MIRACLE Academy

التعقيم والتصنيع المعقم

لجان الدُّفعات

اللهم وفقني في دراستي، ونوّر بالكتاب بصري، واشرح به صدري، واستعمل به بدني، واطلق به لساني، وقوِّ به عزمي بحولك وقوتك، فإنّه لا حول ولا قوة إلّا بك يا أرحم الراحمين.

وفقكم الله...

Factors influencing the activity of biocidal agents

- **Concentration:**
- **Concentration exponent (n): describes the change in rate of kill with a change in concentration**

N بتحكي كيقف يتغير rate of kill مع تغير تركيز
chemical biocides

- **C1 & C2 : concentration of the biocides required to kill a standard inoculum in times t1 & t 2**
- **n= slope of the line when log death time is plotted against log concentration**

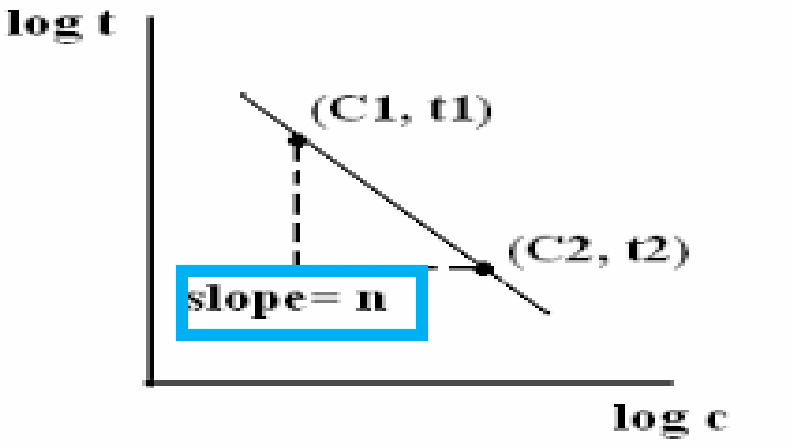
Remember:

High concentration exponent is a formulation issue in aliphatic alcohol & some phenols

كيف بنعملها بالمختبر؟

على نفس البكتيريا (لازم نكون محددينها) و بنجيب ال chemical biocides وبندرس ال rate of kill اله على C1 وعلى C2 وبنشوف الوقت اللازم لقتل 99% من البكتيريا على كل تركيز فيهم و ناخد العلاقة وبنطلع ال slope منها اللي هو نفسه n

- $n = \frac{\log t2 - logt1}{logC1 - \log C2}$



Higher n so time to kill will decrease

Note :

There is a natural assumption that doubling the concentration of biocide will double the rate of killing or conversely halving the concentration will reduce the activity by a factor of 2 this rule may just apply for simple chemical reactions the interaction of biocide with cellular targets is much more complex

Table 16.2 Concentration exponents of commonly used biocides.

Antimicrobial agent	Concentration exponent
Hydrogen peroxide	صغير 0.5
Mercurials Mercury, phenyl mercury nitrate, phenyl mercury acetate	0.03–3.0
Bronopol Type of alcohol ,but it is not aliphatic alcohol	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

ما في أي مشكلة بـ n لأنه مش Aliphatic alcohol

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 (2^1) which is 2.**
- **A three fold dilution means the biocides activity will be reduced by a value 3^1 (a third of its original activity)**
- **Agents with a concentration exponent of 6:**
- **a doubling in concentration will increase the activity of 2^6=64-fold**
- **And a halving in concentration will reduce their activity by 64-fold**
- **A three fold dilution will mean a decrease in activity of 3^6 or 729 times less active than the original**

Note:

Hence these agnets with n=6 are highly suseptible to losses in activity within a formulation due to adsorption, degredation and so on

- عشان نفهم تأثير n خلينا نتخيل CHEMICAL BIOCIDES اله n =1 هسا
لو عملنا مضاعفة للتركيز و صارت n =2 فبنحكي التغير بالتركيز هو:
2 TO POWER OF CONCENTRATION EXPONENT يعني 2^1
فالناتج 2

- ✓ Doubling will just double the effect
- ✓ Halving will reduce effect
- ✓ 3 fold increase so activity increase by a factor of 3

مشكلة ال n العالية انه مثلا لو عنا n= 6
وعملنا doubling فبصير عنا 2 مرفوعة لقوة 6 يعني تساوي 64 ولو عملنا halving رح تقل 64 مرة و لو عملنا 3 fold dilution رح تقل ال activity بمقدار 3 مرفوعة لقوة 6 يعني 729 مرة

عشان هيك ال aliphatic alcohol لو صارلها adsorption على serum ممكن كمية بسيطة تكون اللي صارلها adsorption لكن انعكاس كبير على الفعالية حيث انها بتقل بشكل كبير او حتى ال phenol كمادة حافظة بحب يرتبط ب polymerبالتركيبة او يرتبط بال container حتى لو كمية بسيطة الانعكاس رح يكون كبير

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**

→ Preservatives and they should be uncharged to be active and should be in acidic media (pH less than 5)

- **Both are active at low pH values, why?**

Note:

At alkaline pH will be highly ionized and this form cannot cross the membrane of the cell

Acridines as preservatives or disinfectants need basic media (high pH) to be active

Factors influencing the activity of biocidal agents

- **Solubility:** Issue in esters (methyl parabens, ethyl parabens, propyl parabens) because their activity increases when chain increase

- An issue with classes of molecules having variable alkyl chain lengths as parabens
- 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
- Care must be taken in multiphase products such as emulsion, what will happen?

