaces, and bathrooms	Chlorine bleach, phenols (e.g., Lysol), glutaraldehyde
Sanitization	Reduces microbial load of an inanimate item to safe public health levels through application of heat or antimicrobial chemicals	Commercial dishwashing of eating utensils, cleaning public restrooms	Detergents containing phosphates (e.g., Finish), industrial-strength cleaners containing quaternary ammonium compounds
Sterilization	Completely eliminates all vegetative cells, endospores, and viruses from an inanimate item	Preparation of surgical equipment and of needles used for injection	Pressurized steam (autoclave), chemicals, radiation
For Use on Living Tissue			
Antisepsis	Reduces microbial load on skin or tissue through application of an antimicrobial chemical	Cleaning skin broken due to injury; cleaning skin before surgery	Boric acid, isopropyl alcohol, hydrogen peroxide, iodine (betadine)
Degerming	Reduces microbial load on skin or tissue through gentle to firm scrubbing and the use of mild chemicals	Handwashing	Soap, alcohol swab


Antisepsis
Sanitization
Disinfection
Sterilization
Log (microbial population)
0
Time



Time (min)	Number of cells
0	10^{10}
5	10^{9}
10	10^{8}
15	10^{7}
20	10^{6}
25	10^{5}
30	10^{4}
35	10^{3}
40	10^{2}
45	10^{1}
50	10^{0}

Actions of Microbial Control Agents

1. Alteration of Membrane Permeability
2. Damage to Proteins and Nucleic Acid

Control of Microbial Growth

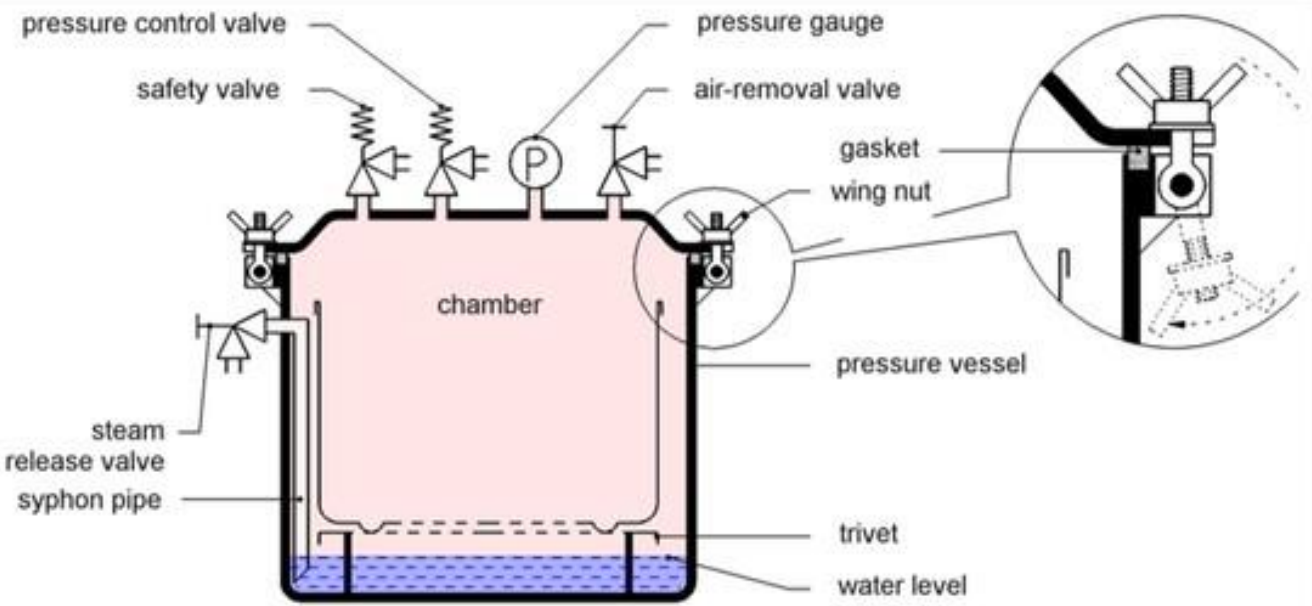
Terminologies

1. *Thermal death point* - lowest temperature at which all the microorganisms in a liquid suspension will be killed in 10 minutes (constant time)
2. *Thermal death time* - the minimal length of time for all bacteria in a liquid culture to be killed at a given temperature (constant temperature)
3. *Decimal reduction time* - is the time, in minutes, in which 90% of a population of bacteria at a given temperature will be killed

Physical Methods of Control			
Method	**Conditions**	**Mode of Action**	**Example Uses**
Heat			
Boiling	100 °C at sea level	Denatures proteins and alters membranes	Cooking, personal use, preparing certain laboratory media
Dry-heat oven	170 °C for 2 hours	Denatures proteins and alters membranes, dehydration, desiccation	Sterilization of heat-stable medical and laboratory equipment and glassware
Incineration	Exposure to flame	Destroy by burning	Flaming loop, microincinerator
Autoclave	Typical settings: 121 °C for 15 minutes at 15 pounds per square inch (psi)	Denatures proteins and alters membranes	Sterilization of microbiological media, heat-stable medical and laboratory equipment, and other heat-stable items
Pasteurization	Can vary. One type is 72 °C for 15 seconds (HTST)	Denatures proteins and alters membranes	Prevents spoilage of milk, apple juice, honey, and other ingestible liquids
Cold			
Refrigeration	0 °C to 7 °C	Inhibits metabolism (slows or arrests cell division)	Preservation of food or laboratory materials (solutions, cultures)
Freezing	Below −2 °C	Stops metabolism, may kill microbes	Long-term storage of food, laboratory cultures, or medical specimens

