تفريغ تعقيم

محاضرة: Antiseptics, disinfectants and preservatives

الصيدلاني\ة: ياسمين خليل

لجان الدُّفعات

Soul Academy

MO: micro-organisms

اللهم علمنا ما ينفعنا، وانفعنا بما علمتنا، وزدنا علما

المطهرات: تقليل/قضاء على MO على الأسطح غير الحية (أرضيات، أدوات طبية، أثاث)

مُعقمات: تقليل/قضاء على microorganism على جلد الكائنات الحية

Antiseptics, disinfectants and preservatives

مواد حافظة: مواد كيميائية تُضاف إلى المنتجات لمنع نمو MO أثناء التخزين (الأدوية + الطعام)

Chapter 16

← هذول الثلاثة هم مواد كيميائية مُصنعة (صنعهم هاي العلوم)

Introduction:

- Chemical biocides are used as antiseptics, preservatives or disinfectants

بالموبه الأخضر معلومات عن المضادات antibiotics

اختصاري ← الـ 3 ← قصدي فيه

antiseptic + preserv + Disinfec

عكسهم في هاي الأشياء

- Differ from antibiotics:
  - Chemically synthesized → المضادات الحيوية تصنع من كائنات حية عادةً زي فطريات
  - Cause more general damage to cell)unlike antibiotics which have specific targets)
  - Are not used therapeutically
  - Resistance to biocides tends to occur less readily

هدول هم وظائفهم
anti septic + Dis infectants + preservatives

- الـ 3 تسبب ضرر لخلايا الكائنات بينما antibiotic تستهدف أهدافاً محددة في البكتيريا.
- الـ 3 لا تستخدم كعلاج مثل antibiotic بس بنستعملهم تعقيم + تنظيف...
- مقاومة الـ 3 أقل من مقاومة المضادات.

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)

سبحان الله العظيم وبحمده

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 16.1 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	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)

لا إله إلا أنت سبحانك إني كنت من الظالمين

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

العوامل المؤثرة على فعالية المواد القاتلة

Factors influencing the activity of biocidal agents

لسه تتذكروا ؟

- Temperature:
  - ↓ بنزيد الحرارة ← يزداد القتل
- Concentration
  - ← مضاعفة التركيز ← تزيد نشاط القاتل
- pH
  - حسب المادة : مثلاً حمض البنزويك يعمل بشكل أفضل في بيئة حمضية
- Solubility
  - كل ما زادت الكربونات قلت الذائبية في الماء
- Interaction with excipients and packaging materials
  - المواد إلي إلها شحنة زي NH_4^+ ممكن ترتبط بمكونات المنتج أو الباكيج فتقلل فعاليتها.

صحيحا مقطع MO القاتلة وليس المثبطة.

اللهم ارحم أبي واغفر له وتجاوز عنه وجازه الجنة بحسناته واجمعه وأهله عند حوض نبيك صلى الله عليه وسلم

Q10 → هاد معامل درجة الحرارة، يوضح كيف تتغير سرعة القتل مما تتغير الحرارة بمقدار ١٥ درجات.

Factors influencing the activity of biocidal agents

- Temperature
- Temperature coefficient (Q10): describes the change in rate of kill for a 100C change in temperature
- Example:
- Phenols have a Q10 of around 4 معامل تغير الحرارة بالنسبة للفينول = ٤ طيب شو يعني؟!
- Means: their rate of kill increases by a factor of 4 for every 100C rise in temperature (limits: above 400C□ temperature have

Effect of temperature on the efficacy of an antimicrobial chemical

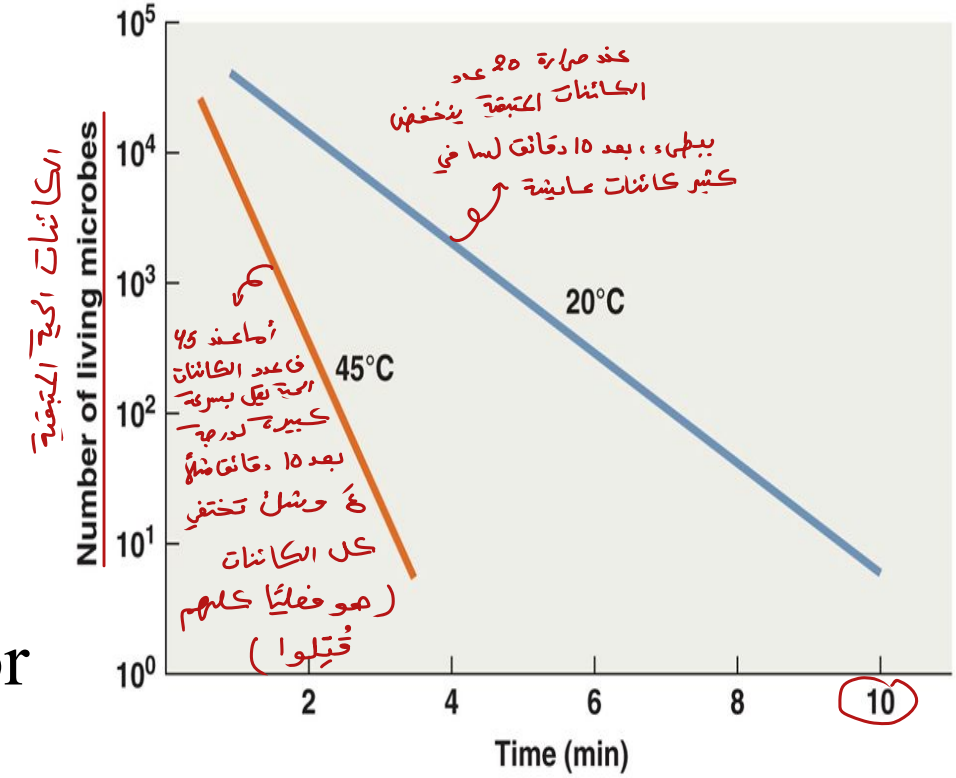


Figure 9.3

يعني سرعة قتل البكتيريا تزيد بمقدار 4 أضعاف لما ترتفع الحرارة بمقدار 10 درجات مئوية

← لو الفينول بده 40 دقيقة لقتل البكتيريا عند حرارة 20 ، فإنه رح يحتاج إلى $\frac{40}{4}$ = 10 دقائق بس عند حرارة 30

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

نسبة الوقت اللازم لقتل الكائنات عند درجة حرارة [T] مقارنة بـ بالوقت عند درجة حرارة أعلى بـ 10 [T+10]

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.

الحمد لله رب العالمين

- The general relationship for how the rate changes with temperature is:

$$\frac{k_2}{k_1} = Q_{10}^{\frac{T_2 - T_1}{10}}$$

$$\frac{k_2}{k_1} = \frac{Ae^{\frac{-E_a}{RT_2}}}{Ae^{\frac{-E_a}{RT_1}}}$$

$$\frac{k_2}{k_1} = e^{\frac{-E_a}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)}$$

- Where:
- k1 = reaction rate at temperature T1
- k2 = reaction rate at temperature T2
- Q10 = temperature coefficient
- T2 - T1 = temperature difference in °C

Explanation of Temperature Coefficient (Q_{10}) and Its Implications in Preservative Activity

- The **temperature coefficient (Q_{10})** describes how the rate of an antimicrobial agent's activity (such as its ability to kill microorganisms) changes with a **10°C change in temperature**. It varies depending on:
- The **preservative** being used,
- The **type of microorganism** it is acting against, and
- The **temperature range** being considered.
- **Interpreting the Given Example:**
- If an antimicrobial agent has a **Q_{10} value of 3**, this means that for **every 10°C increase**, its killing rate increases **3-fold**.
  - However, if we consider only a **3°C increase**, the effect follows an **exponential relationship**:

$$3^{(3/10)}$$

$$\frac{k_2}{k_1} = Q_{10}^{\frac{T_2 - T_1}{10}} \qquad \frac{k_2}{k_1} = 3^{\frac{3}{10}}$$

    - which approximately equals **1.44** (a 44% increase in activity).
  - Over a full **10°C increase**, the increase in activity would be: $3^1 = 3$ (3-fold increase)
  - Over a **3°C increase**, the total increase would be: $3^3 = 27$ meaning the antimicrobial activity increases **27-fold** in this case.
- **Effect of Decreasing Temperature:**
  - If **phenol** has a **Q_{10} value of 5**, it means that its activity changes **5-fold** for a **10°C temperature shift**.
  - A **drop in temperature from 30°C to 20°C** (a **10°C decrease**) would **reduce** the antimicrobial activity **by a factor of 5**.
  - This means the killing rate **slows down significantly** when the temperature drops.

- **Why Is This Important in Preservative Testing?**
- When testing preservatives, **temperature control is crucial** because even small variations can **greatly affect antimicrobial effectiveness**.
- A high **Q_{10} value** means the preservative is **highly sensitive to temperature changes**, while a lower **Q_{10}** indicates a **more stable effect**.
- In **challenge testing** (where products are deliberately contaminated to evaluate preservative efficacy), **temperature consistency** is necessary to obtain **accurate and reproducible** results.

نفس الجدول

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

Factors influencing the activity of biocidal agents

- Concentration

- معامل التركيز (n) هو مقياس يُظهر مدى تأثير تغيير تركيز المادة القاتلة على سرعة قتل الكائنات الحية
ومعادلة تربط بين الوقت والتركيز

$$\log t = n \cdot \log c + \text{constants}$$

slope

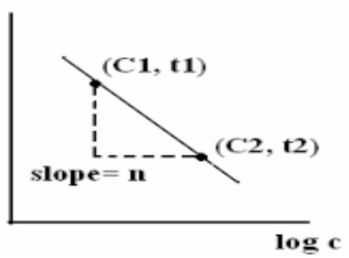
$$n = \frac{\log t_2 - \log t_1}{\log c_2 - \log c_2}$$

طيب شو استفدنا من حساب n !؟

← استفدنا إنه لو كانت n تساوي 1 فإن تغيير التركيز له تأثير بسيط على الوقت اللازم للقتل ، أما لو كانت 6 تعني كبيرة ، فالتركيز له تأثير كبير على الوقت

للتوضيح أكثر

- Concentration:
- Concentration exponent (of kill with a change in con
- $n = \frac{\log t2 - \log t1}{\log C1 - \log}$
- C1 & C2 : co



1.إذا كان (n = 1):

- مضاعفة التركيز (مثل من 1 إلى 2) تقلل الوقت اللازم للقتل مرتين فقط ((2^1 = 2)).
- تقليل التركيز إلى النصف يزيد الوقت مرتين.
- مثال: إذا كان الوقت 10 دقائق عند تركيز 1، فسيصبح 5 دقائق عند تركيز 2.

2.إذا كان (n = 6):

- مضاعفة التركيز تزيد السرعة بشكل كبير جدًا ((2^6 = 64))، أي الوقت يقل 64 مرة.
- تقليل التركيز إلى النصف يزيد الوقت 64 مرة.
- تقليل التركيز 3 أضعاف يزيد الوقت بمقدار (3^6 = 729) مرة.
- مثال: إذا كان الوقت 10 دقائق عند تركيز 1، فسيصبح 10 ÷ 64 = 0.156 دقيقة (حوالي 9 ثوانٍ) عند تركيز 2.

Table 16.2 Concentration exponents of commonly used biocides.

Antimicrobial agent	Concentration exponent
Hydrogen peroxide	0.5
Mercurials	0.03–3.0
Bronopol	0.7
Iodine	0.9
Acridines	0.7–1.9
Quaternary ammonium compounds	0.8–2.5
Polymeric biguanides	1.5–1.6
Chlorhexidine	2
Parabens	2.5
Sorbic and benzoic acids	2.6–3.2
Phenols	4.0–9.9
Aliphatic alcohols	6.0–12.7

Factors influencing the activity of biocidal agents

- Agents with a concentration exponent around one
- doubling of their concentration will increase activity of kill by a power of one (2¹) which is 2.
- A three fold dilution means the biocides activity will be reduced by a value 3¹ (a third of its original activity)
- Agents with a concentration exponent of 6:
- a doubling in concentration will increase the activity of ~~26=64-fold~~ $2^6 = 64$
- And a halving in concentration will reduce their activity by 64-fold
- A three fold dilution will mean a decrease in activity of ~~36~~ $3^6 = 729$ or 729 times less active than the original

Factors influencing the activity of biocidal agents

- pH:

- pH of the formulation may have effect on the activity of some biocidal agent
- Benzoic acid
- Sorbic acid
- Both are active at low pH values, why?