- بالمستحضر يتم استخدام more than one type of parabens هاد عشان يحلوا مشكلة ال solubility لانه ال ethyl parabens بتروح على الزيت لكن لما يكون في كمان methyl parabens بتضل بالمي فهون mixture of parabens حل مشكلة ال solubility لكن لو بس استخدمنا methyl parabens رح تكون الفعالية مش قوية ، فخيار ال mixture of parabens مناسب في مستحضر ال Emulsion

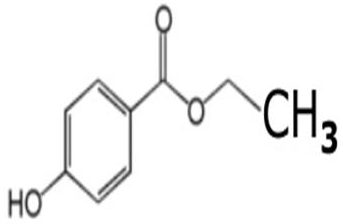
Note:

The longer chain compounds may preferentially dissolve in the organic phase and the aqueous phase is the most likely to be susceptible to contamination left unprotected

Methyl Paraben

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

Structure:



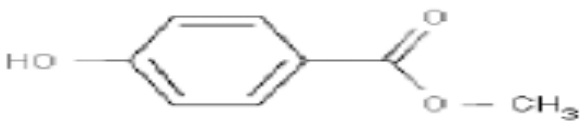
- **Physical properties**

Description: Colorless crystals or white powder.

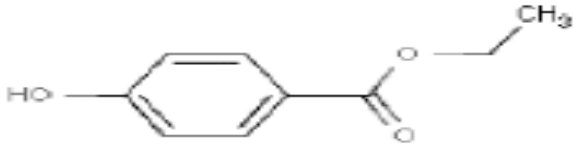
Solubility: soluble in water, ethanol, slightly soluble in benzene and acetone.

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

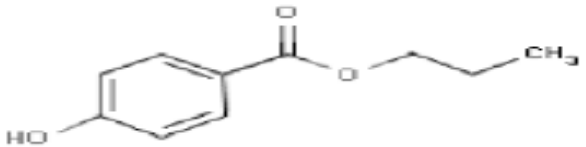
يستخدموا ضمن التركيز المسموح فيه



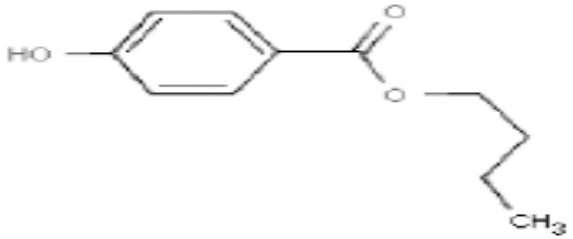
Methyl-4-hydroxybenzoate
(methyl paraben)



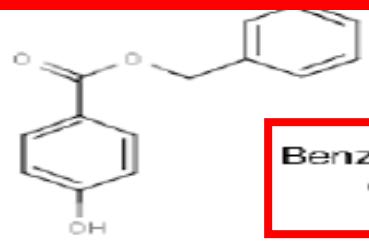
Ethyl-4-hydroxybenzoate
(ethyl paraben)



Propyl-4-hydroxybenzoate
(propyl paraben)



Butyl-4-hydroxybenzoate
(butyl paraben)



Benzyl-4 hydroxybenzoate
(benzyl paraben)

Source: Dermatitis © 2005 American Contact Dermatitis Society

لا يستخدموا في الصناعات (ممنوع استخدامهم) لأنهم
Very lipid soluble فبصيرلهم adsorption
بالجسم و تخزين بال fatty tissue

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

لا يتم استخدامهم مع Thiceniers
charged biocidal agents

- Adsorption to plastic or rubber components of the packaging
- Partition into the non-aqueous phase (in parabens)
- all of the above decrease biocidal activity especially with biocides with high concentration exponent, why?

