T& Total
V& Viable
C& count

Detection of objectionable MOs

- Non sterile dosage forms may contain some MOs
- The quality of non sterile products is controlled by pharmacopeia in two ways:

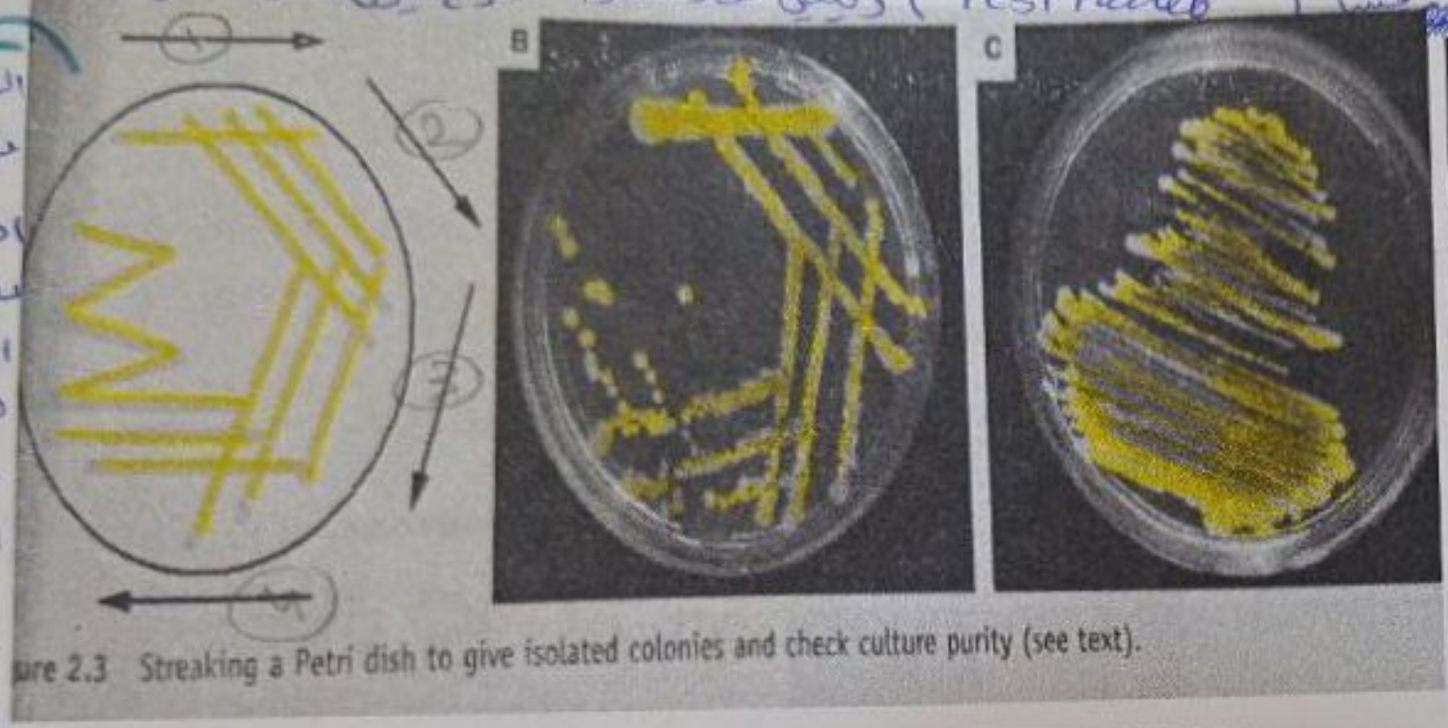
1. Limit on total number or concentration of MO that may be present
2. Particular objectionable organisms must be absent in a specified weight of material:

e.g. EP quality gelatin → Salmonella should be absent in a 10g sample and E.coli absent in 1 g sample