فعاليتهم بتكون أقوى في بيئة حامضية يعني pH قليلة ليه؟!

بكونه تواجد الحمض في وسط حمضي رح يكون موجود بشكله غير المتأين إلي رح يسهله يخترق جدار الخلية البكتيرية على فرض ف فعالية بتكون أفضل .

أستغفر الله العظيم وأتوب إليه

Factors influencing the activity of biocidal agents

- **Solubility:**

مجموعة مركبات تستخدم كمواد حافظة لمنع نمو MO في المنتجات الصيدلانية او تجميلية

- An issue with classes of molecules having variable alkyl chain lengths as parabens

تختلف الفعالية للمرغب بحسب طول السلسلة الألكيلية
كيف؟

- As the alkyl chain length increases from methyl paraben to butyl paraben: activity inc. but aq. Sol. Dec. → this why parabens are usually used as mixture

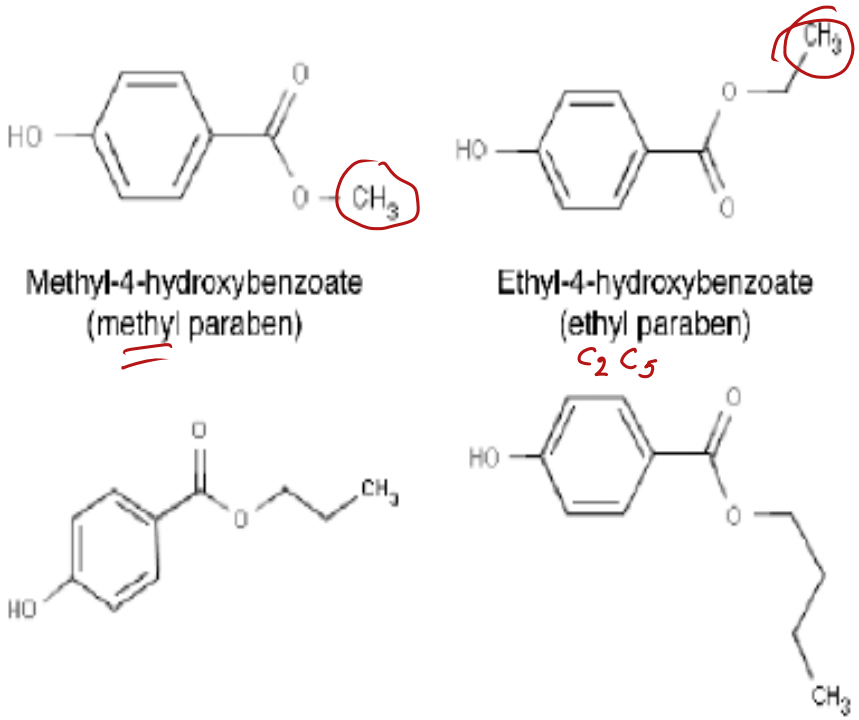
* كلما زادت السلسلة، ح يقل الذوبان بالماء
بالتالي فعالية أفضل

- Care must be taken in multiphase products such as emulsion, what will happen?

← تستخدم Parabens كمزيج لتحقيق توازن بين الفعالية والذوبانية

كونه biocidal agents ممكن تتوزع في 2 phases بالتالي تركيزها ح يقل
في الزيت لأنها حسب وين كانه أكثر قبل مايتوزع ع ف فعاليته ح تقل في phase إلي
بدي إياها تكون متركزة فيه

Medscape® www.medscape.com

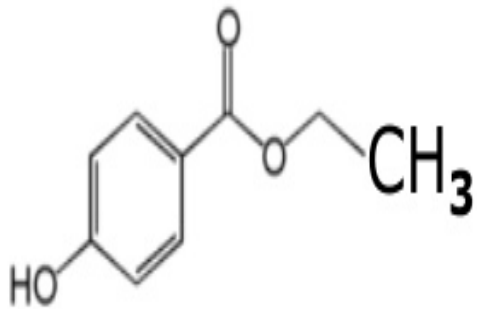


Source: Dermatitis © 2005 American Contact Dermatitis Society

Methyl Paraben

- **Chemistry:** It is the methyl ester of parahydroxybenzoic acid.

  Structure:



- **Physical properties**

  Description: Colorless crystals or white powder.

  Solubility: soluble in water, ethanol, slightly soluble in benzene and acetone.

- **Uses:** It is used as preservative in pharmaceutical formulation to inhibit the growth of microorganisms.

Factors influencing the activity of biocidal agents

- Interaction with excipients and packaging materials:
  - Charged biocidal agents → interacts with oppositely charged excipients in the formulation: e.g. quaternary ammonium compounds bind to alginates
  - Adsorption to plastic or rubber components of the packaging
  - Partition into the non-aqueous phase
  - all of the above decrease biocidal activity especially with biocides with high concentration exponent, why?

قياس نشاط المضادات

Measurement of antibacterial activity:

- Evaluate the inhibitory effect of the formulation on live cultures:

والهدف زيادة فعاليتها في تثبيط MO

- Broth-dilution methods
- Agar diffusion methods
- Kill curves

فيها يتم تحديد أقل تركيز للتثبيط وضع نمو MO [MIC]

مثلاً كان A عنده MIC = 1.0 Mg/ml

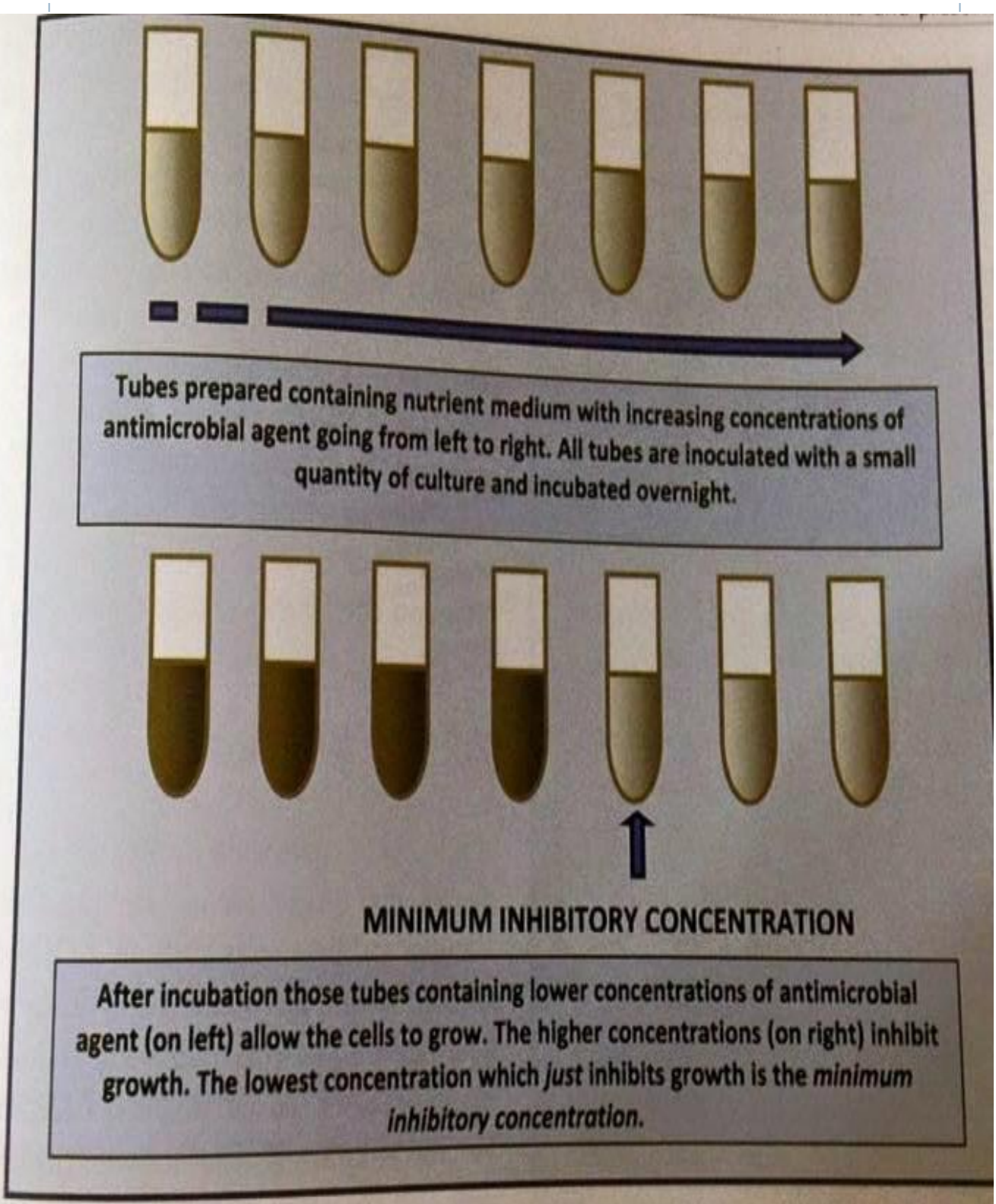
وكان B عنده MIC = 16 Mg/ml

← بنعرف هيك إنه B عنده مقاومة أعلى.

لتقييم فعالية biocidal agents; and anti-biotics، وبيد أعماله: بنحط قرص عليه المادة إلي بدنا نتأكد من فعاليتها على agar فيه بكتيريا فارح بيكون حوله دائرة نظيفة كأنها حطلو البكتيريا

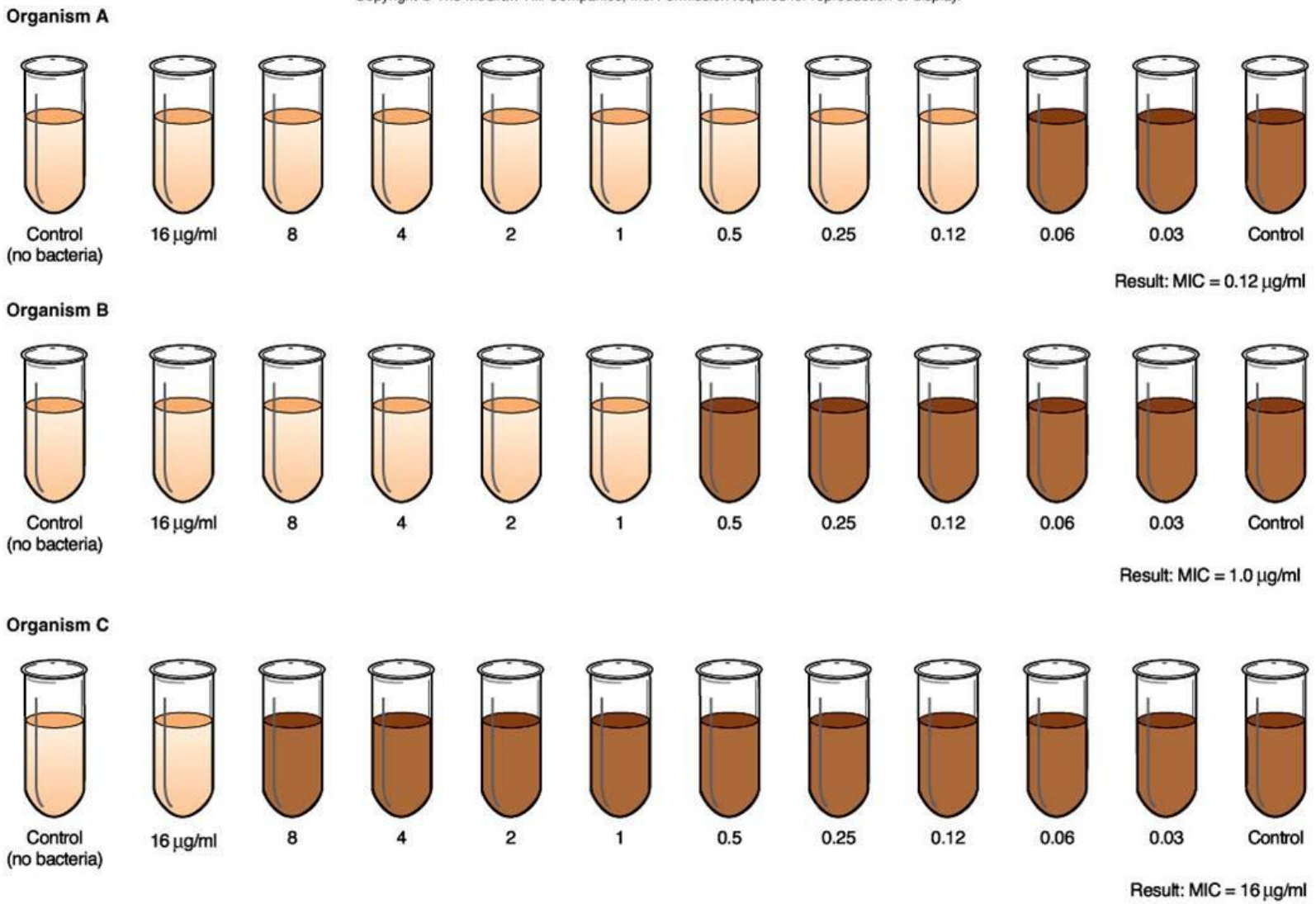
وفي كذا عوامل بتأثر على حجم منطقة الحظر مثل: ذوبانية ومقاومة البكتيريا لـ حركة الأقراص على الانتشار وغيرها.