Note:

Relatively small reduction in conc with high conc exponent could sever consequences in terms of its ability to preserve the product

بدنا ما يصير ال interaction عشان لا يقل ال concentration و بالتالي يقل ال effect

Measurement of antibacterial activity:

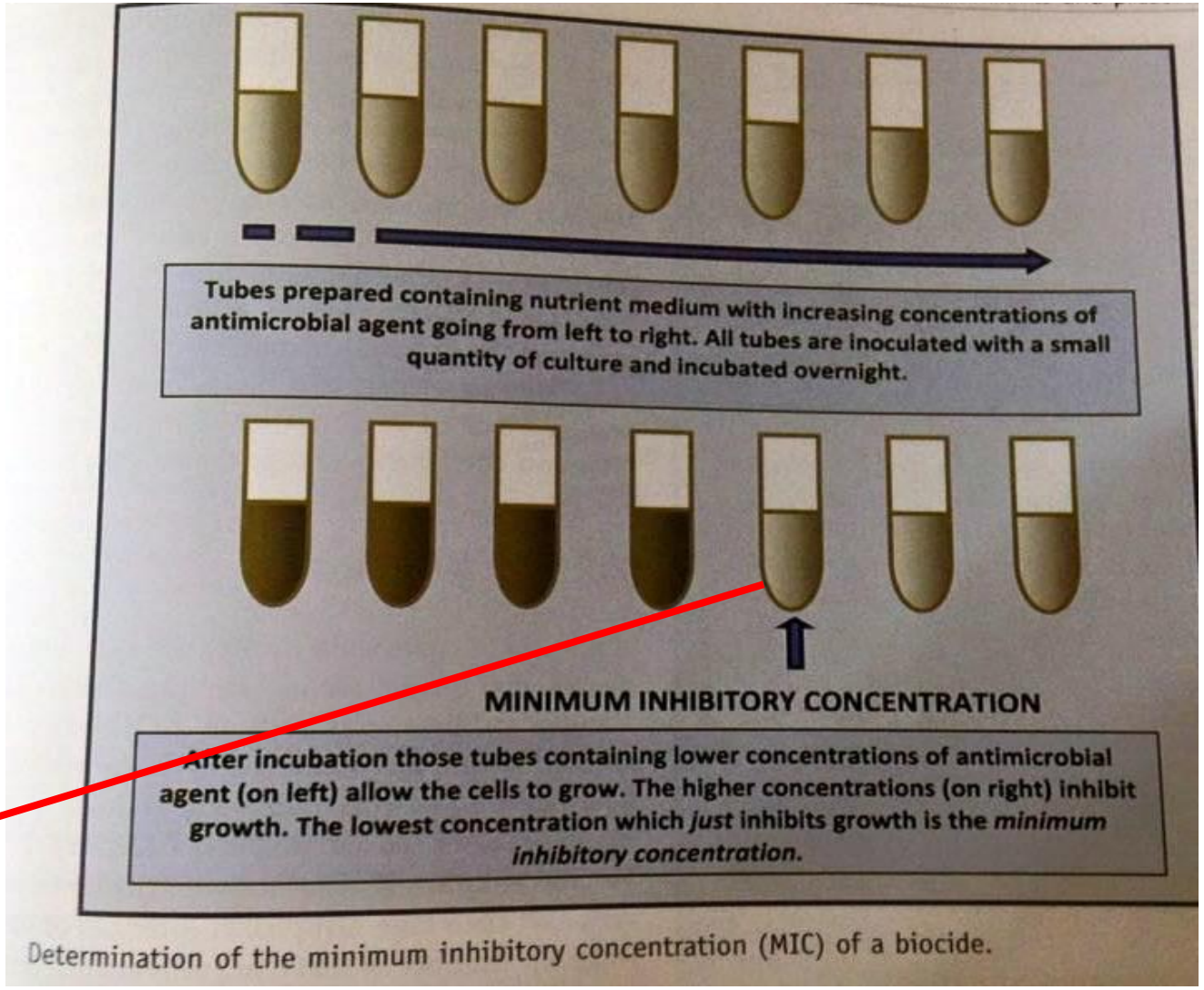
- Evaluate the inhibitory effect of the formulation on **live cultures**:

1. Broth-dilution methods
2. Agar diffusion methods
3. Kill curves

Bacteria or fungi that we want to study it

Note:

The MIC values will vary with the incubation conditions, growth medium, strain of cultures…



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

No turbidity

- نستخدم (liquid media(broth بـ test tube اللي بتلف فيهم هو تركيز ال chemical biocides اللي بدنا نشوف قدرته على قتل البكتيريا المزروعة بكل test tube لكن بدنا ننتبه تركيز البكتيريا ثابت وبعدها بنعمل incubation ونشوف اول تركيز ما صار فيه growth of bacteria وبكون هو ال MIC