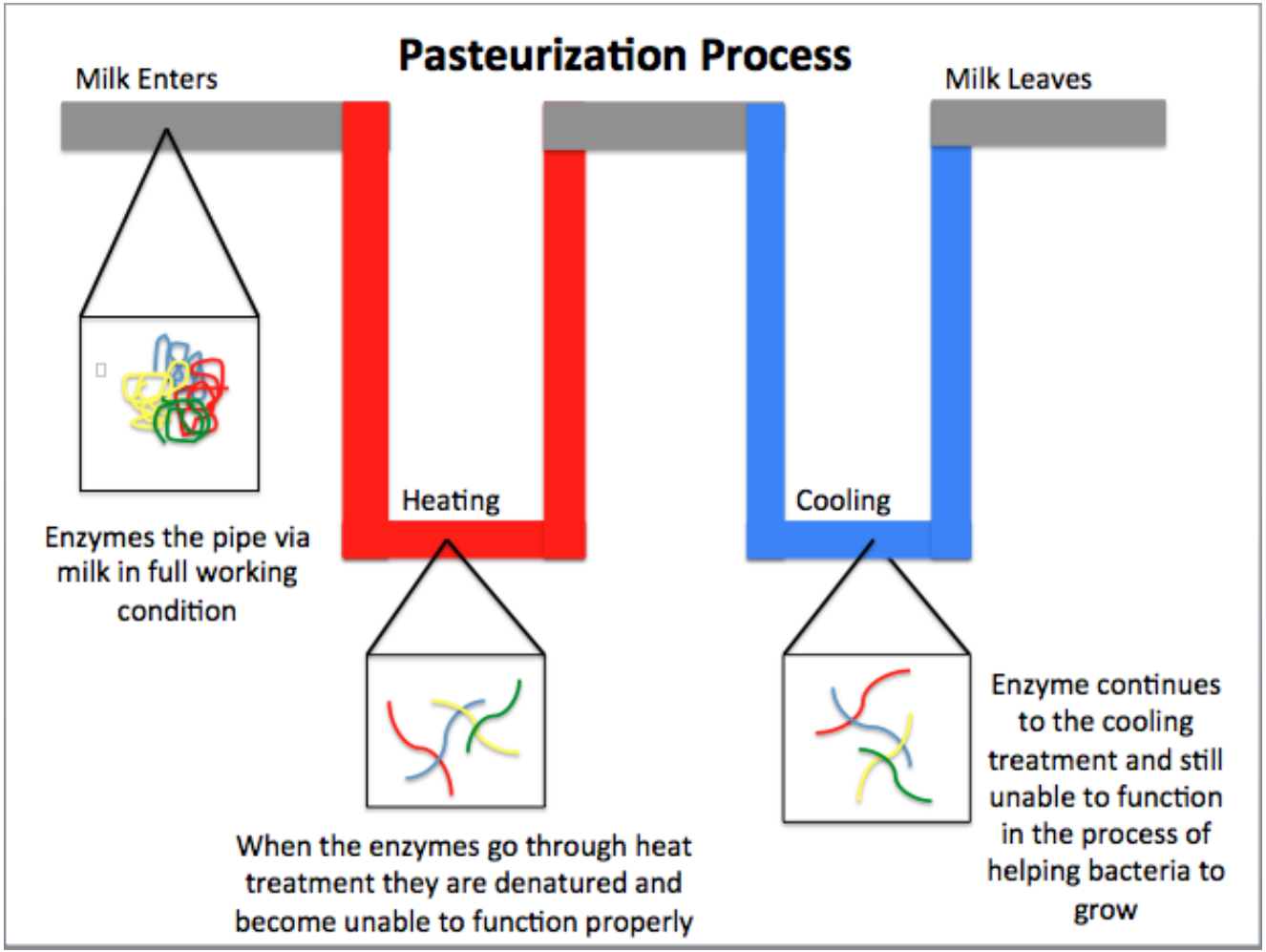

180
MENU
0%
UN 30
memmert


alamy
Image ID: HAC5E1
www.alamy.com

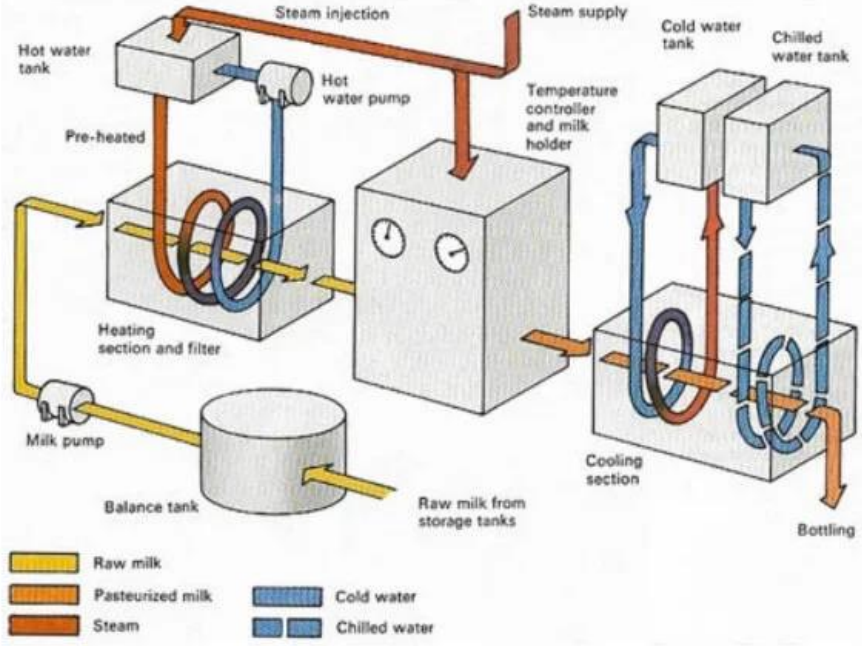

pressure control valve
safety valve
pressure gauge
air-removal valve
gasket
wing nut
chamber
pressure vessel
steam
release valve
syphon pipe
trivet
water level

The Effect of Container Size on Autoclave Sterilization Times for Liquid Solutions		
Container Size	Liquid Volume	Sterilization Time (min)
Test tube: 18 x 150 nm	10 ml	15
Erlenmeyer flask: 125 ml	95 ml	15
Erlenmeyer flask: 2000 ml	1500 ml	30
Fermentation bottle: 9000 ml	6750 ml	70