هاي الطريقة بتقدم بيانات كمية عن معدل قتل الخلايا وتأثير العوامل البيئية اللي بيتم فيها تدريج محلول بتركيز معروف من الخلايا الحية، وبيؤخذ عينات في أوقات متباعدة لعد الخلايا



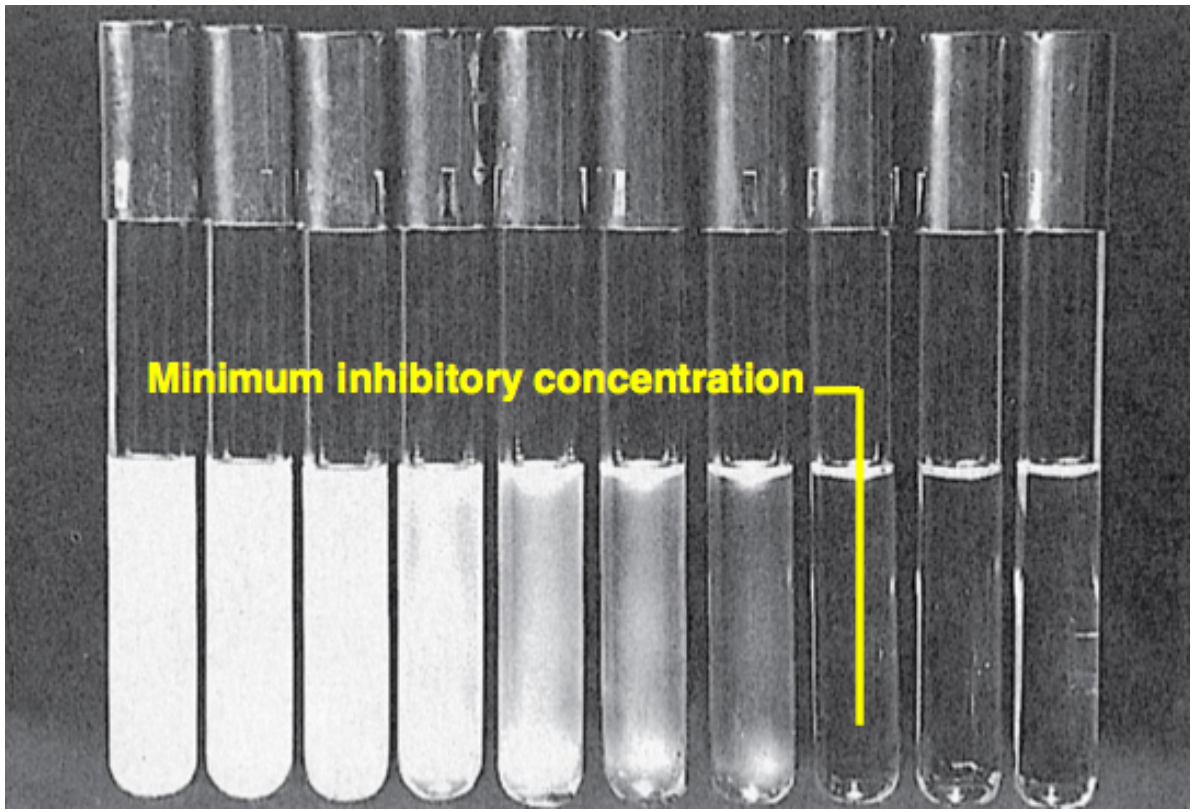
Determination of the minimum inhibitory concentration (MIC) of a biocide.