Measurement of antibacterial activity: 1. broth-dilution methods

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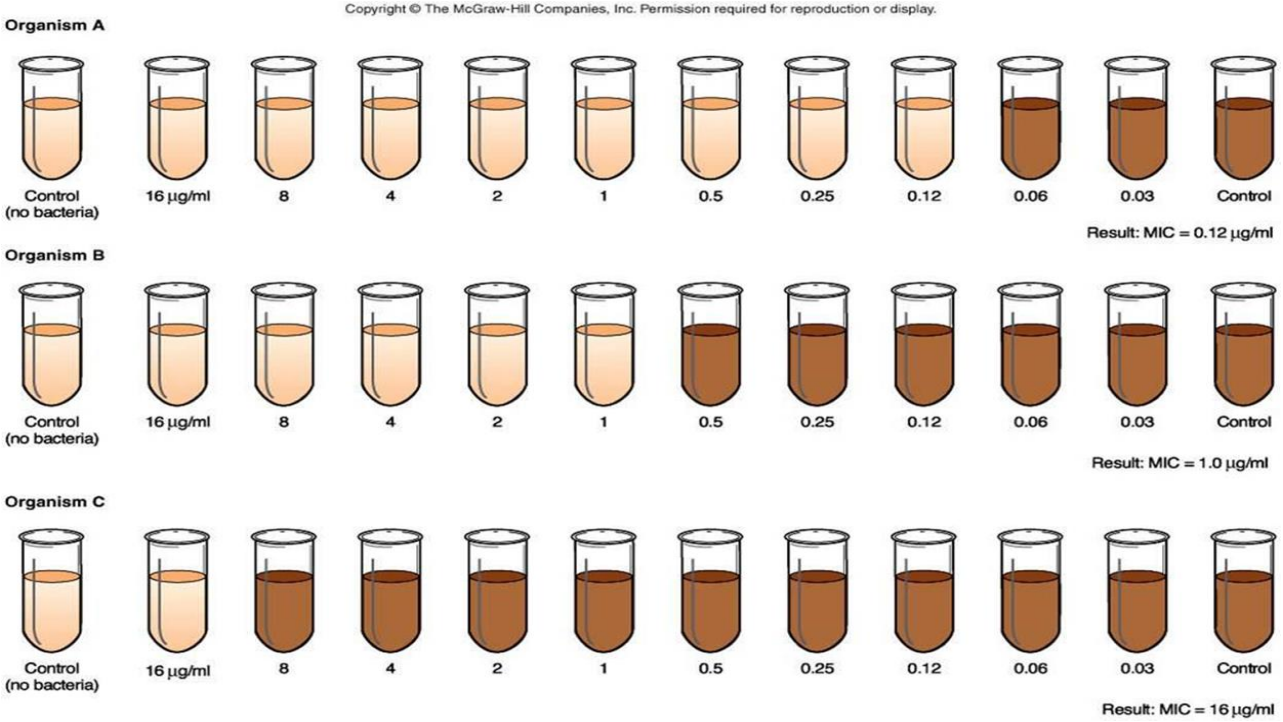


MIC for:

A is equal 0.12 , it is the most sensitive for this microorganism

B is equal 1

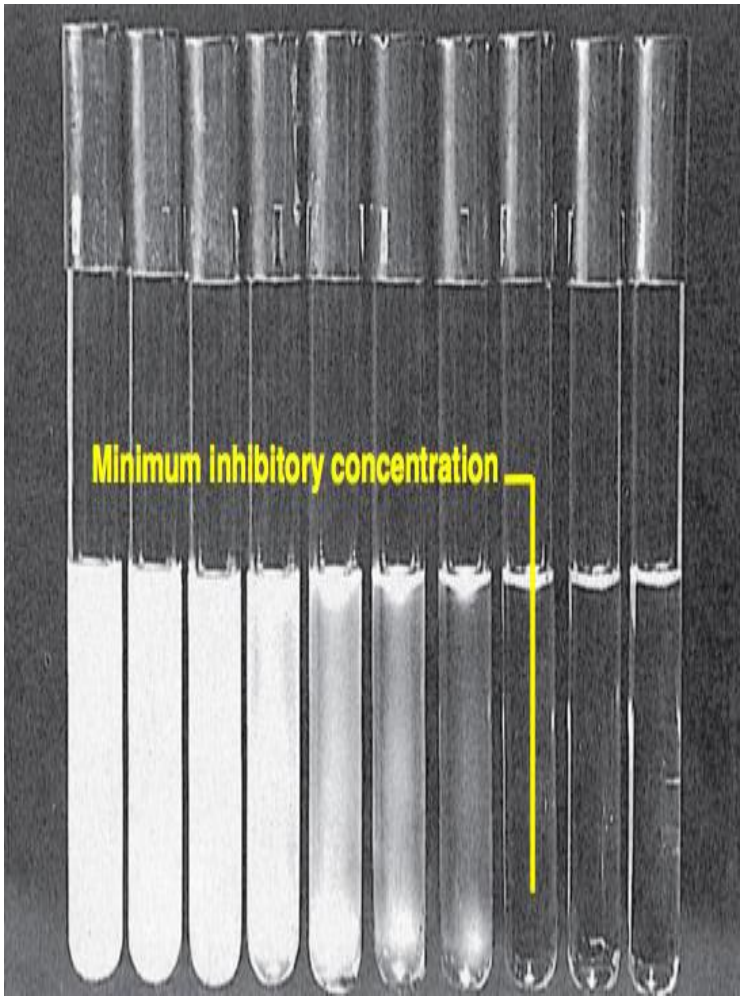
C is equal 16 , it is resistant

هون رح نشوف كيف MIC بعطي فكرة عن ال antibacterial activity of chemical biocides

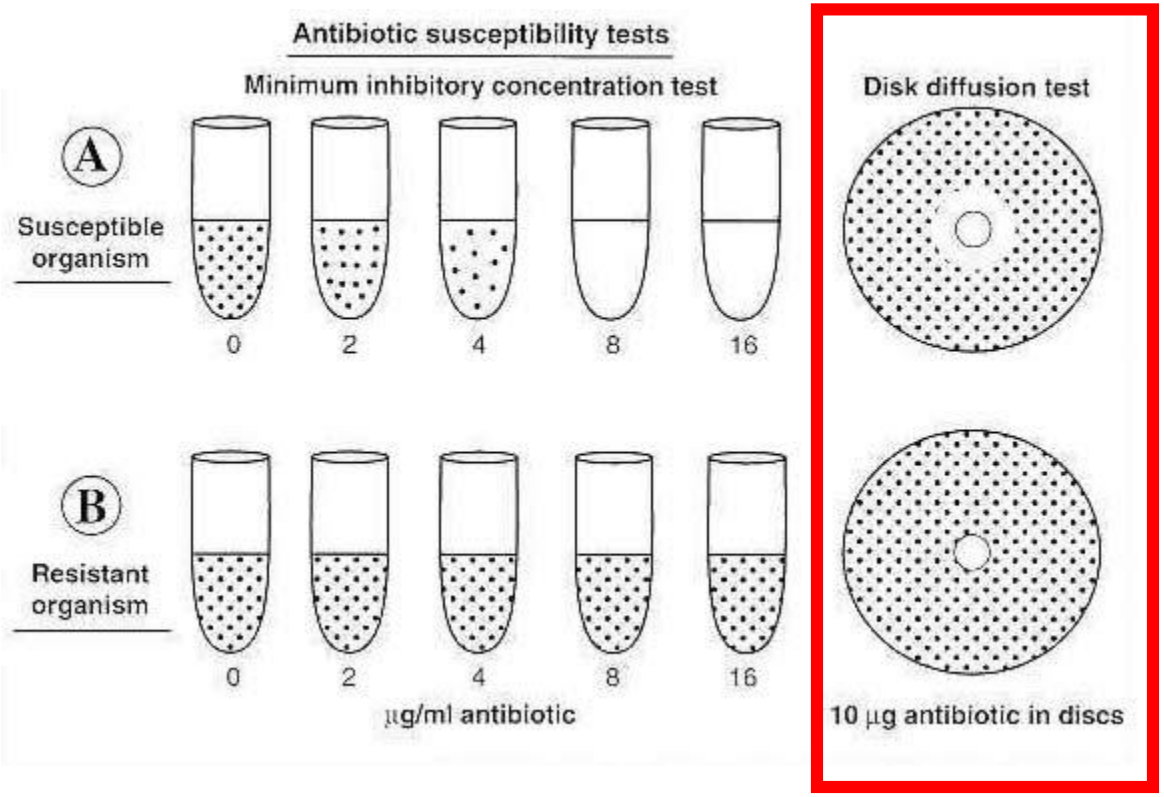
هسا عنا 2 control واحد بس بحتوي على chemical biocides و واحد بس على bacteria ، ليه؟

عشان نتأكد توافر البيئة المناسبة لنمو ال microorganism و نتطمن ان ال killing من ال chemical biocides مش من ال condition وكمان لما يصير growth نعرف شو ال color لنقدر نقارن لو ما صار نمو شو رح يكون اللون و مرجعنا ال Broth وقتها

Measurement of antibacterial activity:
1. broth-dilution methods



broth-dilution methods



هذا ال chemical biocides نستخدمه 8 µg/ml لمعالجة A وليس لمعالجة B لأن B هو resistant لهاد ال Chemical biocides

لو بدنا نعمله هاد ال test على شكل Diffusion بنجيب ال agar وبنزرع عليها ال microorganism وبنحط filter paper (disc) بكون مغمس Saturated بال chemical biocides و بنعمل incubation و بنشوف النتائج بعدها

اسمها disc diffusion لأنه ال solution المغمور بال filter رح يصيرله diffusion بالمنطقة حوالين ال filter فهي المنطقة لو قتل البكتيريا فيها رح يتكون ما يسمى بـ zone of inhibtion

Measurement of antibacterial activity:

2. Agar diffusion methods بكون مزروع عليه البكتيريا Solid agar

Very common method

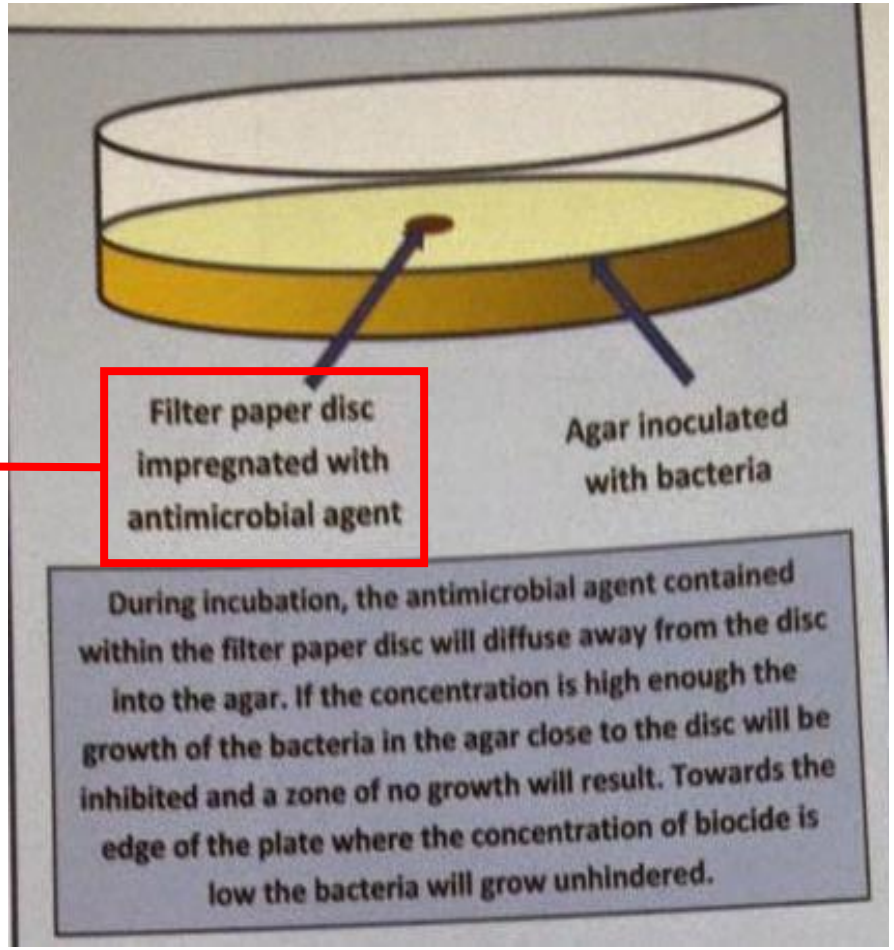
- **Used commonly to:**

1. **Assay activity of biocides**
2. **Assay antibiotics**
3. **Determine the sensitivity of clinical isolates to a particular antibiotic prior to treatment**