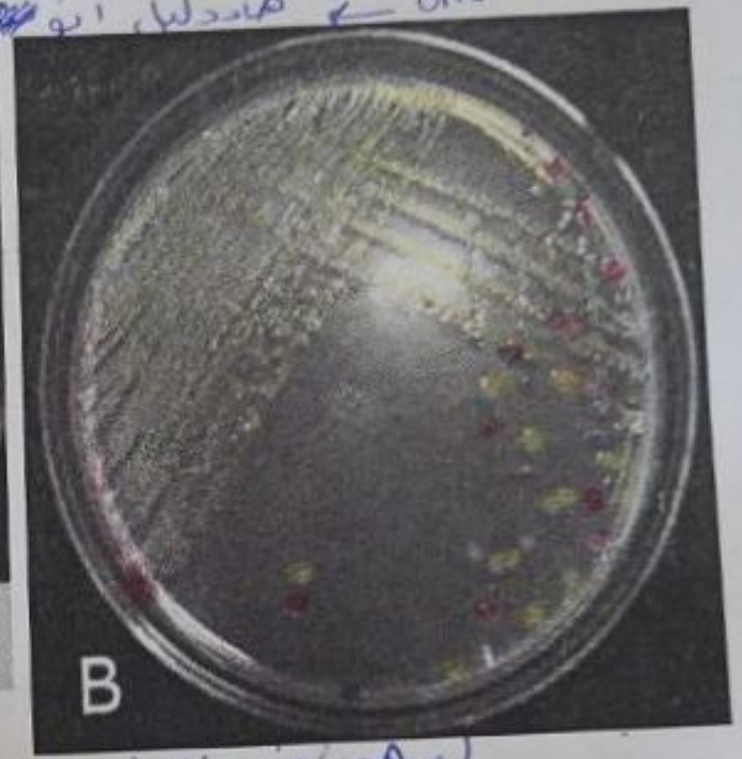
Detection of objectionable MOs

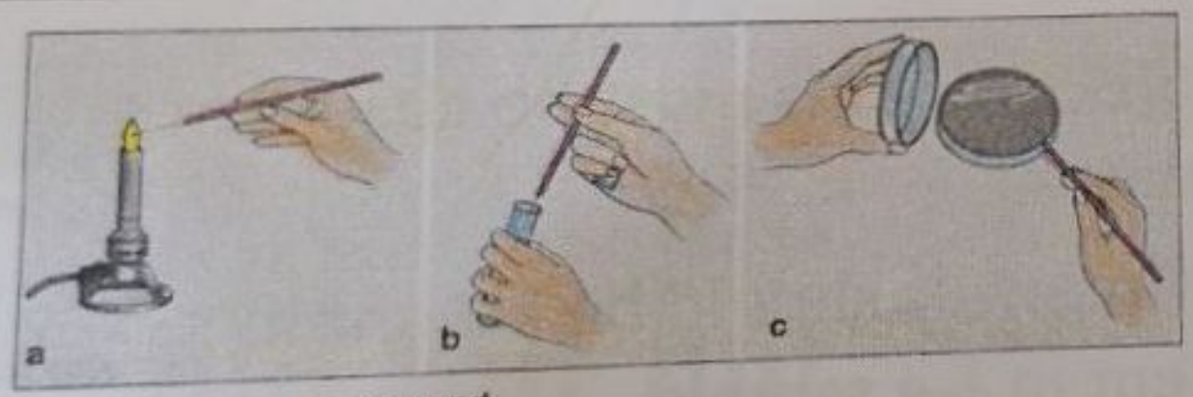
1. Dissolution or dispersal of the sample in a suitable liquid culture medium and , where necessary, inactivation of any substances that might inhibit the growth of the organism under test
2. Enrichment: increasing the relative concentration of the test organism by growing in a liquid medium that inhibit other contaminants but allows free multiplication of the organism of interest
3. Streaking liquid cultures from step 2 onto **selective agar media** that usually permit easy recognition of any colonies of the test organism that might arise
4. The use of specific biochemical or immunological confirmatory tests (test kits)