Measurement of antibacterial activity:
1. broth-dilution methods

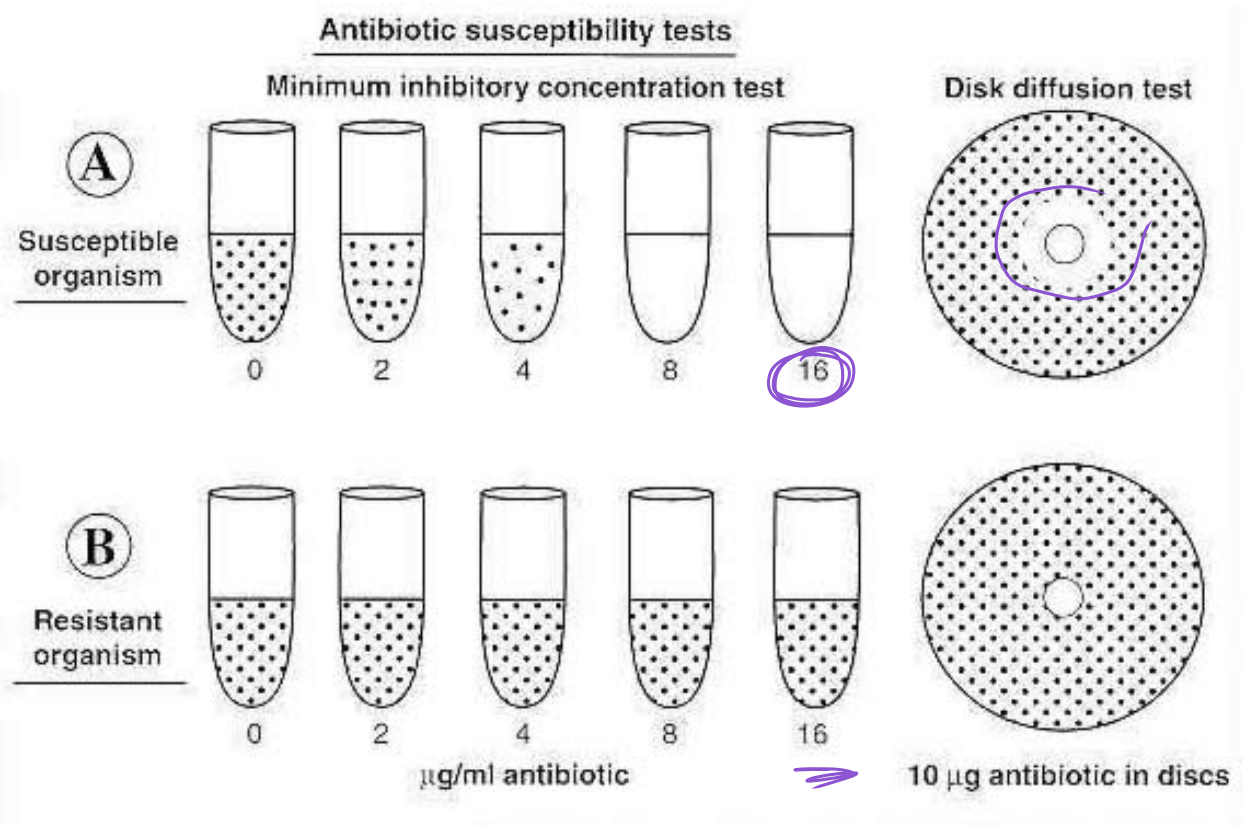


ربِّ إني لما أنزلت إلي من خيرٍ فقير

Measurement of antibacterial activity: 1. broth-dilution methods



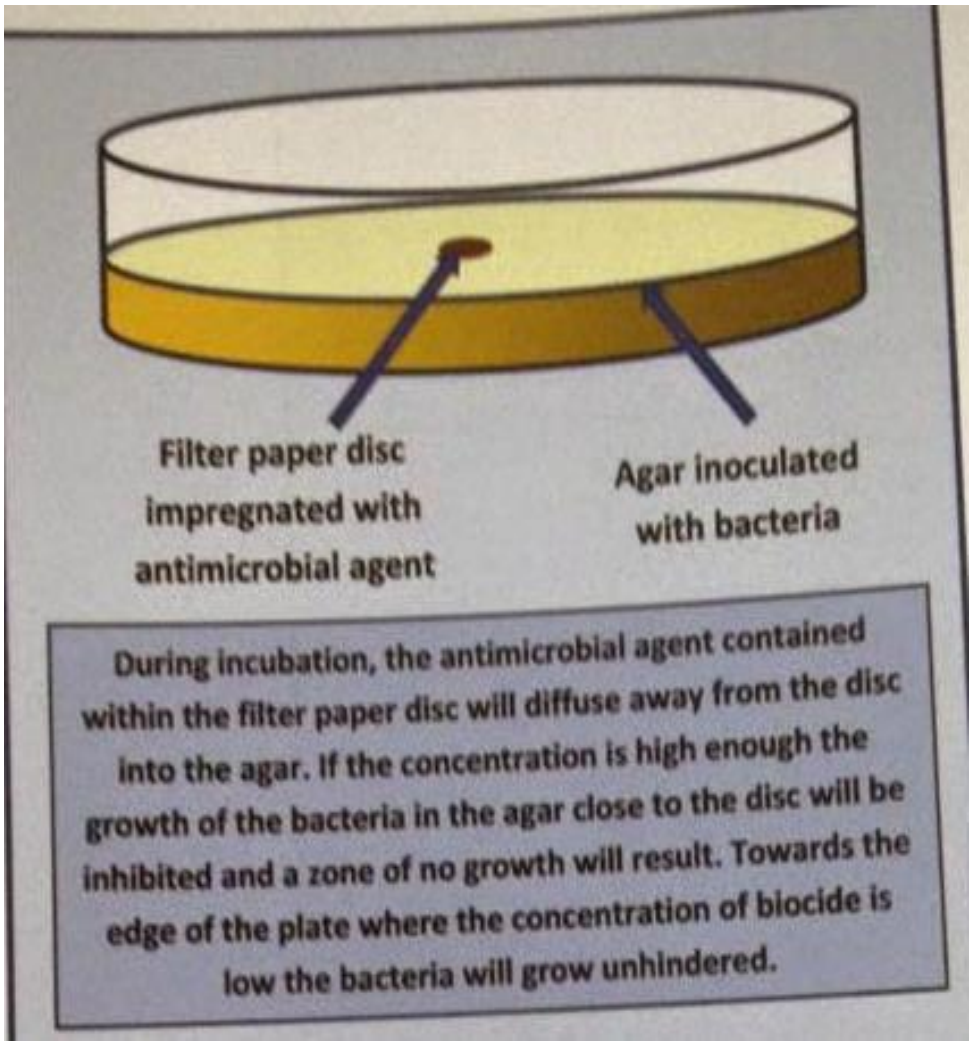
broth-dilution methods

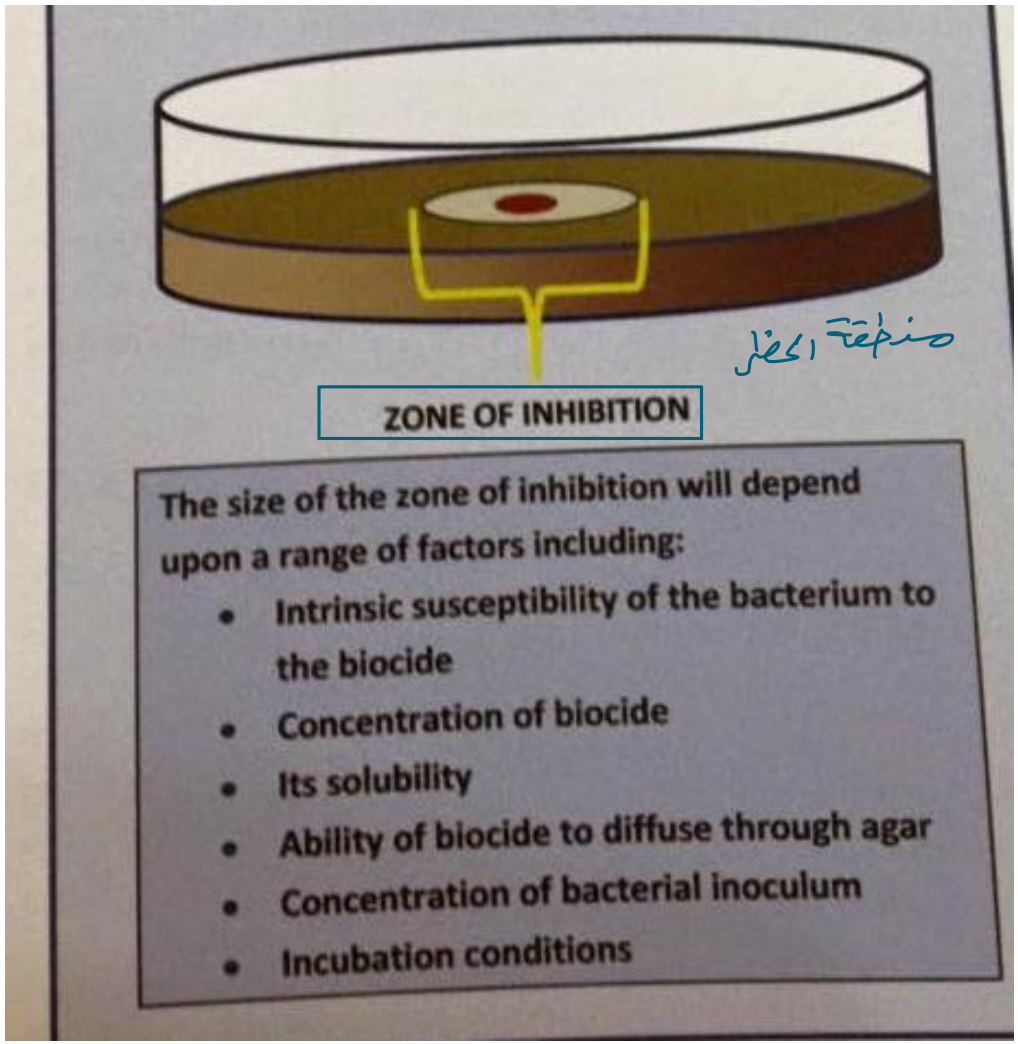


Measurement of antibacterial activity:
2. Agar diffusion methods

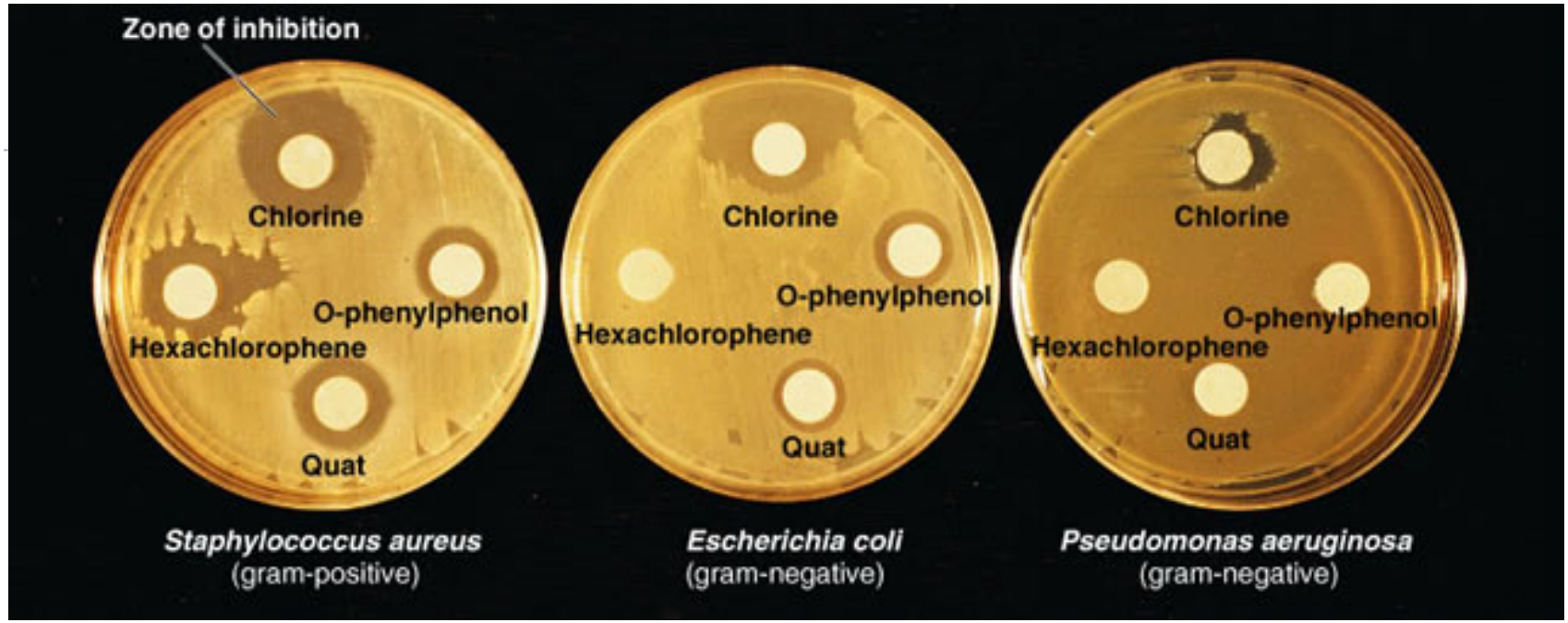
- Used commonly to:
  - Assay activity of biocides
  - Assay antibiotics
  - Determine the sensitivity of clinical isolates to a particular antibiotic prior to treatment

Measurement of antibacterial activity:
2. Agar diffusion methods





اللهم اغفر لي ولوالدي وللمسلمين والمسلمات الأحياء والأموات


Zone of inhibition
Chlorine
O-phenylphenol
Hexachlorophene
Quat
Chlorine
O-phenylphenol
Hexachlorophene
Quat
Chlorine
O-phenylphenol
Hexachlorophene
Quat
Staphylococcus aureus
(gram-positive)
Escherichia coli
(gram-negative)
Pseudomonas aeruginosa
(gram-negative)

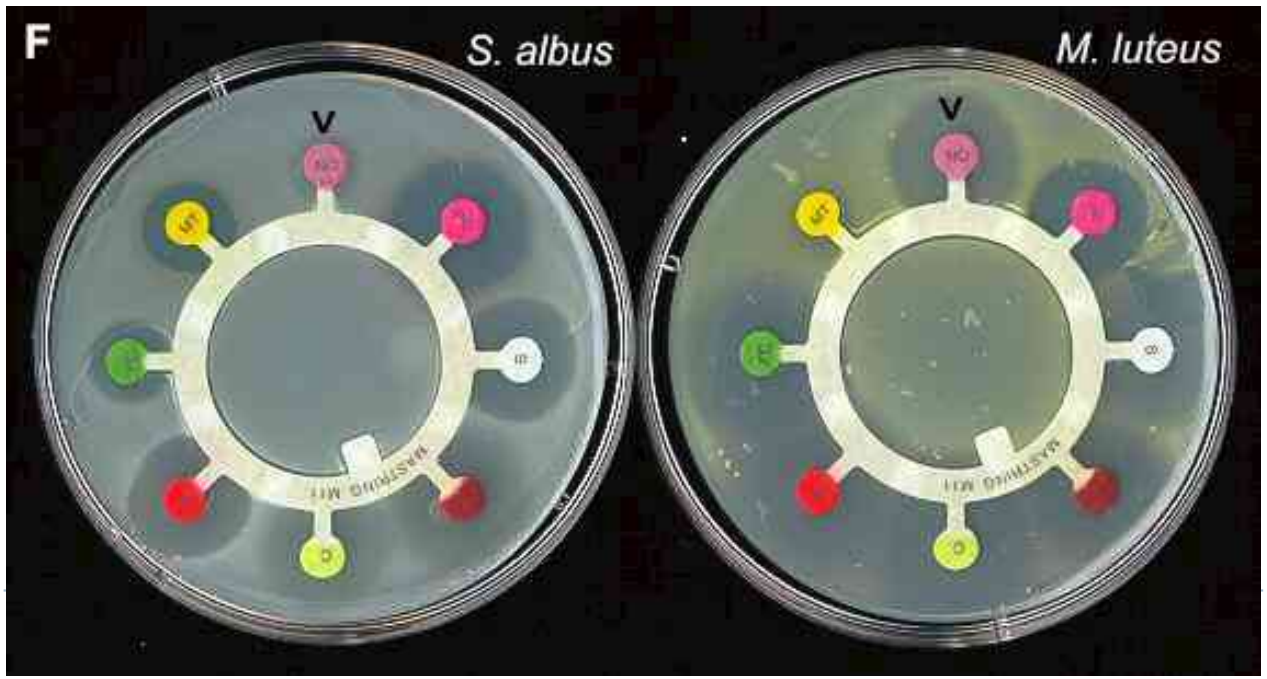
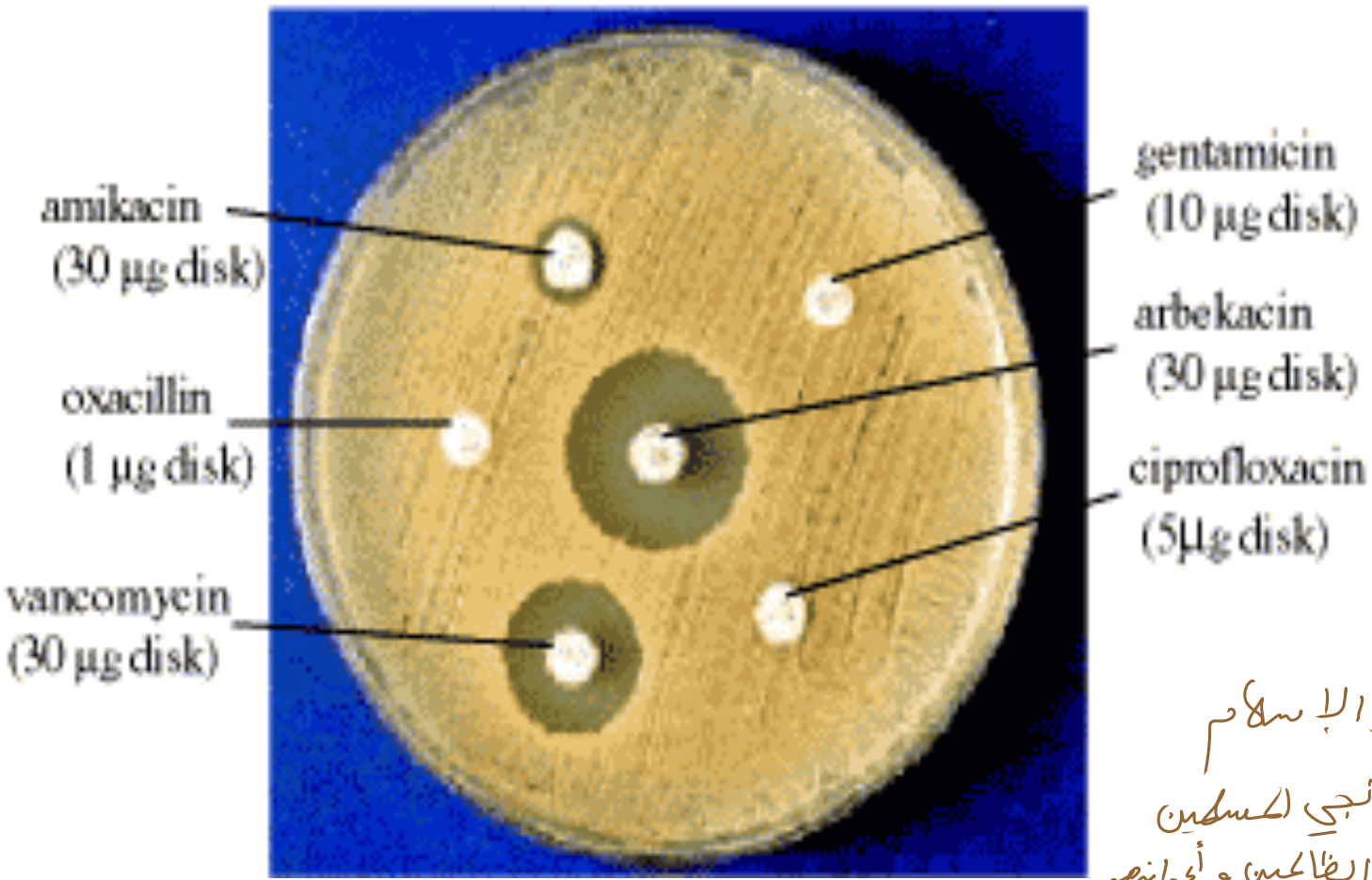