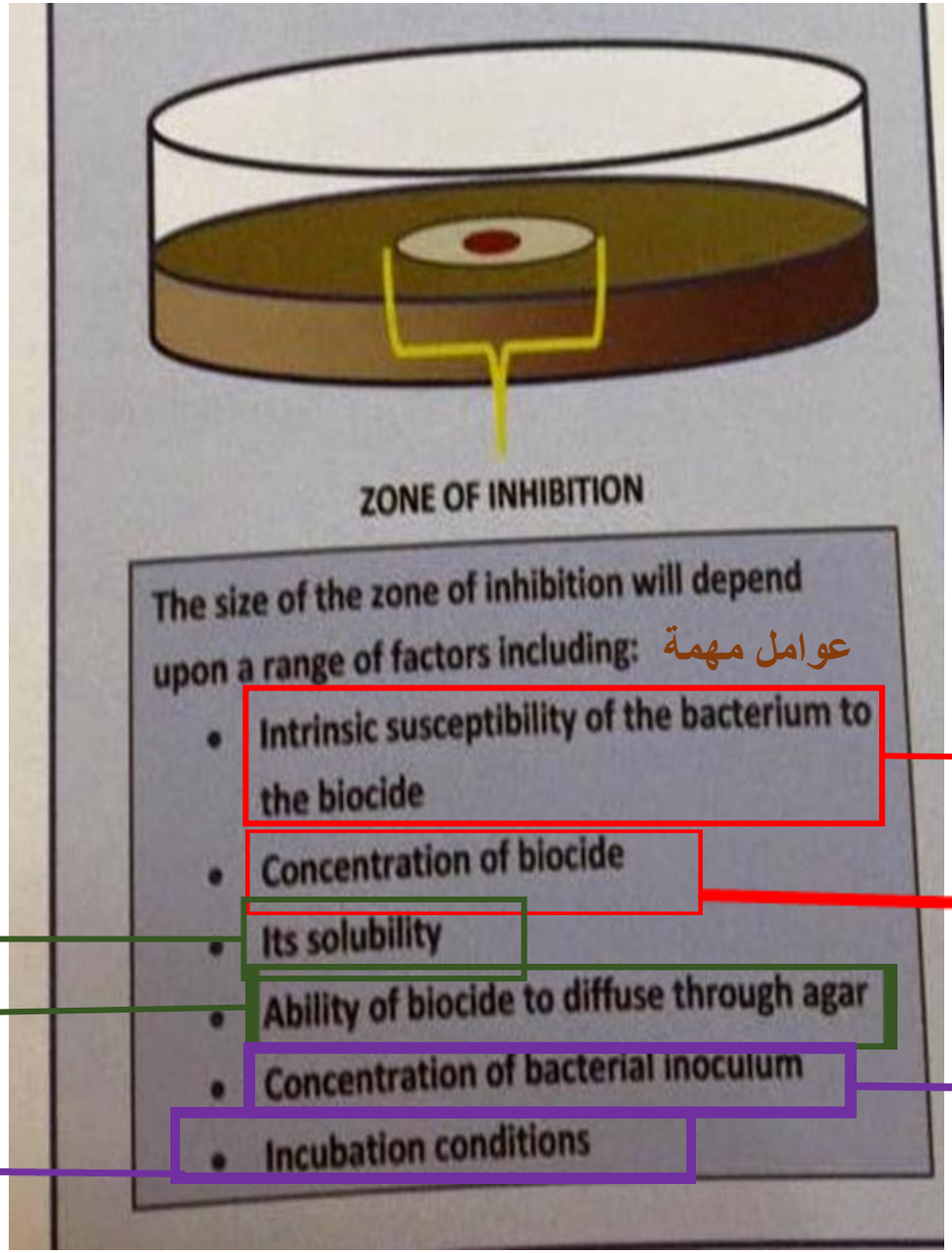
تستخدم كثير بالمختبرات الطبية ليعملوا زراعة urine or blood or throat sample و
يشوفوا أي antibiotic قادر على isolate أو لنعرف ال sensitivity

Measurement of antibacterial activity:
2. Agar diffusion methods

ال filter paper بتكون معبية و مليانة Antimicrobial agent



بزرعوا بكتيريا بنفس تركيزها بال broth و يشوفوا متى صار ال inhibition zone و يقيسوا اكم ال diameter تاعه



اوقات بتكون الفعالية قليلة بسبب انه بكون ال chemical biocides غير ذائب

قدرة ال diffusion

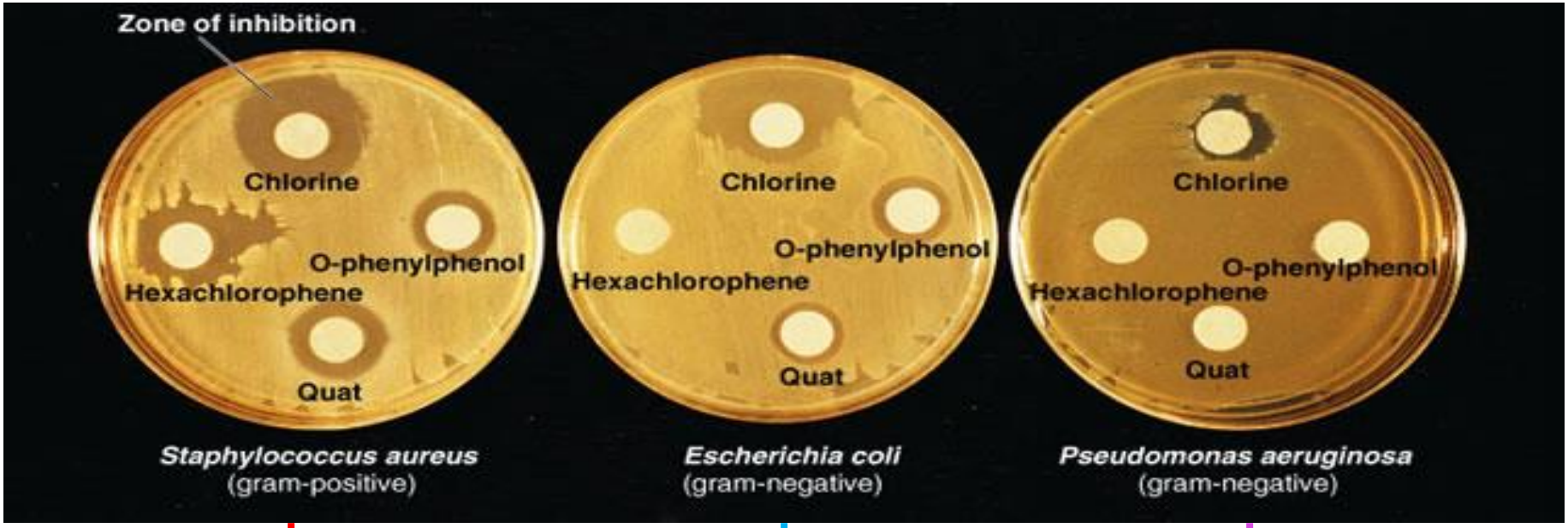
حسب ال condition اذا سمحت للبكتيريا بالنمو