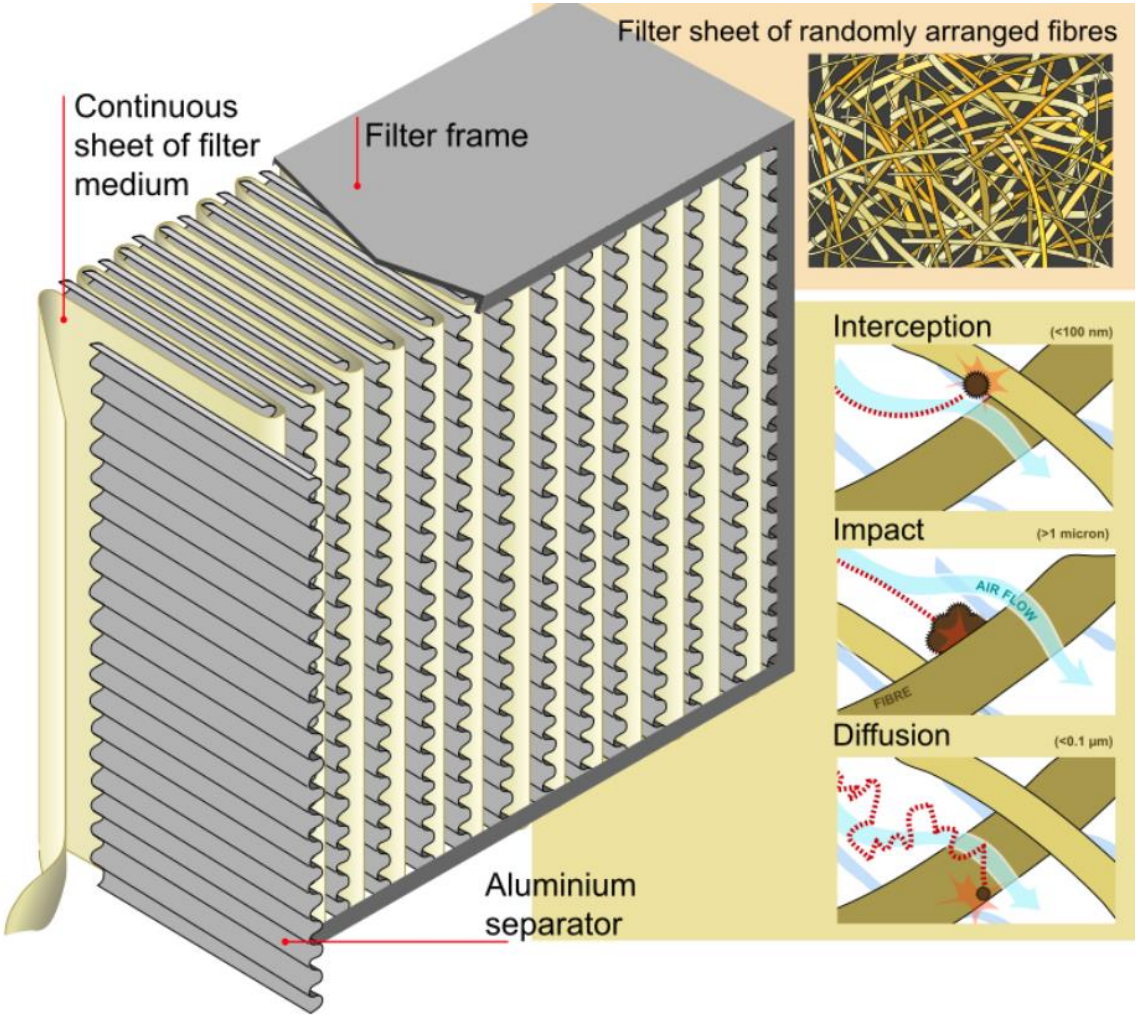
Pasteurization Process

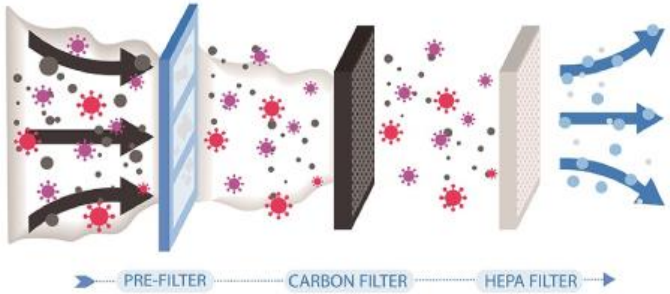


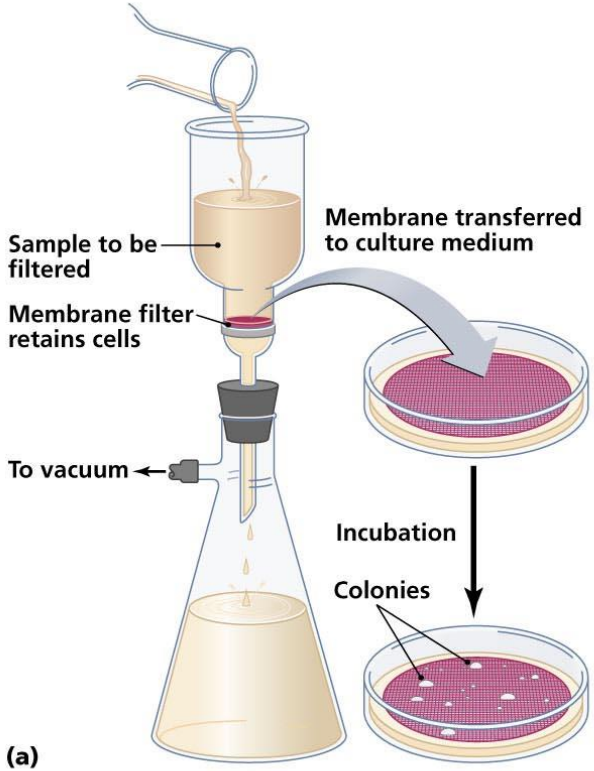
Pasteurization

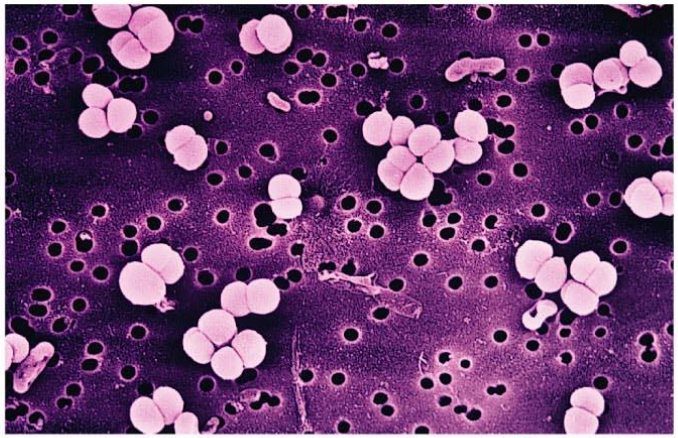


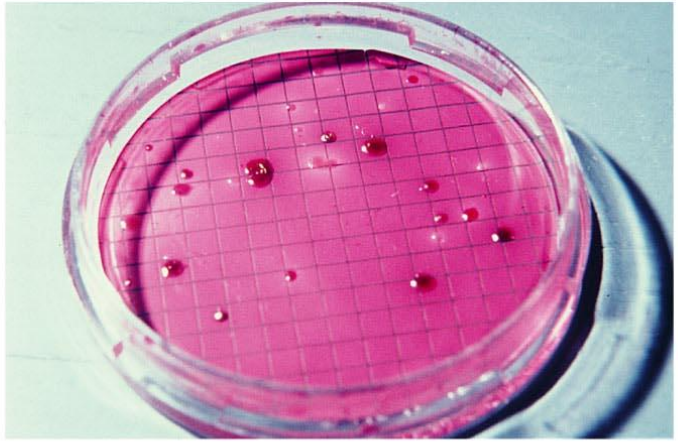
Pasteurization Type	Typical Product	Typical Storage	Temperature (°C)	Holding Time
Batch, vat	Milk	Refrigerated	63	30 min
	Viscous products, or products with more than 10 % fat or added sweetener	Refrigerated	66	30 min
	Egg nog, frozen dessert mixes	Refrigerated	68	30 min
Continuous, high temperature short time (HTST)	Milk	Refrigerated	72	15 s
	Viscous products, or products with more than 10 % fat or added sweetener	Refrigerated	74	15 s
	Egg nog, frozen dessert mixes	Refrigerated	79	25 s
Continuous, higher heat shorter time (HHST)	Milk	Refrigerated	88 90 94 96 100	1 s 0.5 s 0.1 s 0.05 s 0.01 s
Continuous, Ultrapasteurization	Milk and cream	Refrigerated, extended storage	138	2 s
Aseptic, ultra high temperature (UHT)	Milk	Room Temperature	135-150	4-15 s
Sterilization	Canned products	Room Temperature	116	20 min


Filter sheet of randomly arranged fibres
Continuous sheet of filter medium
Filter frame
Interception
(<100 nm)
Impact
(>1 micron)
AIR FLOW
FIBRE
Diffusion
(<0.1 µm)
Aluminium separator


PRE-FILTER
CARBON FILTER
HEPA FILTER



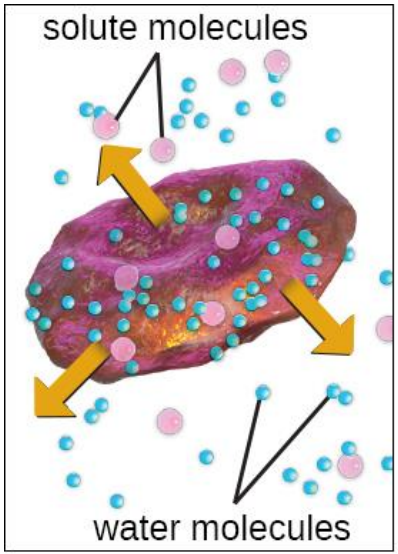




Pressure			
High-pressure processing	100–800 MPa	Denatures proteins and can cause cell lysis	Preservation of food
Hyberbaric oxygen therapy	Air pressure three times higher than normal	Inhibits metabolism and growth of anaerobic microbes	Treatment of certain infections (e.g., gas gangrene)
Desiccation			
Simple desiccation	Drying	Inhibits metabolism	Dried fruits, jerky
Reduce water activity	Addition of salt or water	Inhibits metabolism and can cause lysis	Salted meats and fish, honey, jams and jellies
Lyophilization	Rapid freezing under vacuum	Inhibits metabolism	Preservation of food, laboratory cultures, or reagents
Radiation			
Ionizing radiation	Exposure to X-rays or gamma rays	Alters molecular structures, introduces double-strand breaks into DNA	Sterilization of spices and heat-sensitive laboratory and medical items; used for food sterilization in Europe but not widely accepted in US
Nonionizing radiation	Exposure to ultraviolet light	Introduces thymine dimers, leading to mutations	Surface sterilization of laboratory materials, water purification