F
S. albus
M. luteus
V
V

Figure 2. ORSA isolate showing in vitro susceptibility only to arbekacin and vancomycin



اللهم انصر الإسلام
والمسلمين ونجي المسلمين
المستضعفين من الظالمين وأعوانهم

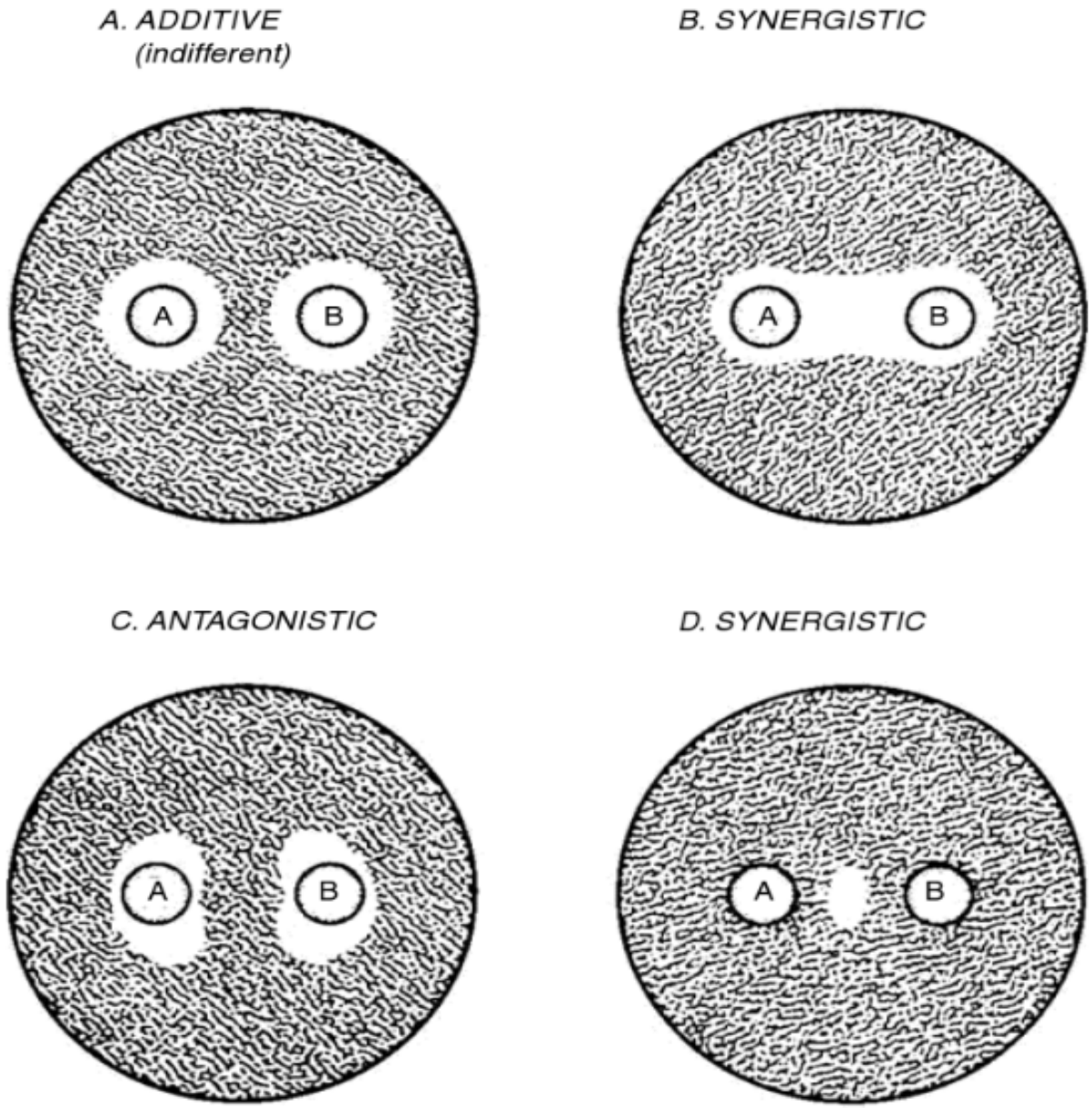


FIGURE 12.1 Assessing drug interactions using disk-diffusion. Shaded areas represent bacterial growth and clear areas represent zones of inhibition. (Adapted and reprinted with permission from Eliopoulos, G.M. and Moellering, R.C., Jr. *Antibiotics in Laboratory Medicine*, Lorain, V., Ed., Williams Wilkins, Baltimore, 1996, figure 9.10, p. 344.)

12.2.2.6 Interpretation

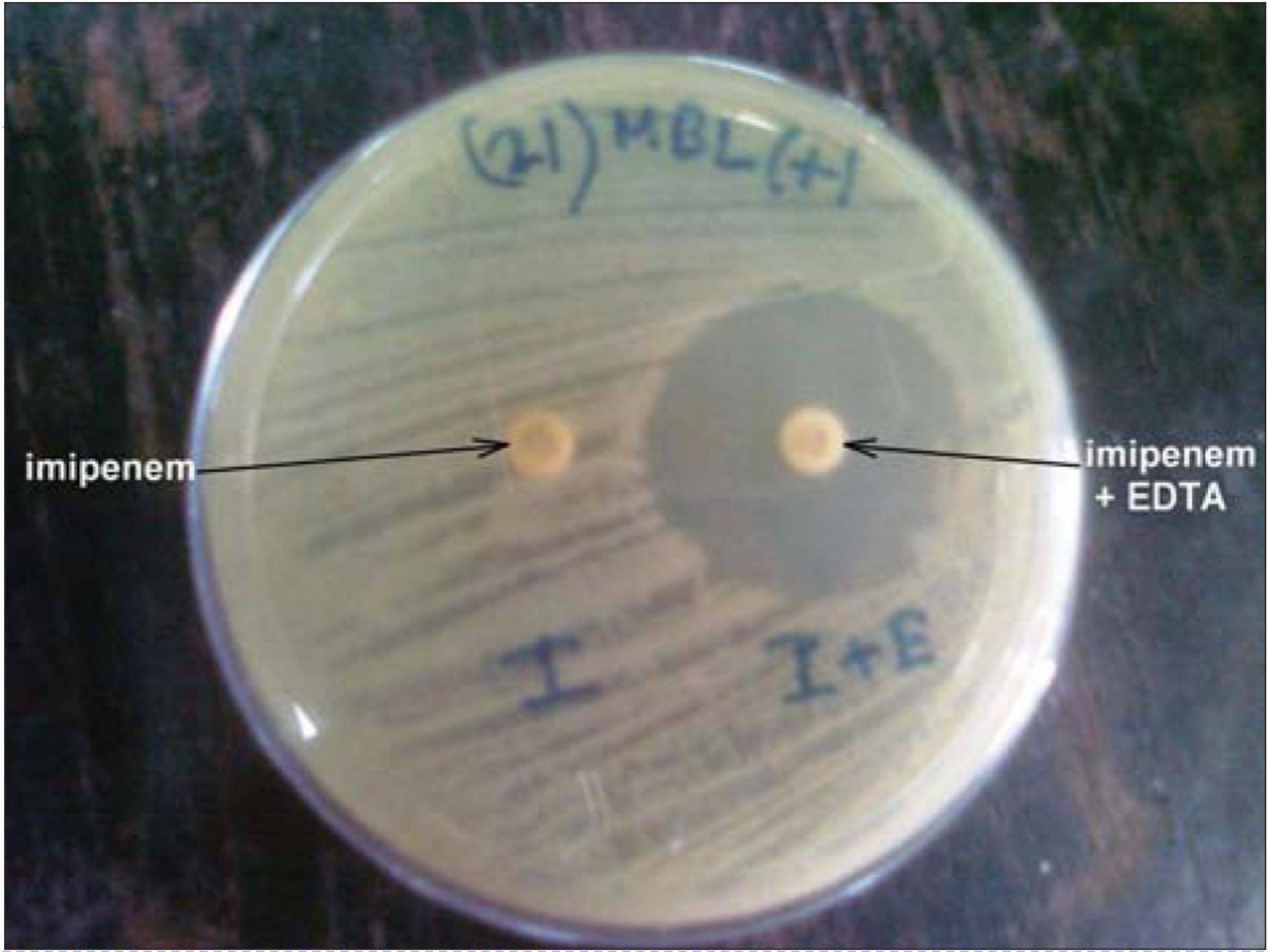
If the quality control is within limits, record the pattern of the zones of inhibition as follows (see Figure 12.1):

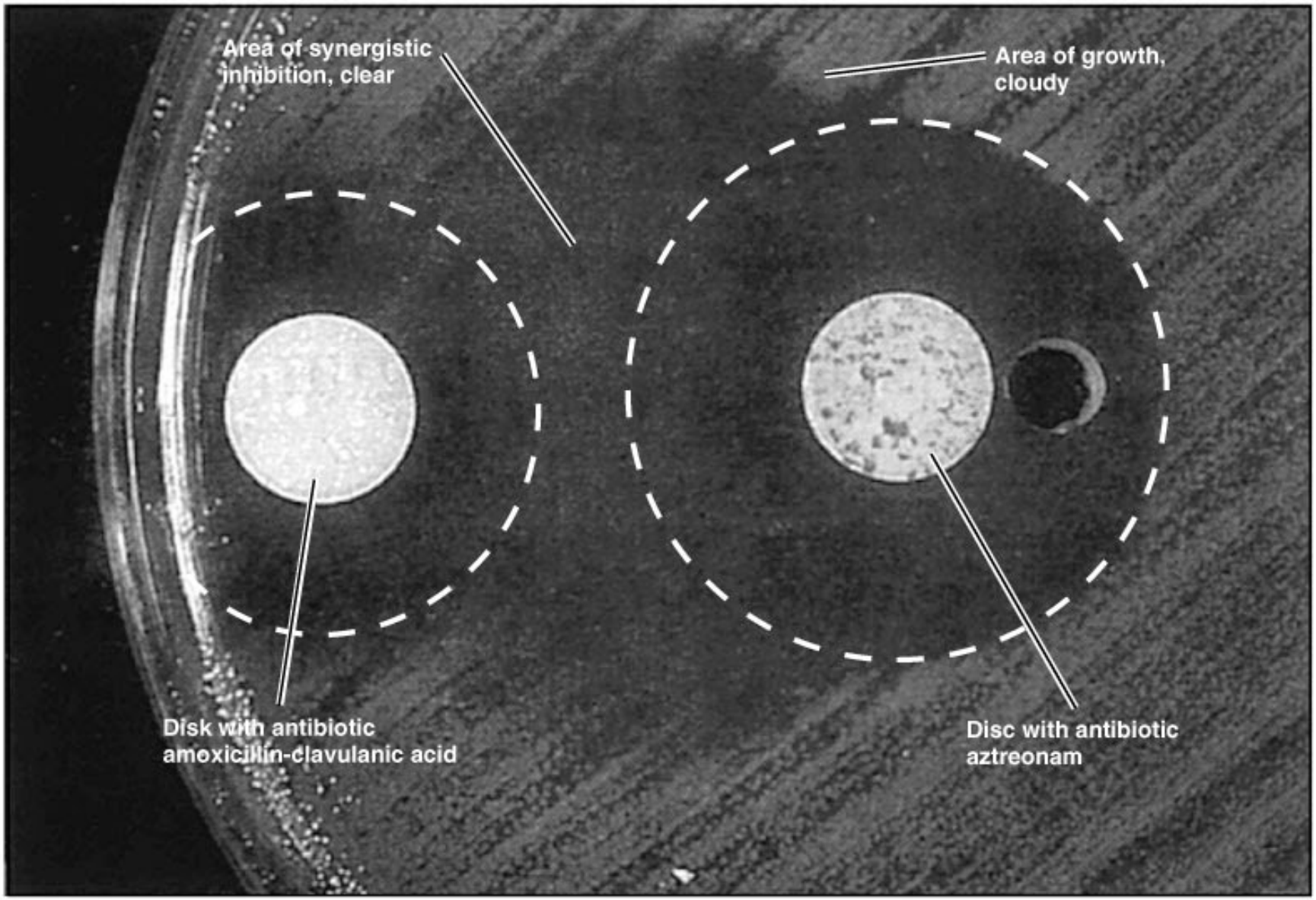
1. Indifference: Two independent circles
2. Synergism: Enhancement or bridging at or near the junction of the two zones of inhibition or inhibition of growth only due to the combined effects of both antibiotics A and B.
3. Antagonism: Truncation of zones is observed near the junction of the two zones.