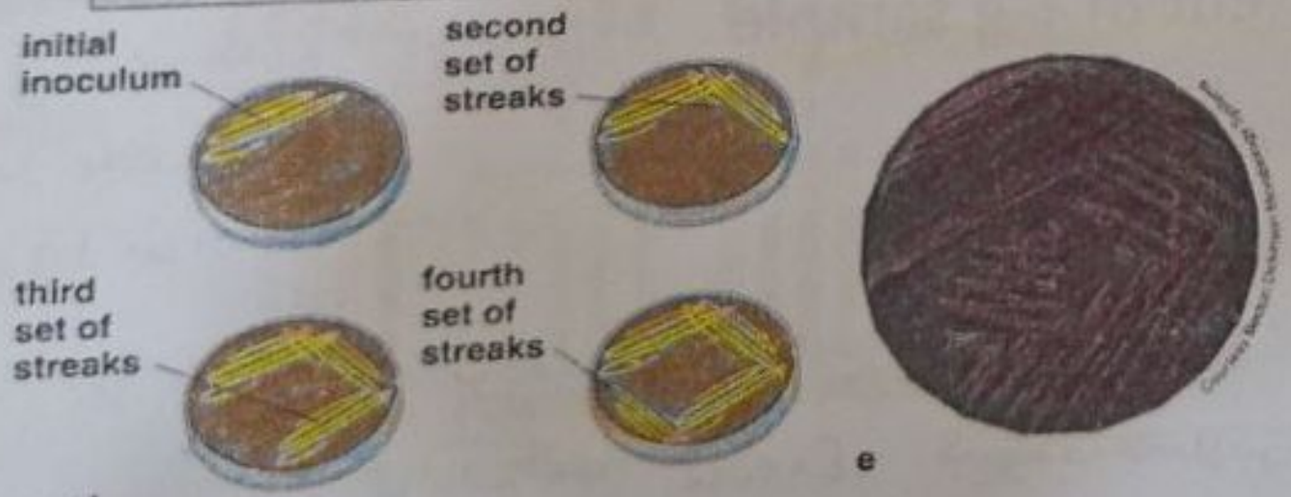
الصدق من اني اسوي اشي هاد الشكل هو عشان اشوف single colony كيف شكلها ولونها

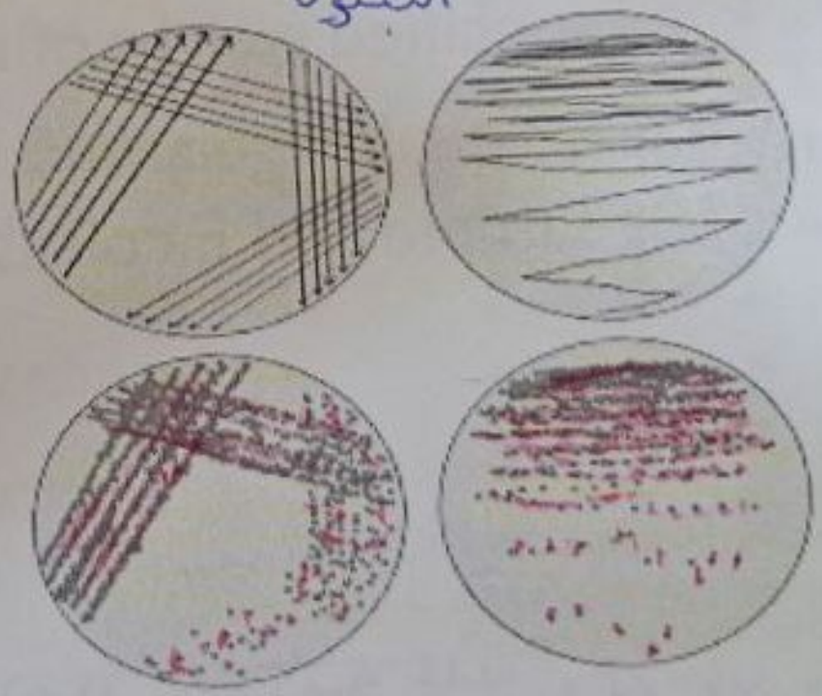


ure 2.3 Streaking a Petri dish to give isolated colonies and check culture purity (see text).