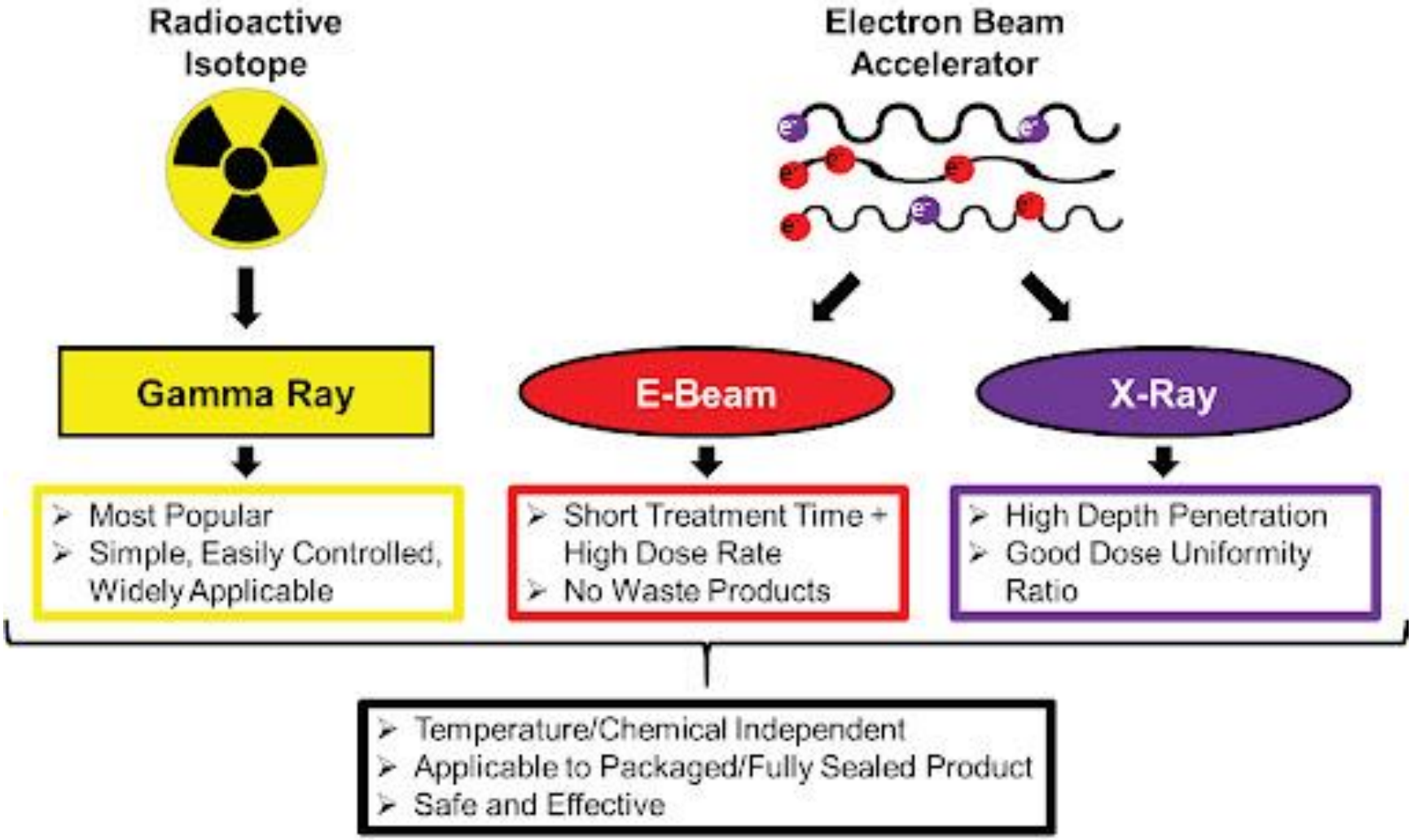
Desiccation - absence of water to the microbial cell

Osmotic Pressure



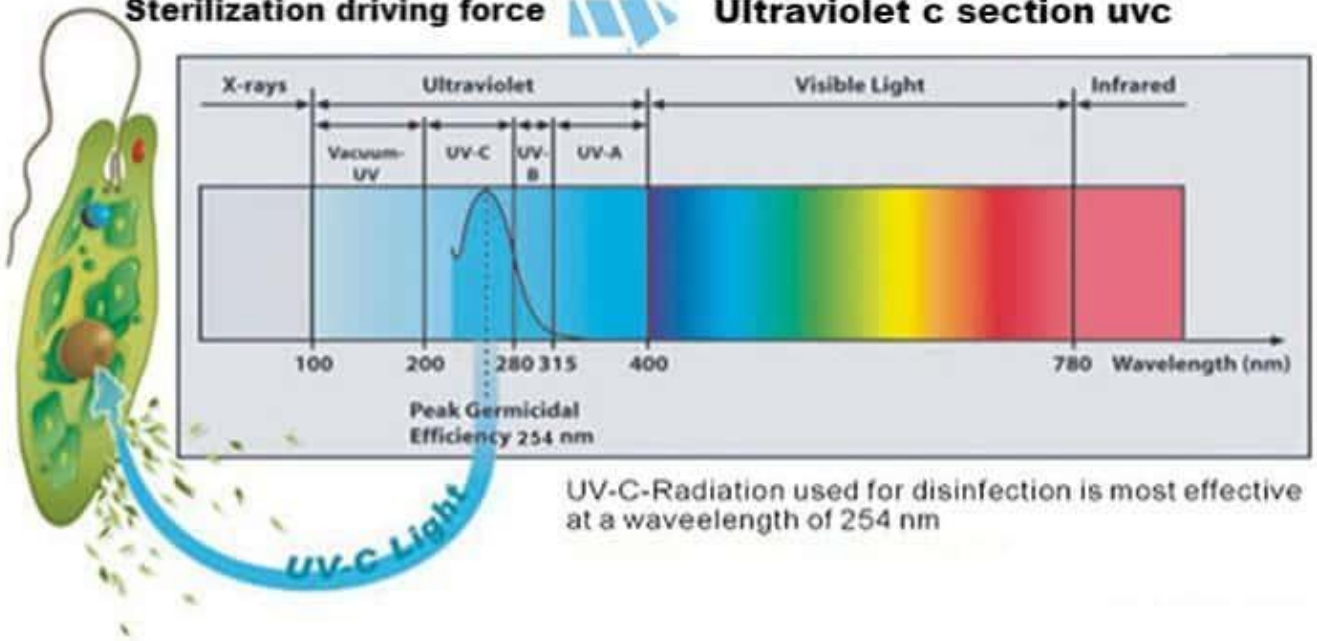
(a)