12.2.2.7 Limitations

This methodology yields only qualitative information about the antimicrobial agent combination. Using this technique, it may be difficult to distinguish indifferent from synergistic interaction [1,2,5].

- **Synergistic effect**: The effect when chemical substances interact resulting in an overall effect that is greater than the sum of individual effects of any of them


(21) MBL(+)
imipenem
imipenem
+ EDTA
I
I+E



سورة الإخلاص 3 مرات = ختمة كاملة

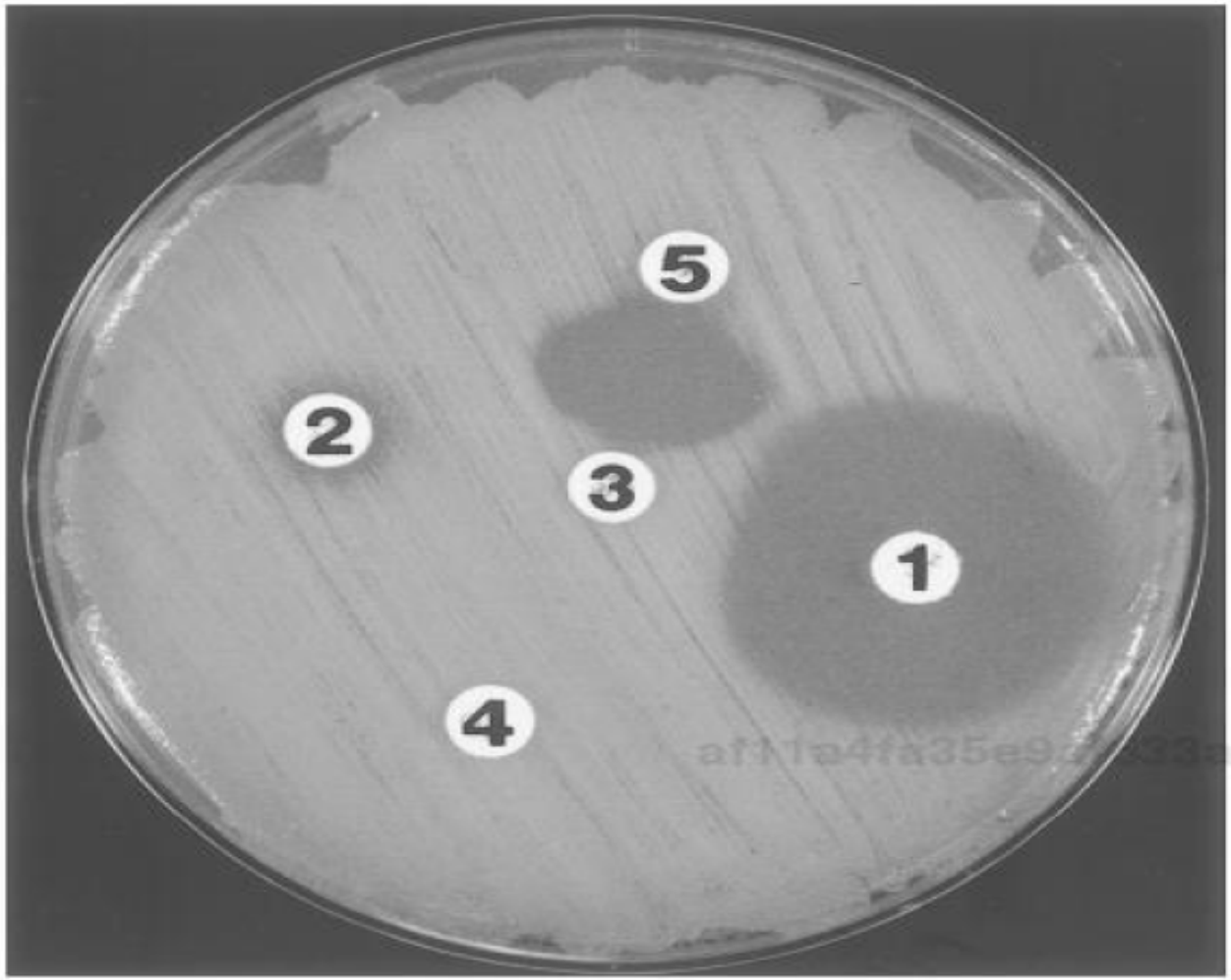
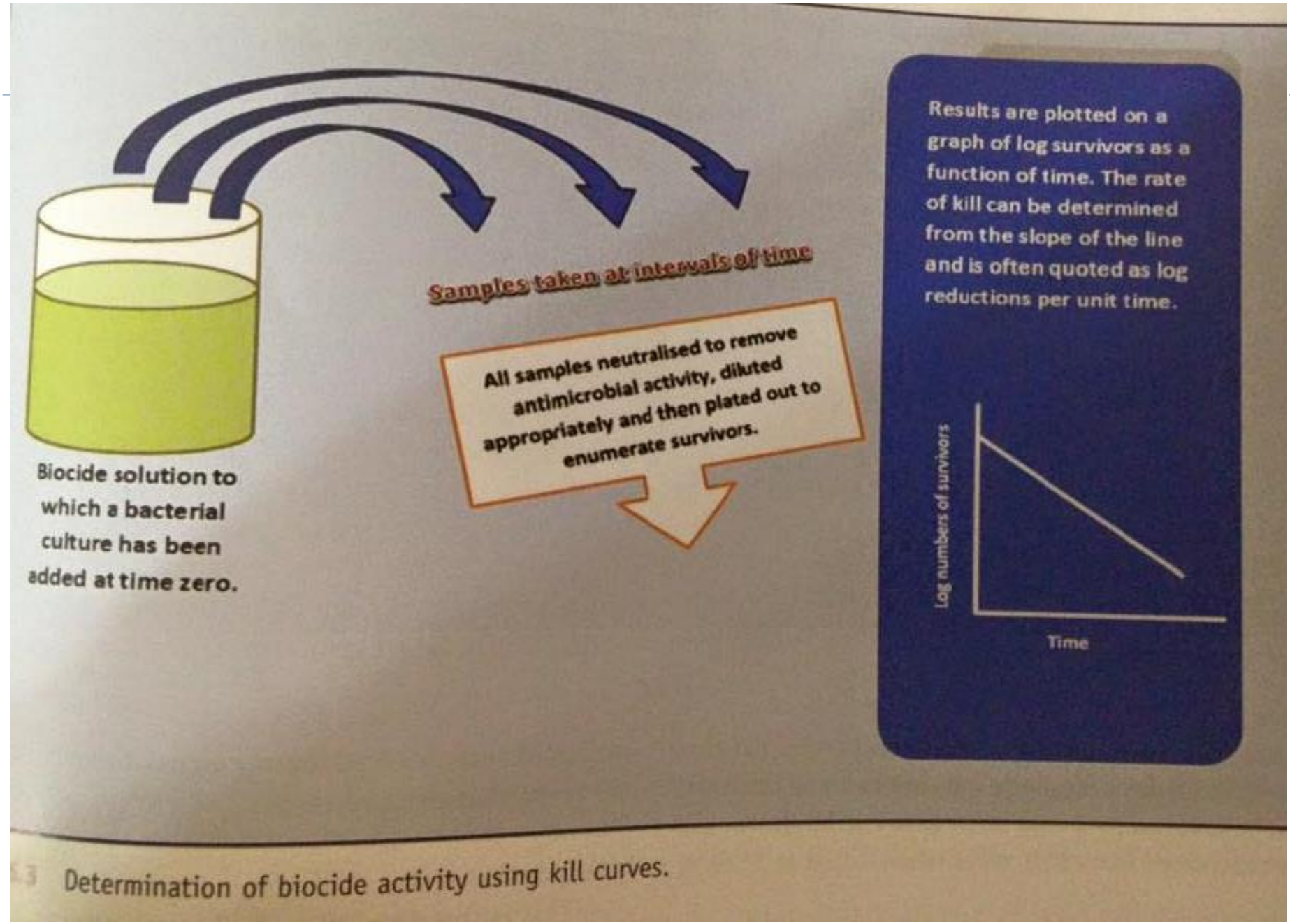


Figure 18.4 Disc test with inhibition zones around two (1, 2) of five discs. The zone around disc 1 is clear and easy to measure, whereas that around disc 2 is indistinct. Although none of the antimicrobials in discs 3, 4 or 5 appear to inhibit the bacterium, synergy (as evidenced by inhibition of growth between the discs) is evident with the antimicrobials in discs 3 and 5. Slight antagonism of the drug in disc 1 by that in disc 3 is evident.

Measurement of antibacterial activity:
3. kill curves

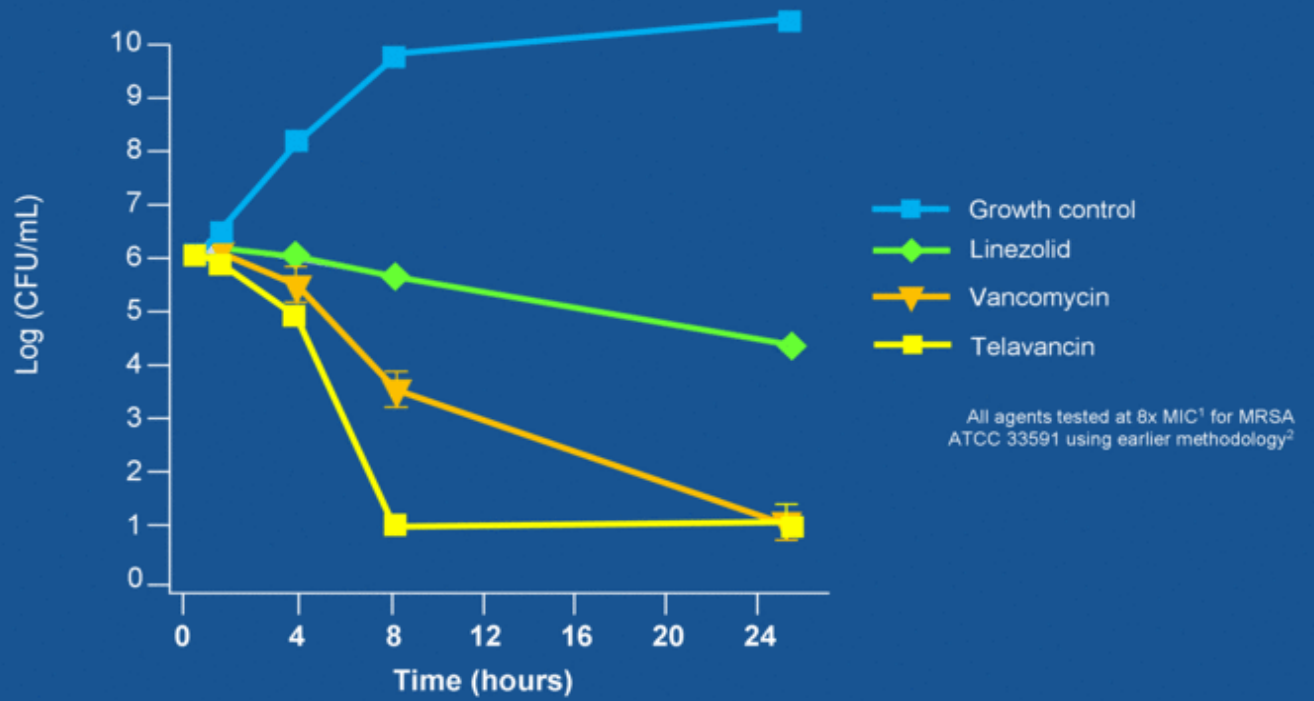
- MIC and agar diffusion methods give qualitative information about antimicrobial activity
- They do not give any quantitative details on the rate at which the agent kills cells and how it might be influenced by environmental factors
- Kill curves: inoculate a biocide solution with a known concentration of viable cells and then take samples at intervals of time to determine the number of surviving cells at each time point
- When the sample is taken the antimicrobial agents must be neutralized:
  - Dilution



Determination of biocide activity using kill curves.

