

* هدف التجربة التعرف على العوامل التي تؤثر على activity للـ disinfectant .
 * متى يقدر استعماله صالغ للاستخدام .

Experiment 6 Testing of disinfectants

Disinfection is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects. Disinfection can be achieved by physical or chemical methods. Chemicals used in disinfection are called disinfectants. Different disinfectants have different target ranges, not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out. Sterilization is an absolute condition while disinfection is not

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemical has very narrow spectrum of activity and some have very wide. Those chemicals that can sterilize are called chemosterilants. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics

An ideal antiseptic or disinfectant should have following properties:

- Should have wide spectrum of activity
- Should be able to destroy microbes within practical period of time
- Should be active in the presence of organic matter
- Should make effective contact and be wettable
- Should be active in any pH
- Should be stable and have long shelf life
- Should have high penetrating power
- Should be non-toxic, non-allergenic, non-irritant or non-corrosive
- Should not have bad odor
- Should not leave non-volatile residue or stain
- Efficacy should not be lost on reasonable dilution
- Should not be expensive and must be available easily

Such an ideal disinfectant is not yet available. The level of disinfection achieved depends on contact time, temperature, type and concentration of the active ingredient, the presence of organic matter, the type and quantum of microbial load.

عملية إزالة most M.O.
 متى كلها .
 عن المواد الغير حية .

"عنا نوعين من disinfection method"

- ① physical ⇒ Filtration يتضمن الـ steril M.O.
- ② chemical ⇒ Disinfectant عبارة عن not all can Kill M.O. method

بشكلها جاز range narrow wide
 في مفهوم
 و في

انالة لكل M.O.

يقدر استعمالها
 على الجلد

مميزات antiseptic and disinfectant

activity تكون انه ضمن وقت معقول (ما استنى ساعات) حتى يشتغل

يقدر احتزنها

تكون رائحة معتوية

تصنيف الـ disinfectants

Classification of disinfectants:

1. Based on consistency

- a. Liquid (E.g., Alcohols, Phenols)
- b. Gaseous (Formaldehyde vapor, Ethylene oxide)

2. Based on spectrum of activity

- a. High level *can kill all type of M.O*
- b. Intermediate level *can kill all M.O except spores*
- c. Low level *weak activity against Mycobacteria as well as spores.* → *مقاومة* resistance.

Spectrum of activity

	Vegetative cells	Mycobacteria	Spores	Fungi	Viruses	Examples
High level	+	+	+	+	+	Ethylene Oxide, Gluteraldehyde, Formaldehyde
Intermediate level	+	+	-	+	+	Phenolics, halogens
Low level	+	-	-	+	+/-	Alcohols, quaternary ammonium compounds

كيف يشغلوا ؟

1. Based on mechanism of action

- a. Action on membrane (E.g., Alcohol, detergent)
- b. Denaturation of cellular proteins (E.g., Alcohol, Phenol)
- c. Oxidation of essential sulphhydryl groups of enzymes (E.g., H₂O₂, Halogens)
- d. Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Ethylene Oxide, Formaldehyde) *in protein and DNA*
- e. Damage to nucleic acids (Ethylene Oxide, Formaldehyde)

Factors Influencing the Action of Disinfectants

العوامل التي تؤثر مع disinfectants

wide narrow

حسب هو

1. Type of disinfectant
2. Concentration of disinfectant
3. Contact time: the longer the contact time, the greater the kill
4. Temperature
5. Type of organisms
6. Concentration of organisms
7. Nature of suspending liquid

← خليه وقت اكثر
more M.O رح يقتل

نوع M.O

كمية M.O

نوع الـ solvent

* بشو بنعمنا هاي Factors ؟

مثلا اذا كان عندي disinfectant ضعيف
فروح اضطر اخليه لوقت اكثر (contact time)
او احسن منه تركيز اعلى.

Suspension tests: (Comment)

In these tests, a sample of the bacterial culture is suspended into the disinfectant solution and after exposure it is verified by subculture whether this inoculum is killed or not. Suspension tests are preferred to other test as the bacteria are uniformly exposed to the disinfectant.

بعضو بسبب M.O
موزونة بشكل
uniformly
الdisinfectant
في كل الجوانب

There are different kinds of suspension tests:

1. Test for the determination of the phenol coefficient (Rideal and Walker). → activity of disinfectant or phenol
2. The qualitative suspension tests. → effective or not at specific time.
3. The quantitative suspension tests.

1. Determination of phenol coefficient:

Phenol coefficient (PC or PE) of a disinfectant is calculated by dividing the dilution of test disinfectant by the dilution of phenol that disinfects under predetermined conditions.

Rideal Walker method: Phenolic disinfectant is diluted from 1:400 to 1:800 and the phenol is diluted from 1:95 to 1:115. Their bactericidal activity is determined against *Salmonella typhi* suspension. Subcultures are performed from both the test and phenol at intervals of 2.5, 5, 7.5 and 10 minutes. The plates are incubated for 48-72 hours at 37°C. Then PC is calculated.