(b)


Radioactive Isotope
Electron Beam Accelerator
Gamma Ray
E-Beam
X-Ray
Most Popular
Simple, Easily Controlled, Widely Applicable
Short Treatment Time + High Dose Rate
No Waste Products
High Depth Penetration
Good Dose Uniformity Ratio
Temperature/Chemical Independent
Applicable to Packaged/Fully Sealed Product
Safe and Effective

Ultraviolet sterilization

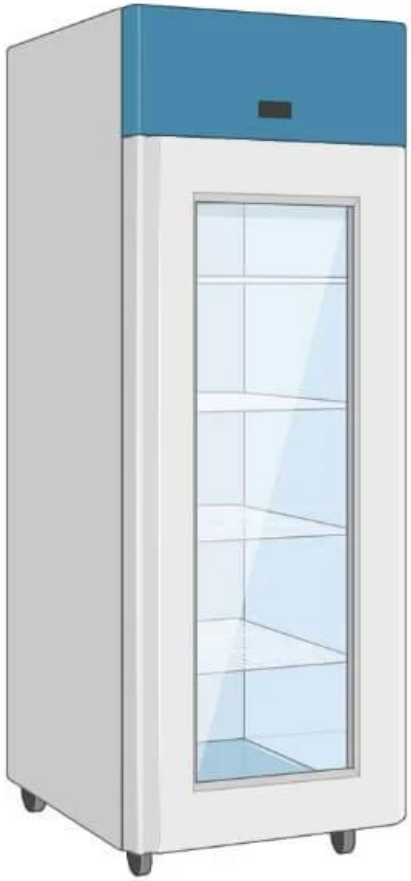
Sterilization driving force **Ultraviolet c section uvc**

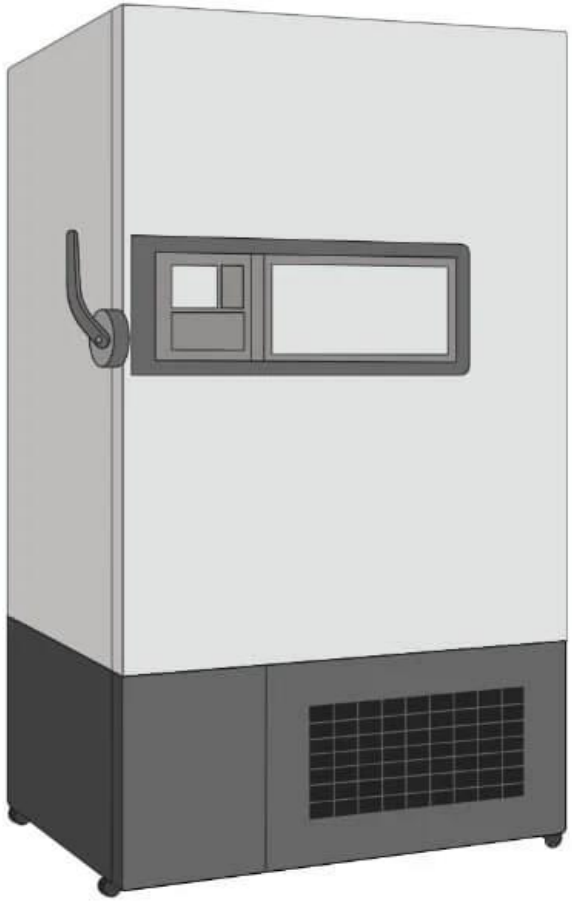


UV-C-Radiation used for disinfection is most effective at a waveelength of 254 nm

Ultraviolet light is divided into A, B, C, D four bands, with sterilization efficacy is the C band of which 254nm is the strongest ultraviolet bactericidal power.