Bactericidal Activity against MRSA

In Vitro Time-Kill Curves



Pace, AAC, 2003;47(11):3602-3604; CLSI. M7 A9

41

ربنا آتنا في الدنيا حسنة وفي الآخرة حسنة وقنا عذاب النار

اختبار فعالية المواد الحافظة .

Preservative efficacy testing

- The activity of the preservative influenced by:
  - pH
  - Multiphase systems
  - Suspended solids
  - Adsorption on packaging
- Final product should be tested when the product is first manufactured and also during storage, why?

الهدف من الاختبار التأكد من عدم تدهور هاي المواد الحافظة مع الوقت بسبب التفاعلات أو الظروف البيئية

to show there is no deterioration of preservative action over time

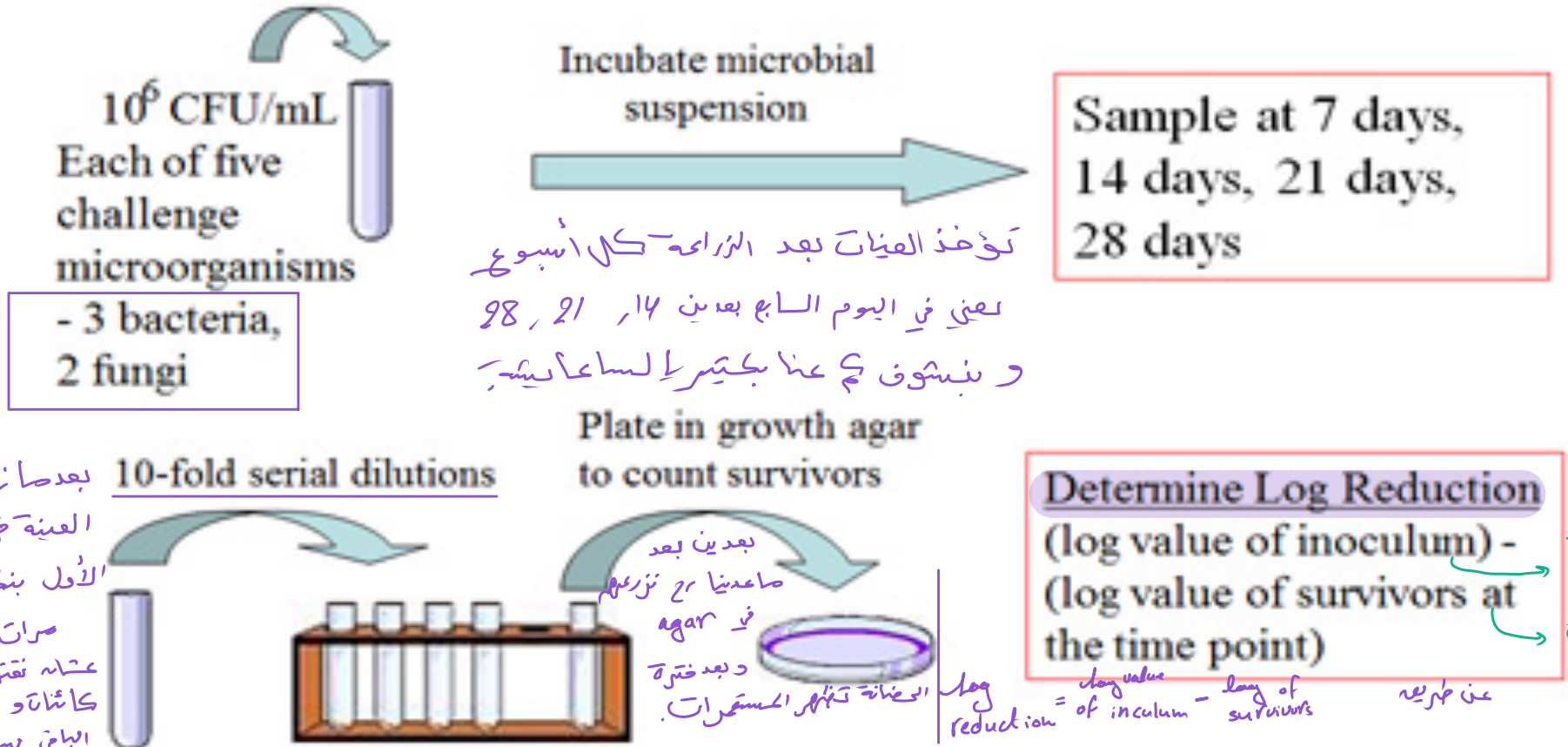
Preservative efficacy testing

- Pharmacopeia testing procedure for the final product (pharmaceutical and cosmetic products)
- The product is tested in its final container and different samples are inoculated with a range of different cultures (described in details in various pharmacopeias)
- The organisms used are:

Pseudomonas aeruginosa	Candida albicans
Staphylococcus aureus	Aspergillus braziliensis
Escherichia coli	Zygosaccharomyces rouxii

Preservative efficacy testing

Antimicrobial Efficacy Test



تؤخذ العينات بعد الزراعة كل أسبوع
يعني في اليوم السابع بعدين ١٤، ٢١، ٢٨
و بنشوف كم عنا بكتيريا لسا عايشة

بعدها نأخذ العينة في الاسبوع الأول بنخففها ٥ مرات ١٠ عشانه نقتل شوية كائنات و نقدر نعد الباقي بسهولة.

بعدين بعد ما عدينا رح نزرعهم في agar و بعد فترة الحضانة تظهر المستعمرات.

عدد الكائنات الأولي

عدد الكائنات الناجية

عن طريقه

بنقدر نحسب انخفاض عدد MO عشانه نقيم فعالية المواد الحافظة

Preservative efficacy testing

- After inoculation of the products in their final containers they are stored under specified conditions for 28 days
- During this period samples are removed at intervals of time and neutralized before enumerating the survivors using plate counts
- Bacteria are incubated at 30-35C for 18-24 hours
- Candida and Zygosaccaromyces at 20-25C for 48 hours
- Aspergillus at 20-25C for one weeks

Table 16.3 EP performance criteria for preservative efficacy tests.

Product type	Challenge organism	Acceptance criteria	Log reductions specified					
			6 h	24 h	48 h	7 days	14 days	28 days
Parenteral/ophthalmic	Bacteria	A	2	3				NR
		B		1		3		NI
	Fungi	A				2		NI
		B					1	NI
Topical, nasal, ear and inhaled products	Bacteria	A			2	3		NI
		B					3	NI
	Fungi	A					2	NI
		B					1	NI
Oral and rectal products	Bacteria						3	NI
	Fungi						1	NI

NI = no increase; NR = no recovery.

- Acceptance criteria are given the pharmacopeias

- A vs B criteria: A more demanding, generally applied and B less stringent if there is a risk of toxicity if conc. of preservative is too high

Disinfectant testing

- Disinfectants are intended for use within diverse environments thus there is requirements that they possess bactericidal, sporicidal, fungicidal, and virucidal properties
- The basic approach is to add MOs to a disinfectant and remove samples at interval of time, neutralizes the biocides and assess the survivors

اللهم صلِّ وسلم وبارك على محمد

Non-sterile Regulations: Microbial Control

- 21CFR 211.113 – Control of Microbiological Contamination
  - "Appropriate written procedures, designed to prevent objectionable organisms on drug products not required to be sterile, shall be established and followed."

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

اللهم إنك عفو تحب العفو فاعف عنا

- 21CFR 211.84
  - "Each lot of component, drug product container, or closure with potential for microbiological contamination that is objectionable in view of its intended use shall be subject to microbiological tests before use."
- 21CFR 211.165
  - "There shall be appropriate laboratory testing, as necessary, of each batch of drug product required to be free of objectionable microorganisms"

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

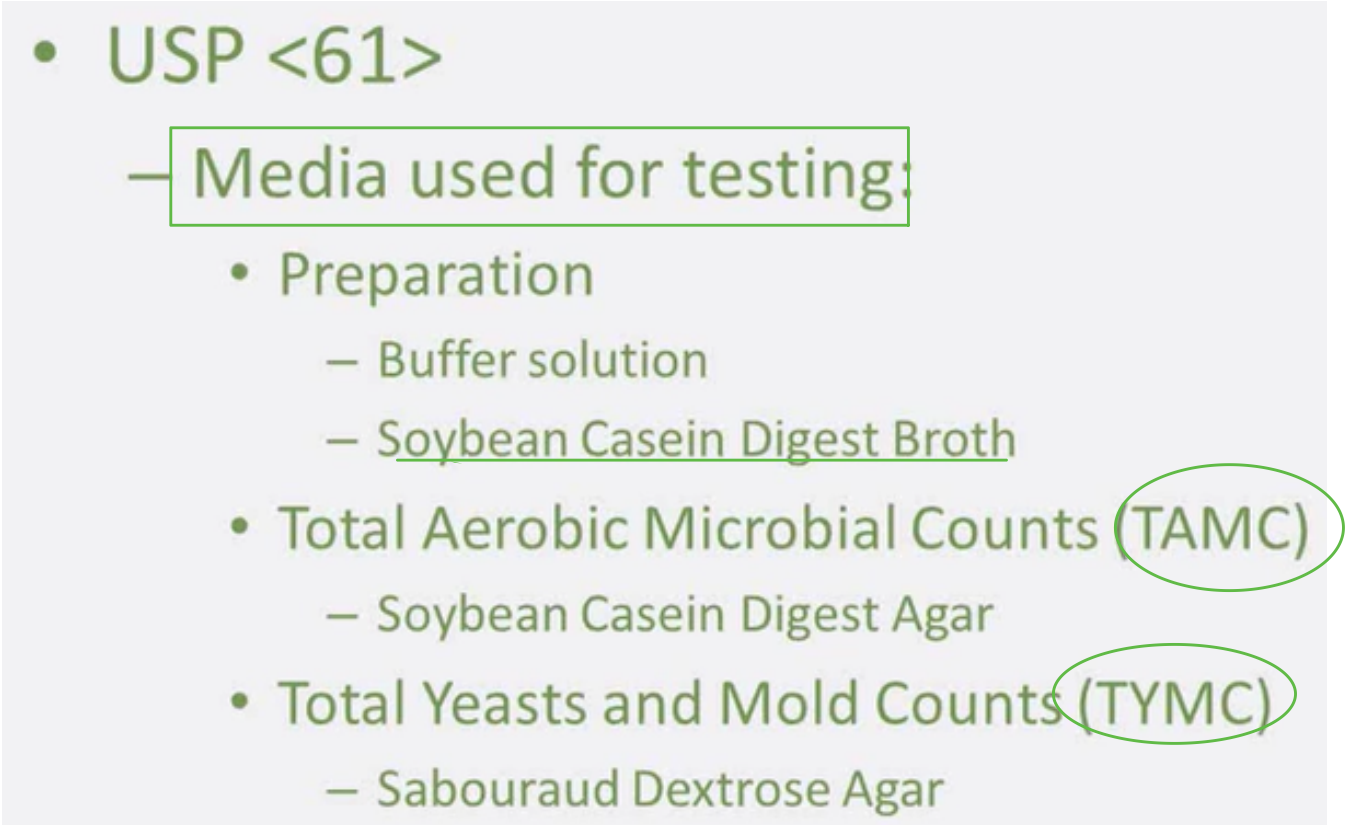
USP Testing methods:

- <61> - Microbiological Examination of Non-sterile Products - Microbial Enumeration
- <62> - Microbiological Examination of Non-sterile Products - Tests for Specified Organisms
- <51> - Antimicrobial Effectiveness Test