higher susceptibility so higher zone

لأنه مش شرط يكون كل ال Chemical biocides تركيز واحد

تركيز البكتيريا

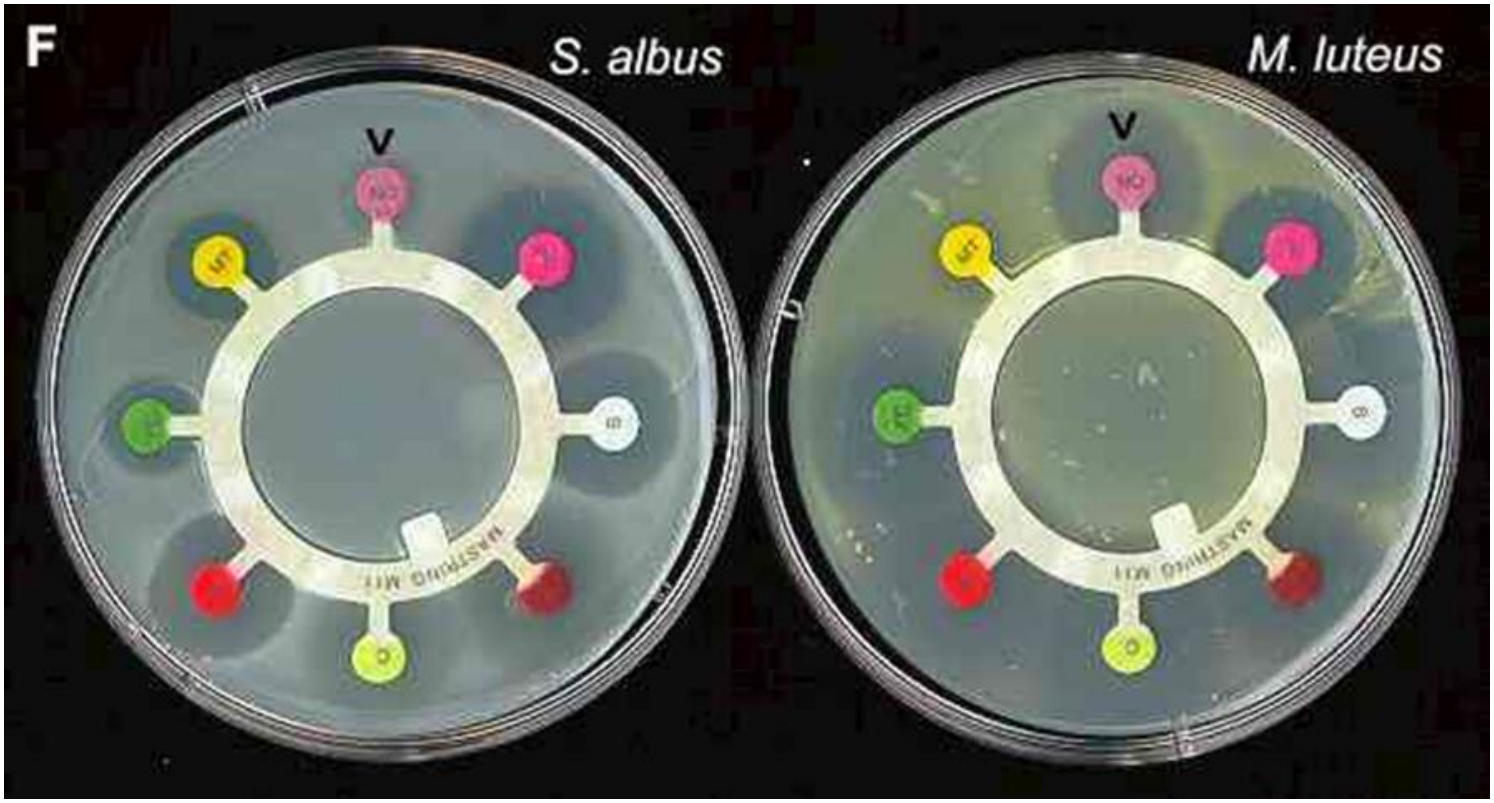

Resistant to Antibiotics
Zone of inhibition
Antibiotic disc
Bacterial
Growth



أي واحد بقدر يقتل

فقط Hexachlorophene اللي ما بقتل

فقط chlorine اللي بقتل



شوفوا كيف في منهم اله inhibition zone
و اله و قتل و منهم ما اله و ما بقتل

نهاية المحاضرة الثالثة