Detection of objectionable MOs

- Selective agar media recommended in the pharmacopeias:
- MacConkey's agar for E. coli
- XLD agar for Salmonella species
- Mannitol salt agar for Staphylococcus aureus
- Cetrimide agar for Pseudomonas aeruginosa

Students are required to read about each type of agar mentioned

عشان نشوف وظيفه

MacConkey's → E. coli

XLD → salmonella

حرف لاتيني حرف L يعني تقاطع زي حرف X

Mannitol salt → staphylococcus aureus

cetrimide → psedomonas aeruginose

ماكونات ايظها

Detection of objectionable MOs

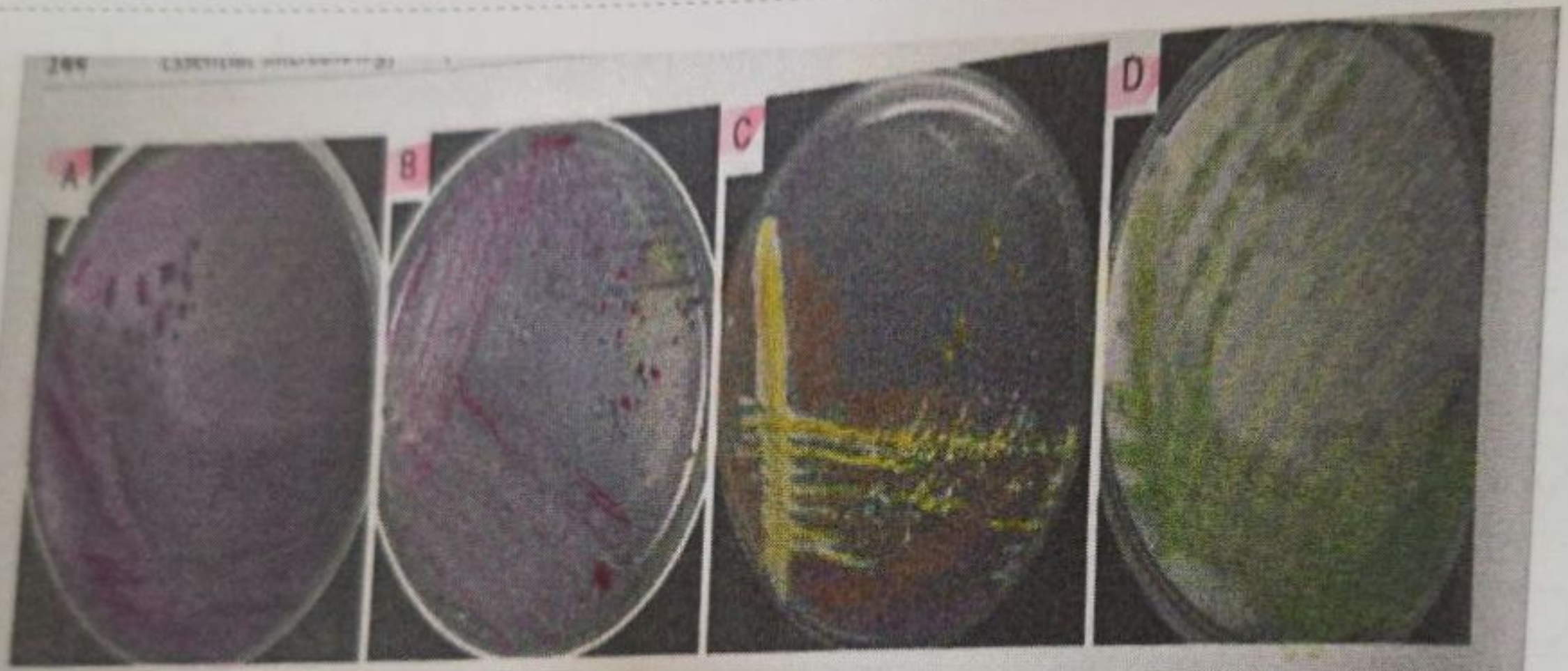


Figure 15.6 *The appearance of 'objectionable organisms' on selective media. A. E. coli on MacConkey's agar, showing characteristic red colonies and precipitation of bile as a result of acid production; B. Salmonella species on XLD agar, showing alkali and hydrogen sulphide production indicated by pink agar and black precipitate in the colonies respectively; C. Staphylococcus aureus on mannitol salt agar showing yellow colonies that have produced acid as a result of mannitol fermentation; D. Pseudomonas aeruginosa on cetrimide agar, showing the characteristic green pigmentation.*