Laboratory Refrigerators and Freezers





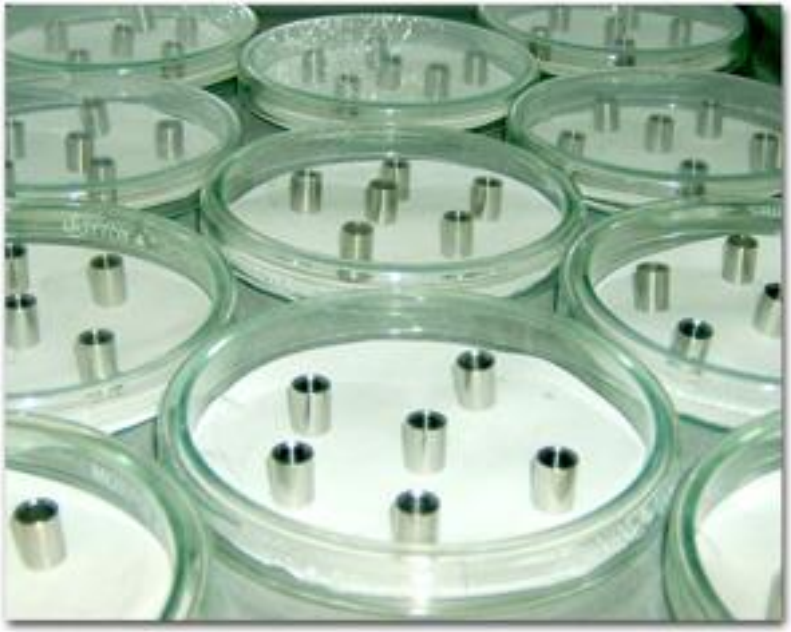
Chemical Methods of Microbial Control

Principles of Effective Disinfection

1. Concentration
2. Nature of the material being disinfected
3. pH
4. Contact with the microbe

Evaluating Disinfectants

Use-Dilution Tests

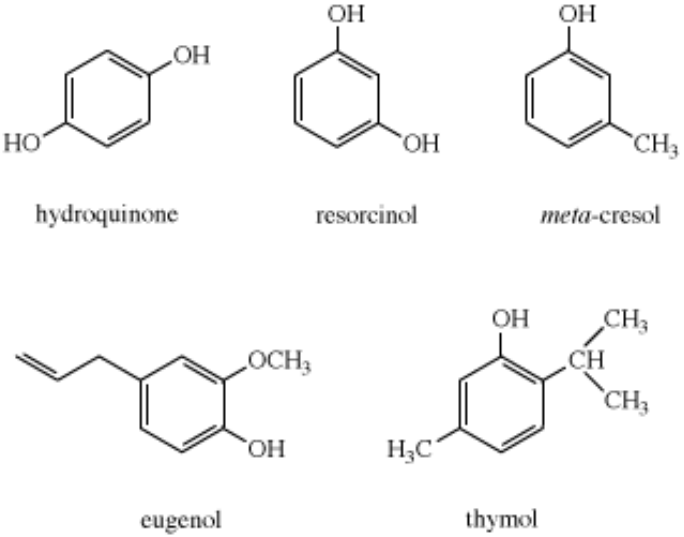


Disk-Diffusion Method

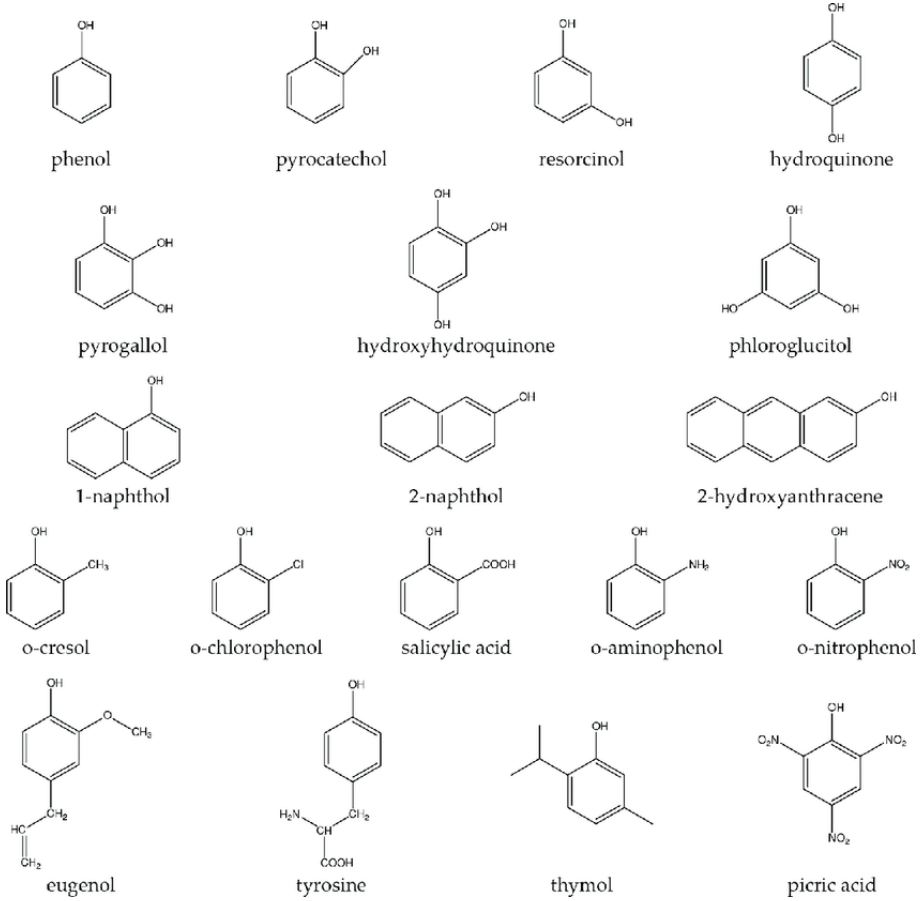
Types of Disinfectants

Phenol and Phenolics

- Also called **carbolic acid**
- **Phenolics** - derivative of phenol



Phenol



Phenolics

Active

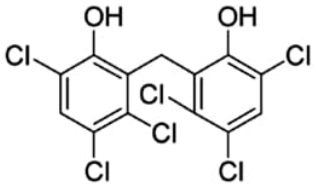
- o-Benzyl-p-chorophenol

Intentionally Added

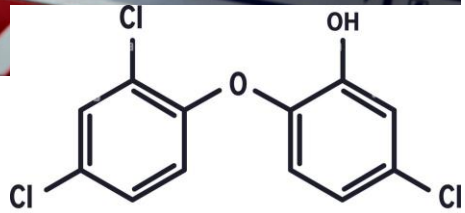
- Water
- Coconut Oil, Fixed
- Potassium Hydroxide
- Ethanol DL
- Tetrasodium EDTA
- p-Ethylphenol
- 2,4-Xylenol DL
- m-Cresol DL
- m-Ethylphenol DL
- p-Cresol DL
- 2,5-Xylenol
- 2,3-Dimethylphenol
- 3,5-Dimethylphenol
- 2-Ethylphenol
- 3,4-Xylenol
- 2,6-Xylenol
- FD&C Red 4
- D&C Red No. 33

Bisphenol

- Derivative of phenol that contains two phenolic groups connected by a bridge (hexachlorophene and triclosan)



hexachlorophene



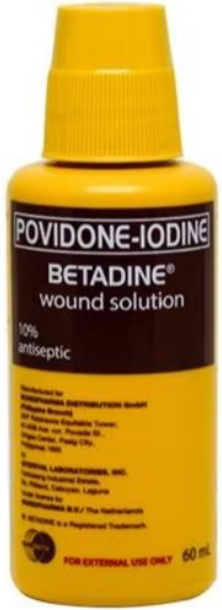
TRICLOSAN

Biguanides

- Includes chlorhexidine
- Broad spectrum for skin and mucous membranes

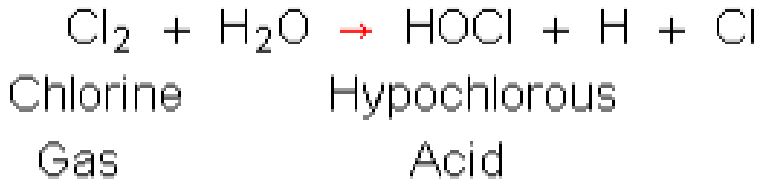
Halogens

- Refers to iodine and chlorine



Halogen

- Chlorine



- Forms: calcium hypochlorite (Ca(OCl)2 (chloride of lime), sodium hypochlorite (NaOCl) and chloramine (Cl and ammonia)


Active ingredients:
Calcium oxychloride
Inert ingredients: 1.6%
Provides 65% available chlorine
Calcium hypochlorite
Ca(OCl)2 65%
For SHOCK treatment of pool water
SPT pools
CALCIUM HYPOCHLORITE
Hypochlorite de calcium
Calciumhypochlorit
5 Kg
20 Kg
50 Kg
For swimming pool use only
Store in a cool, dry, well ventilated place.
Keep out of reach of children
made in China
distributed by SPTpools
TOLL FREE 180 030 0180
swimmingpoolsthailand.com


For Stainless & HE Machines
ACTIVE INGREDIENT:
Sodium Hypochlorite.....7.5%
OTHER INGREDIENTS:.....92.5%
TOTAL:.....100.0%
(Yields 7.1% available chlorine)
CONTAINS NO PHOSPHORUS.
2.53 QT
CLOROX
DISINFECTING BLEACH

PETSHOP.PH
OFFICIAL STORE

SEA QUEST | CHORINE NEUTRALIZER
CHLORINE REMOVAL 120ML

Alcohol

- Kills bacteria and fungi but not endospore and nonenveloped viruses

TABLE 7.6 **Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution Against *Streptococcus pyogenes***

Concentration of Ethanol (%)	Time (sec)				
	10	20	30	40	50
100	–	–	–	–	–
95	+	+	+	+	+
90	+	+	+	+	+
80	+	+	+	+	+
70	+	+	+	+	+
60	+	+	+	+	+
50	–	–	+	+	+
40	–	–	–	–	–