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

- USP <61>
- Purpose of the test:
  - To determine the overall bioburden of the non-sterile product – Bacterial and Mold/Yeast
  - Quantitative result
  - Monitor in-process testing
  - Lot release
  - Harmonization
    - USP/EP/JP Methods

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s



Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

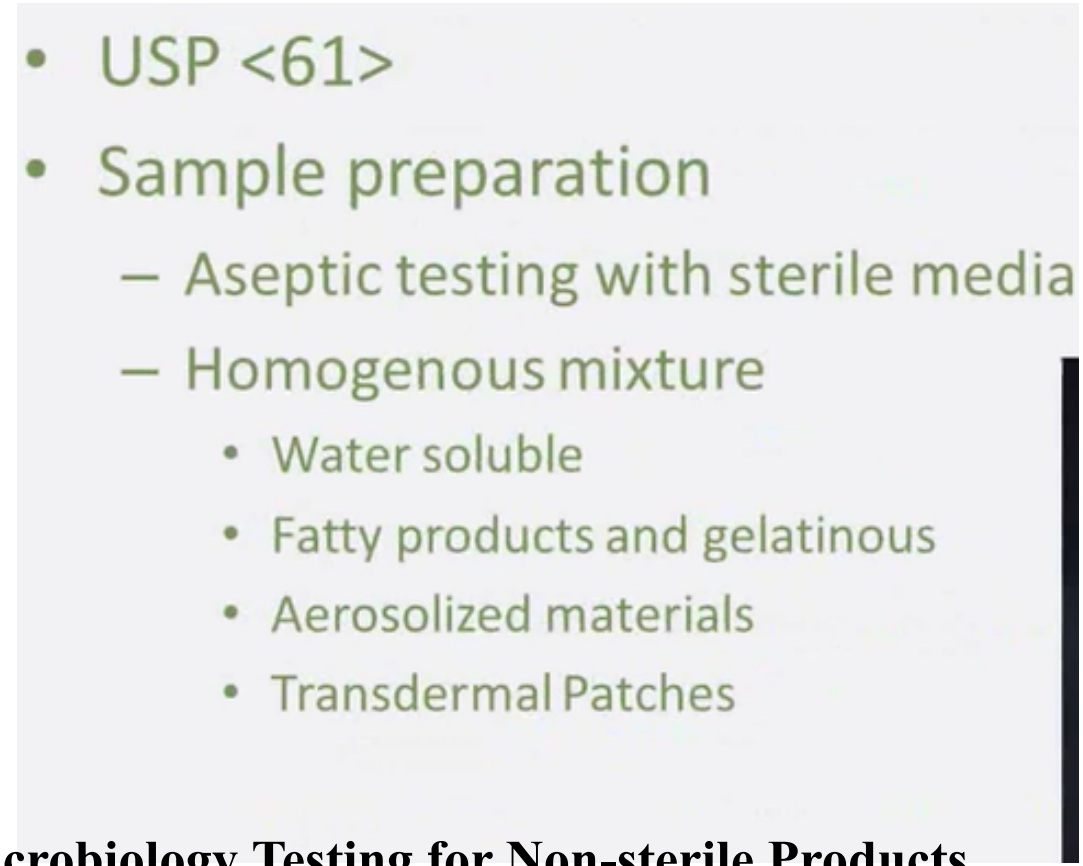
اللهم ارزقنا ووالدينا حُسن الخاتمة والشهادة في سبيلك

- USP <61>
  - Sampling and sample size
    - 10 gram/milliliters/ patches of sample recommended
    - 1% for smaller batches
    - Amount tested should have a justification and included in sampling scheme
    - Must represent the entire lot tested

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

- The sampling scheme should include:
  - Follow 21CFR 211.84 requirements
    - What and where to sample –
      - Beg, mid, end
      - Top, mid, bottom
    - Amount of sample needed
    - How often the sampling should be performed
    - Very specific sampling methods/procedures (training, precautions to avoid cross contamination)
      - Equipment use – Sterile
    - Sampling labeling, handling, storage and shipping

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s



Microbiology Testing for Non-sterile Products
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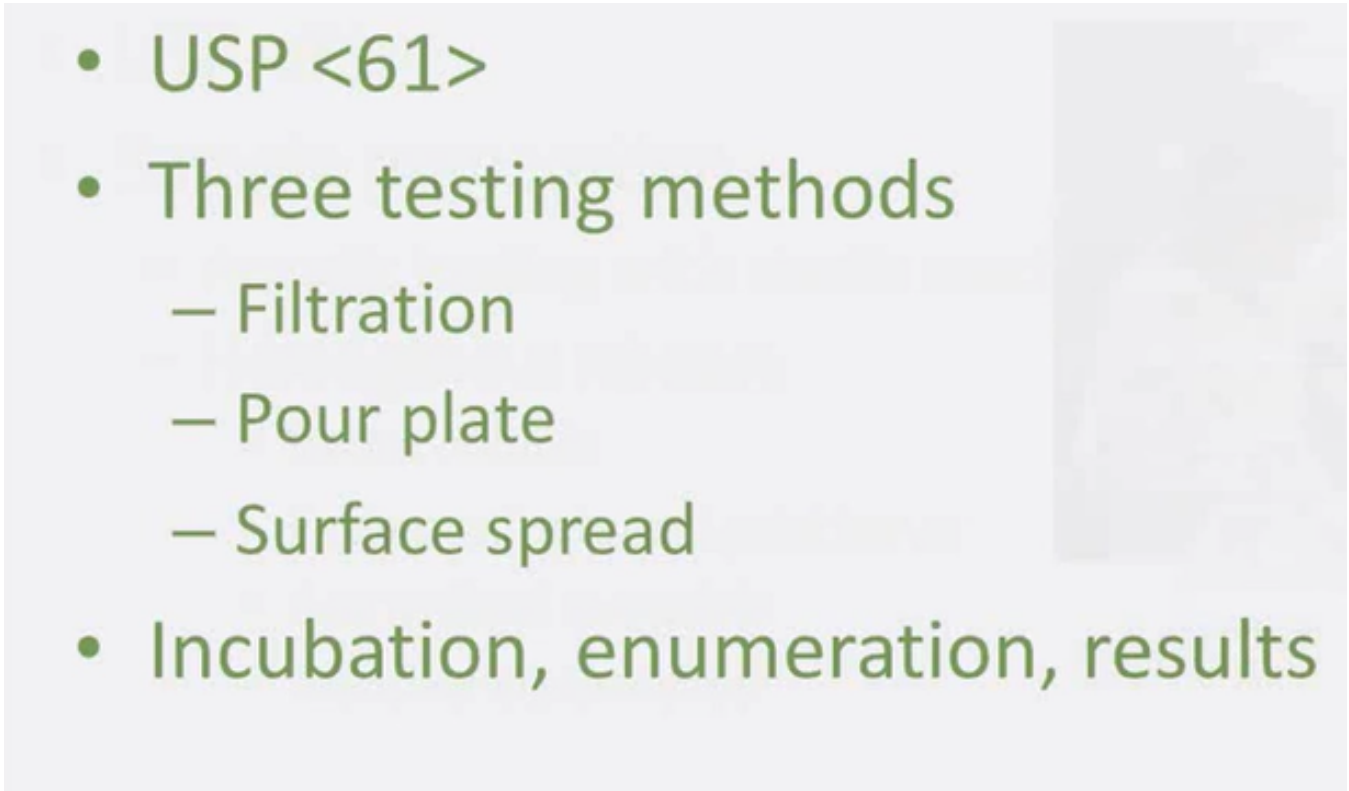


Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

استغفر الله

- USP <61> - Limitations
  - Not all organisms present in the product will grow under testing conditions
    - Viable but non-culturable
  - Microorganisms in samples are not homogenous.
    - Sink to the bottom or create a film on the top
    - Fungal balls floating in different areas

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

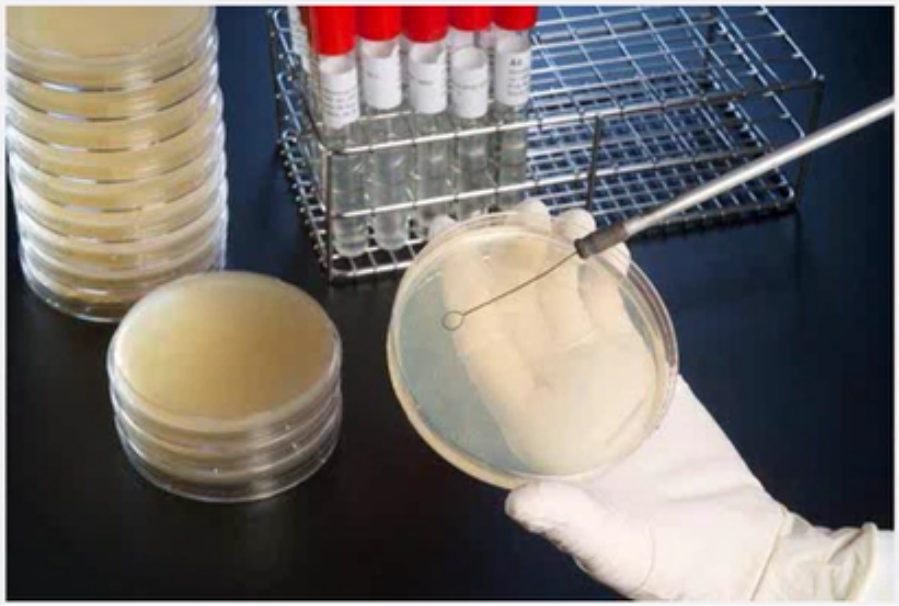
Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

– Limitations

- Screen for specific organisms only
  - Additional specific organism screens may need to be created
  - Identify any growth recovered - Any growth recovered could mean a quality issue

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

USP <51> - Antimicrobial Effectiveness Testing (AET)



Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

- USP <51> - Antimicrobial Effectiveness Testing (AET)
  - Purpose of the test:
  - Testing performed to determine the effectiveness of antimicrobial preservatives by inoculating the sample with a set of challenge organisms and the number of CFU surviving at different time points is recorded.
    - Testing must be performed on sterile and non-sterile products that contain antimicrobial preservatives, even if the active drug intrinsically is antimicrobial.

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

- USP <51>
  - Sterile - Multi-dose products that contain preservatives to inhibit growth during the withdrawing of individual doses
  - Non-sterile - Products that contain preservatives which are used to protect the product from microbiological growth during usage and manufacturing.
  - Testing is performed on the product as it is distributed by the manufacture (finished product)
  - Preservatives used should be below the level that is toxic to the user

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

- USP <51> - Product categories – Based on route of administration

Category	Product description
1	Injections, parenterals including emulsions, otic, sterile nasal and ophthalmic products made with aqueous bases or vehicles
2	Topical (aqueous bases or vehicles), non-sterile nasal and emulsions, including those applied to the mucous membranes
3	Oral products other than antacids with aqueous bases or vehicles
4	Antacids made with an aqueous base

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

- USP <51> - How is testing performed?
  - Inoculum is prepared per USP instructions
  - Sample is inoculated with tested organisms
    - *S. aureus, P. aeruginosa, E. coli, C. albicans* and *A. brasiliensis*
    - Categories 1, 2, and 3 – final inoculum concentration is between 1 x 10^5 and 1 x 10^6 CFU/ mL
    - Category 4 – final inoculum concentration is between 1 x 10^3 and 1 x 10^4 CFU/ mL
  - Incubated and plated on specific days per product category requirements

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

Category	Organism Type	Result Criteria
1	Bacteria	Not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days.
2	Bacteria	Not less than 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days.
3	Bacteria	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days.
1-4	Mold and yeast	No increase from the initial calculated count at 14 and 28 days

- Additional criteria required for EP testing for parenteral, ophthalmic, topical and oral liquid based preparations.

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s

- Determining Acceptance criteria
  - USP <1111> - Non-sterile products: Acceptance criteria for pharmaceutical preparations
  - Chapter recommendations for acceptance criteria
    - Cutaneous
      - » TAMC <200 CFU/g/mL
      - » TYMC <20 CFU/g/mL
      - » Absent of *S. aureus* and *P. aeruginosa*

Microbiology Testing for Non-sterile Products
https://www.youtube.com/watch?v=2RsuPBZK058&t=966s