Automated bioburden determinations

- Bases of the methods
- Limited application
- Look for pg 144

بس بحل ذاتية للبكتريا من plat خاصه ، بدخلها بالجهاز ويحل deduction discount ، بس تحل مشكله انو هاي automated ... are accept from pharmacopia or not

يعني ادوية سريعة وقادرة تحقق pharmacopia standerd تقدر تدخل ادويتها لأعلان البلدان

Bioburden specifications in the pharmacopeias

Table 15.4 Quality criteria for nonsterile medicines.

Route of administration[a]	Maximum total aerobic microbial count CFU/g or ml	Maximum total yeast and mould count CFU/g or ml	Specified microorganisms absent in 1 g or 1 ml
Nonaqueous oral products	10^3	10^2	Absence of *E. coli*
Aqueous oral products	10^2	10^1	Absence of *E. coli*
Rectal products	10^3	10^2	
Oral mucosal, gingival, cutaneous, nasal and ear products	10^2	10^1	Absence of *Staph. aureus* and *Ps. aeruginosa*
Vaginal products	10^2	10^1	Absence of *Staph. aureus*, *Ps. aeruginosa* and *Candida albicans*
Inhalation products (excluding nebulized liquids)	10^2	10^1	Absence of *Staph. aureus*, *Ps. aeruginosa* and bile-tolerant Gram-negative bacteria

كل دولة إلها standerd معين

Introduction:

- Chemical biocides are used as antiseptics, preservatives or disinfectants

- Differ from antibiotics:

- Chemically synthesized
- Cause more general damage to cell (unlike antibiotics which have specific targets)
- Are not used therapeutically
- Resistance to biocides tends to occur less readily

Definitions

- **Antiseptics:**
  - Have broad spectrum of antimicrobial activity
  - Sufficiently non toxic
  - Can be used on broken skin or mucosal surfaces

- **Disinfectants:**
  - Have broad spectrum activity
  - Have toxicity issues
  - Limit uses to inanimate surfaces (worktops, floors, sinks, and drains)

- Antiseptic
  - limit surface
  - تستخدم على الاسطح الحية
  - not toxic
  - Broad spectrum

- Disinfectant
  - not limit surface
  - تستخدم على الاسطح الميتة
  - toxic
  - Broad spectrum

- not toxic
- Broad spectrum

- Toxic
- Broad spectrum

Definitions

- **Preservatives:**
- Broad spectrum antimicrobial agents
- Incorporated into pharmaceutical and other products to prevent the growth of contaminant M.O. which might arise during use
- Bactericidal, virucidal or fungicidal
- Bacteriostatic, virustatic and fungistatic

(*Note : time and concentration dependent*)

Table 16.1 (Continued)

Biocide group	Examples	Spectrum of activity	Mode of action	Formulation issues	Commercial uses
Esters	Methyl, ethyl, butyl, propyl and benzyl parabens	Mainly G + ve bacteria and fungi Less active against G − ve cells	Not well understood Disrupt membrane transport processes; inhibit nucleic acid synthesis and inactivate key enzymes	Activity increases with alkyl chain length but solubility decreases Partition into oil phase of emulsions	Widely used as preservatives in pharmaceutical industry
Halogens	Chlorine Hypochlorites Iodine Iodophors	Broad antimicrobial spectrum Sporicidal	Cause enzyme and protein damage by interacting with amino and thiol groups	Can be irritant and staining	Used in skin disinfection and as general disinfectants
Isothiazolones	Range of commercial mixtures	Broad spectrum antibacterial, fungicidal	Inhibit active transport and glucose oxidation by binding to thiol groups on enzymes	Water soluble, pH stable and biodegradable	Mainly used as preservatives
Metals	Copper Mercury Silver Phenylmercuric nitrate (PMN) and Phenylmercuric acetate (PMA) Thiomersal	Phenylmercuric nitrate (PMN) active against G + ve and G − ve cells and fungi Not sporicidal	Silver binds with thiol groups on proteins and enzymes Interacts with bases on DNA	Toxicity problems with mercurials in particular. PMN incompatible with a number of common excipients Activity of silver depends on presence of Ag^+ ion	PMN and PMA limited use as preservatives Silver used as topical antiseptic and wound treatment
Organic acids	Benzoic acid Sorbic acid	Mainly active against fungi More limited activity against bacteria	Uncoupling agents Prevent uptake of substrates requiring proton motive force for transport	Activity highly pH dependent Only active at pH lower than 5	Used as preservatives particularly in the food industry
Peroxygens	Hydrogen peroxide Peracetic acid	Broad spectrum activity Sporicidal	Oxidation of functional groups on proteins	Hydrogen peroxide unstable	Used as antiseptics and disinfectants