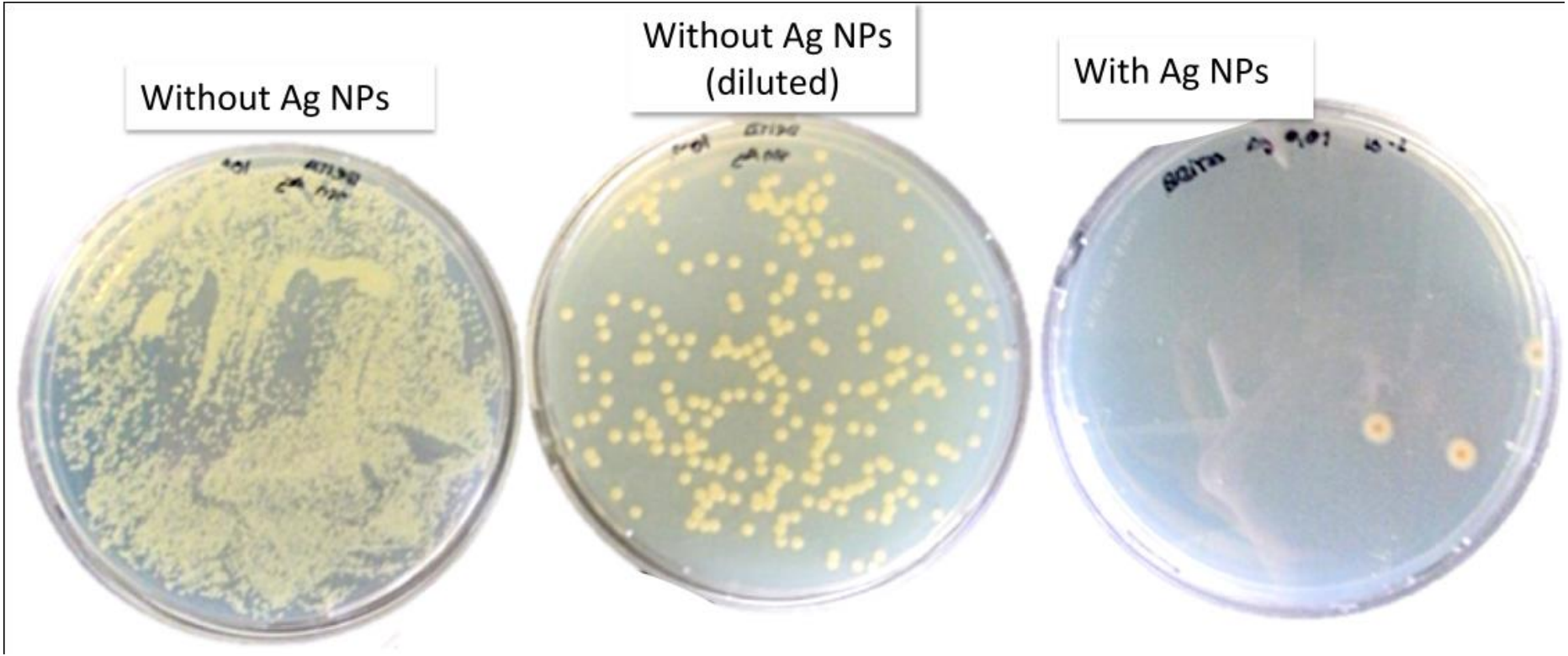
NOTE: A minus sign indicates no biocidal action (bacterial growth); a plus sign indicates biocidal action (no bacterial growth). The highlighted area represents bacteria killed by biocidal action.

Heavy Metals and their Compounds

- Ag, Hg, and Cu

  Oligodynamic action - refers to the ability of very small amount of heavy metals to exert antimicrobial activity


67%
more zinc
chloride
CLINICALLY PROVEN TO REDUCE GUM BLEEDING, REDNESS, AND INFLAMMATION
RÉDUIT LE SAIGNEMENT, LA ROUGEUR ET L'INFLAMMATION DES GENCIVES, PREUVES CLINIQUES À L'APPUI
ANTISEPTIC MOUTHWASH | RINCE-BOUCHE ANTISEPTIQUE
LISTERINE
CLINICAL SOLUTIONS™MC
GUM THERAPY • SOIN DES GENCIVES
Treats gingivitis for 3X healthier gums in 1 week'
Traite la gingivite pour des gencives 3x plus saines en 1 semaine'
ICY MINT • MENTHE GLACÉE
30057009
500 mL
Child Resistant Package
Flacon sécurité-enfants


Without Ag NPs
Without Ag NPs (diluted)
With Ag NPs

Surface Active Agents

- “Surfactants” can decrease surface tension among molecules of a liquid
- Soaps and detergents

Quaternary Ammonium Compounds (QUATS)

- Bactericidal against Gram + bacteria
- Fungicidal, amoebicidal and virucidal against enveloped viruses
- Cleaning ability is related to the positively charged portion - the cation

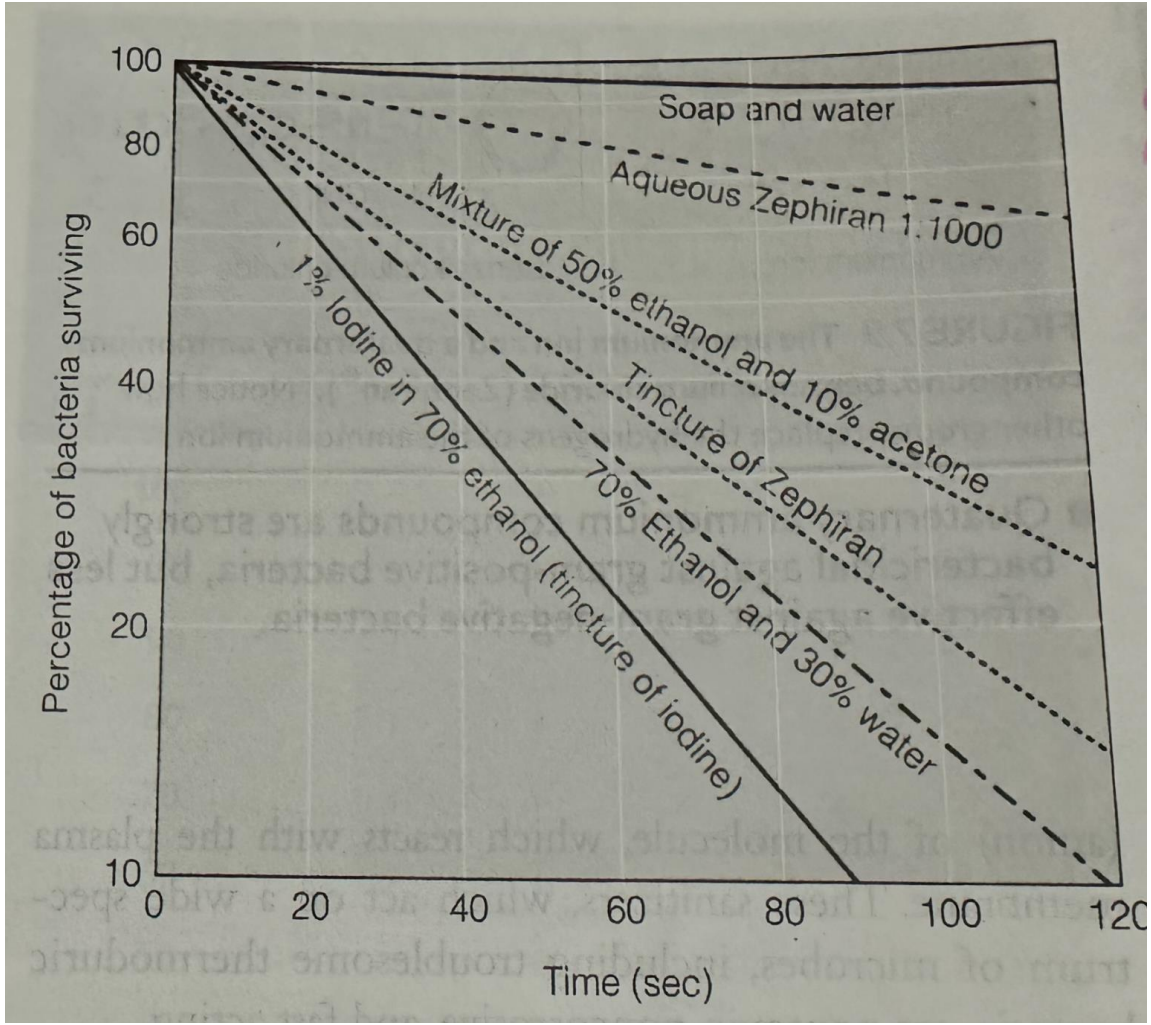


FIGURE 7.10 A comparison of the effectiveness of various antiseptics. The steeper the downward slope of the killing curve of the antiseptic, the more effective it is. A 1% iodine in 70% ethanol solution is the most effective; soap and water are the least effective. Notice that a tincture of Zephiran® is more effective than an aqueous solution of the same antiseptic.