(continued)

Table 18.1 Examples of different chemical biocides and their uses.

Biocide group	Examples	Spectrum of activity	Mode of action	Formulation issues	Commercial uses
Acridines	Aminacrine Acriflavine Proflavine	G + ve and G – ve cells Not sporicidal	Interfere with nucleic acid function	More effective at alkaline pH	Limited use in treatment of infected wounds
Alcohols	Ethanol Isopropanol Benzyl alcohol Bronopol Chlorbutanol Phenethyl alcohol Phenoxyethanol	G + ve and G – ve cells and fungi Not sporicidal and have low virucidal activity	Disrupt cell membranes	High concentration exponents Inactivated by organic matter Flammable	Widely used as antiseptics and preservatives
Aldehydes	Formaldehyde Glutaraldehyde Orthophthalaldehyde	Good activity against G + ve and G – ve cells, endospores, fungi and viruses	Cross-link proteins by interacting with amino and other groups	Relatively high toxicity, particularly glutaraldehyde	Formaldehyde and orthophthalaldehyde used as disinfectants for medical equipment
Amidines	Propamidine Dibromopropamidine	Mainly G + ve cells and fungi Less active against G – ve cells and spore formers	Mode of action uncertain Inhibit oxygen uptake and induce amino acid leakage	Activity reduced by low pH and in blood and serum	Limited use in topical wound treatment
Biguanides	Chlorhexidine Alexidine Polyhexanide	Good activity against G + ve but less against G – ve cells and fungi Not sporicidal	Disrupt cell membranes	Incompatible with negatively charged excipients in formulation	Widely used as medical and veterinary antiseptics
Chelating agents	Ethylenediamine tetra-acetic acid EDTA	G – ve cells only	Increase permeability of cell wall of G – ve bacteria	Potentiates the effects of several antibacterial agents	Limited use as antibacterial agents Used to stabilize formulations

(continued)

Table 16.1 (Continued)

Biocide group	Examples	Spectrum of activity	Mode of action	Formulation issues	Commercial uses
Phenols	Phenol Chlorocresol Chloroxylenol Triclosan	G + ve and G – ve cells. Slowly active against spores and acid-fast bacteria	Disrupt cell membranes Cause general cytoplasmic coagulation	High concentration exponents Some have limited solubility and can be adsorbed to polymers	Used as antiseptics, disinfectants and preservatives
Quaternary ammonium compounds	Benzalkonium chloride Benzethonium chloride Cetrimide Cetylpyridinium chloride	Broad spectrum antibacterials More active against G + ve than G – ve Some antiviral and antifungal activity Not sporicidal	Disrupt cell membranes	Incompatible with negatively charged excipients Benzalkonium chloride can cause sensitization	Widely used as antiseptics, disinfectants and preservatives
Quinolines	8-hydroxyquinoline Dequalinium chloride	Active against G + ve bacteria Less active against G – ve cells Some antifungal activity	Rapid uptake into cells Disrupt nucleic acid function	Some have low water solubility	Used as antiseptics and formulated in lozenges for throat infections
Anionic surfactants	Sodium lauryl sulphate	Weak antimicrobial properties	Disrupt cell membranes	Can interact with positively charged excipients in formulation	Limited use as antibacterial agents Used for detergent properties

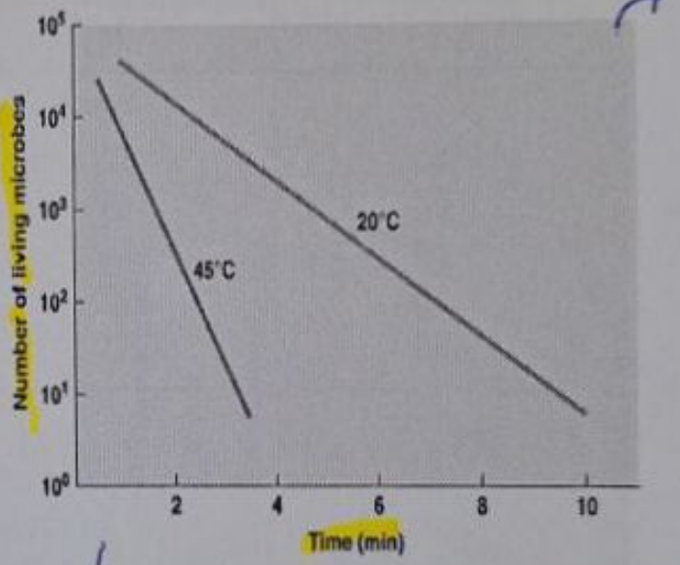
Factors influencing the activity of biocidal agents

- Temperature:
- Concentration
- pH
- Solubility
- Interaction with excipients and packaging materials

agents

- Temperature
  - Temperature coefficient (Q_{10}): describes the change in rate of kill for a 10°C change in temperature
  - Example:
  - Phenols have a Q_{10} of around 4
  - Means: their rate of kill increases by a factor of 4 for every 10°C rise in temperature (limits: above 40°C → temperature have harmful effects on the cell)

Effect of temperature on the efficacy of an antimicrobial chemical



قدرة التغيس في سرعة قتل antimicrobial chemical بكل زيادة 10° degree او نقصان

بقل زادة عن درجة سرعة القتل .. وطلع عن درجة حرارة 45° سرعة kill اسرع

اذا زدنا درجة الحرارة كيش كد يقتل من kill كانا هلا الكائنات Bacterial تقتل

بقل زادة بكتيريا معينة وحط بوكيز معين وبشوف كل شوي كيف بكون التغيس بالقتل

$$Q_{10} = \frac{\text{Time to kill at } T^{\circ}}{\text{Time to kill at } (T + 10)^{\circ}}$$

The value of the temperature coefficient (Q_{10}) may vary with preservative, type of organism, and temperature range (Hugo and Russell, 1987).

Thus, if the value of θ for an antimicrobial agent is 3, the increase in activity for a 3°C rise in temperature is 3^3 or 27-fold. On the other hand, if the value of Q_{10} for phenol is 5, a drop in temperature from 30° to 20°C can result in a 5-fold reduction in the killing rate of the antimicrobial agent. The temperature effect is highly important when evaluating preservative action in challenge-testing procedures.

- not toxic
- Broad spectrum

- Toxic
- Broad spectrum

Definitions

- **Preservatives:**
- Broad spectrum antimicrobial agents
- Incorporated into pharmaceutical and other products to prevent the growth of contaminant M.O. which might arise during use
- Bactericidal, virucidal or fungicidal
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(*Note : time and concentration dependent*)

chemical Biocides للزام احدد اسم و اسم البكتريا

TABLE 1

Bactericidal efficiency of phenol against Staphylococcus aureus at 10° and 20°C.

DILUTION	DISINFECTION TIME		TEMPERATURE COEFFICIENTS
	10°	20°	
	minutes	*minutes*	
1–55	17.5	5	3.5
1–60	40	7.5	5.3
1–65	70	12.5	5.6
1–70	100	20	5.0
1–75	150	30	5.0
Average.....			4.9

http://jb.asm.org/content/43/4/521.full.pdf