Chemical Food Preservatives

1. Sulfur dioxide - wine making disinfectant
2. Sodium benzoate
3. Sorbic acid or potassium sorbate
4. Calcium propionate
5. Sodium nitrate/nitrite

Antibiotics

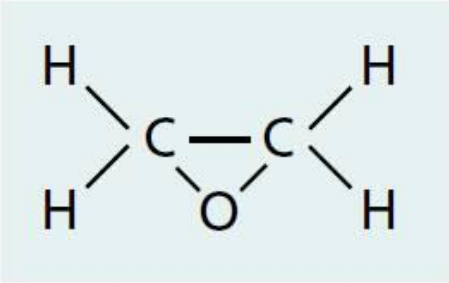
- **Nisin and natamycin**, prevents endospore forming bacteria in cheese and antifungal antibiotic for foods, respectively

Aldehydes

- One of the most effective antimicrobial agents
- Formaldehyde and glutaraldehyde
- Action: inactivate proteins

Gaseous Chemosterilizers

- Chemicals that sterilize in a closed chamber
- Ethylene oxide



Ethylene oxide

Peroxygens

- Exert antimicrobial activity by oxidizing cellular components
- Ozone, hydrogen peroxide, and peracetic acid, and benzoyl peroxide

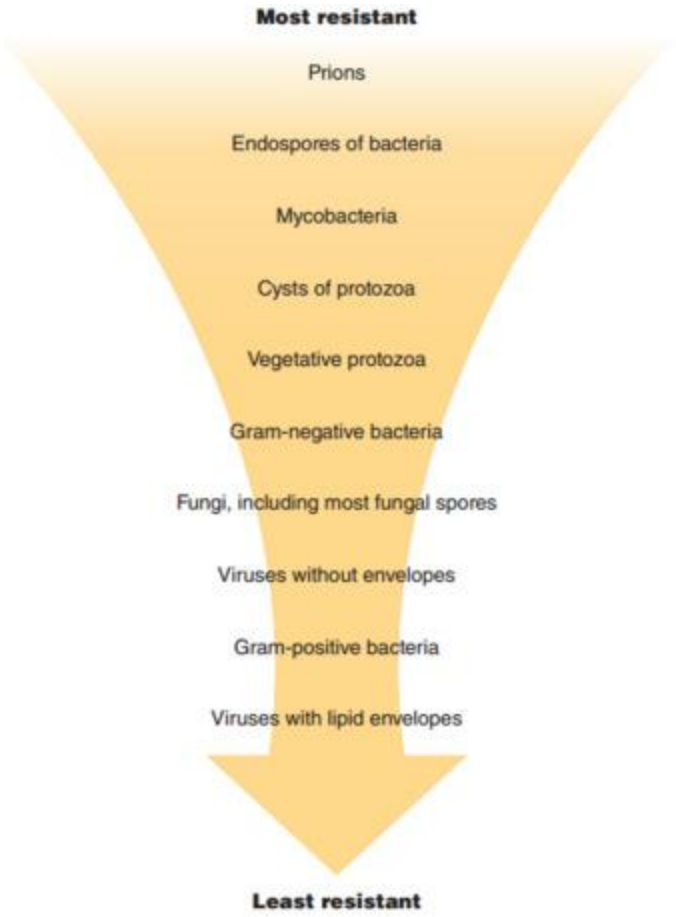


Figure 7.11 Decreasing order of resistance of microorganisms to chemical biocides.

TABLE 7.7 **The Effectiveness of Chemical Antimicrobials Against Endospores and Mycobaceria**

Chemical Agent	Endospores	Mycobacteria
Mercury	No activity	No activity
Phenolics	Poor	Good
Bisphenols	No activity	No activity
Quaternary ammonium compounds	No activity	No activity
Chlorines	Fair	Fair
Iodine	Poor	Good
Alcohols	Poor	Good
Glutaraldehyde	Fair	Good
Chlorhexidine	No activity	Fair

SUMMARY

table 7.8 *Chemical Agents Used to Control Microbial Growth*

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Phenol and Phenolics			
1. Phenol	Disruption of plasma membrane, denaturation of enzymes.	Rarely used, except as a standard of comparison.	Seldom used as a disinfectant or antiseptic because of its irritating qualities and disagreeable odor.
2. Phenolics	Disruption of plasma membrane, denaturation of enzymes.	Environmental surfaces, instruments, skin surfaces, and mucous membranes.	Derivatives of phenol that are reactive even in the presence of organic material; O-phenylphenol is an example.
3. Bisphenols	Probably disruption of plasma membrane.	Disinfectant hand soaps and skin lotions.	Triclosan is an especially common example of a bisphenol. Broad spectrum, but most effective against gram-positives.
Biguanides (Chlorhexidine)	Disruption of plasma membrane.	Skin disinfection, especially for surgical scrubs.	Bactericidal to gram-positives and gram-negatives; nontoxic, persistent.
Halogens	Iodine inhibits protein function and is a strong oxidizing agent; chlorine forms the strong oxidizing agent hypochlorous acid, which alters cellular components.	Iodine is an effective antiseptic available as a tincture and an iodophor; chlorine gas is used to disinfect water; chlorine compounds are used to disinfect dairy equipment, eating utensils, household items, and glassware.	Iodine and chlorine may act alone or as components of inorganic and organic compounds.

Alcohols	Protein denaturation and lipid dissolution.	Thermometers and other instruments; in swabbing the skin with alcohol before an injection, most of the disinfecting action probably comes from a simple wiping away (degerming) of dirt and some microbes.	Bactericidal and fungicidal, but not effective against endospores or nonenveloped viruses; commonly used alcohols are ethanol and isopropanol.
Heavy Metals and Their Compounds	Denaturation of enzymes and other essential proteins.	Silver nitrate may be used to prevent gonorrheal opthalmia neonatorum; mercurochrome disinfects skin and mucous membranes; copper sulfate is an algicide.	Heavy metals such as silver and mercury are biocidal.
Surface-Active Agents			
1. Soaps and acid-anionic detergents	Mechanical removal of microbes through scrubbing.	Skin degerming and removal of debris.	Many antibacterial soaps contain antimicrobials.
2. Acid-anionic detergents	Not certain; may involve enzyme inactivation or disruption.	Sanitizers in dairy and food-processing industries.	Wide spectrum of activity; nontoxic, noncorrosive, fast-acting.
3. Cationic detergents (quaternary ammonium compounds)	Enzyme inhibition, protein denaturation, and disruption of plasma membranes.	Antiseptic for skin, instruments, utensils, rubber goods.	Bactericidal, bacteriostatic, fungicidal, and virucidal against enveloped viruses; examples of quats are Zephiran® and Cepacol®.

END.