كيف يتم ال
best
concn.
1:400 - 1:800
5 best
phenol or alcohol
1:95 - 1:115
5 best
dilution

في best tube
تركيز
phenol
or disinfectant

بعد 5 عين جاري اجن بكتريا Sample (salmonella)
والعملها suspended بلان disinfectant او phenol
بعد اوقات محددة بعد ما نلاحظهم مع أخذ Samples
من هاتي best tube والعملها culture على plate
وتعملها incubation

PC or PE = (highest DF of disinfectant required to eliminate bacteria within 7.5 minutes but not in 5 minutes) / (highest DF of phenol required to eliminate bacteria within 7.5 minutes but not in 5 minutes).

ليحسن حاجين اسبق
عند 7.5 min اي تركيز
ما حاصر فيه growth
مثلا؟ الجواب عند
1:1400
1:1600
1:1800
من طرف عند نفس التركيز عن 5 min
لا يكون فيه growth
- روح اخذ تركيز
1:1400
عند 7.5 min
NG
صا ر فيه
growth

Disinfectant	Dilution	Growth of test organism in subculture after exposure for:			
		2.5 mins	5 mins	7.5 mins	10 mins
Test disinfectant 5 best	1:400	NG	NG	NG	NG
	1:500	G	NG	NG	NG
	1:600	G	G	NG	NG
	1:700	G	G	G	NG
	1:800	G	G	G	G
Phenol 5 best	1:95	G	NG	NG	NG
	1:100	G	G	NG	NG
	1:105	G	G	G	NG
	1:110	G	G	G	NG
	1:115	G	G	G	G

* مثال ثاني
التركيز اللى G عن 7.5 min
وعند نفس التركيز عن 5 min
والجواب هو تركيز 1:100

PC = $\frac{\text{at 7.5 NG at 5 G of disinfectant}}{\text{phenol}}$
 $= \frac{1:600}{1:100} = \frac{600}{100} = 6$

Example:

To calculate phenol coefficient of the tested disinfectant in the above table. After 7.5 minutes, the test organism was killed by the test disinfectant at a dilution of 1:600. In the same period the test organism was killed by phenol at a dilution of 1:100.

$$\text{Phenol coefficient} = \frac{600}{100} = 6 \rightarrow$$

معنى 6؟ يعني :-
disinfectant 6 times more effective than phenol.

This result indicates that the test disinfectant can be diluted six times as much as phenol and still possess equivalent killing power for the test organism.

Test interpretation:

1. If a phenol coefficient no greater than 1 indicates that this agent is equal to or less effective than phenol.
2. If a phenol coefficient greater than 1 indicates that this agent is more effective than phenol.

PC less than 1
have the same or less effective than phenol.

Disadvantages of the Rideal-Walker test are:

← صليان
هاد ال
test.

1. No organic matter is included
2. The microorganism *Salmonella typhi* may not be appropriate
3. The time allowed for disinfection is short also the presence of time skips
4. It should be used to evaluate phenolic type disinfectants only.

فقط لـ disinfectant (اللي بتحتوي على phenol)

2. The qualitative suspension tests

This was done in a qualitative way. A loopful of bacterial suspension was brought into contact with the disinfectant and again a loopful of this mixture was cultured for surviving organisms. Results were expressed as 'growth (+)' or 'no growth (-)'



بس بشوف .
disinfectant مع تركيز معين
وعى وقت معين عمل
Killing (أو لا).

3. The Quantitative Suspension Test:

In quantitative methods, the number of surviving organisms is counted and compared to the original inoculum size. By subtracting the logarithm of the former from the logarithm of the latter, the decimal log reduction or microbicidal effect (ME) or Germicidal Effect (GE) is obtained. An ME of 1 equal to a killing of 90% of the initial number of bacteria, an ME of 2 means 99% killed. A generally accepted requirement is an ME that equals or is greater than 5: at least 99.999% of the germs are killed. Even though these tests are generally well standardized, their approach is less practical.

The Microbicidal Effect (ME):

The Decimal Reduction Rate or the Microbicidal Effect (ME) can be calculated by using the following formula:

$$ME = \log NC - \log ND$$

control ← sterile water
 → after exposure for disinfectant.

NC: Number of colonies forming units (CFU) developed in the control series, in which the disinfectant is replaced by sterile normal saline.

ND: Number of colonies forming units (CFU) developed in the disinfectant series, after exposing the bacterial cells to the disinfectant.

The 5, 5, 5 Test:

The common name for the standard suspension test is the 5, 5, 5 test

- 1st (5): Five test microorganisms are originally tested

Pseudomonas aeruginosa *Staphylococcus aureus* *Escherichia coli*
Bacillus cereus *Saccharomyces cerevisiae*

- 2nd (5): The exposure time is 5 minutes
- 3rd (5): Determination of the bacterial activity (after the 5 minutes exposure to disinfectant) will be by decimal reduction rate (5 logarithms). The criterion for activity is a germicidal effect of 5 logarithms

رجل 5 dilution

المعاني له test
 الي هو 5,5,5 test
 * بقدر اختبار 5 انواع من M.O
 * بستن 5min بـ ما اظلم disinfe مع M.O
 * رجـل 5 dilution

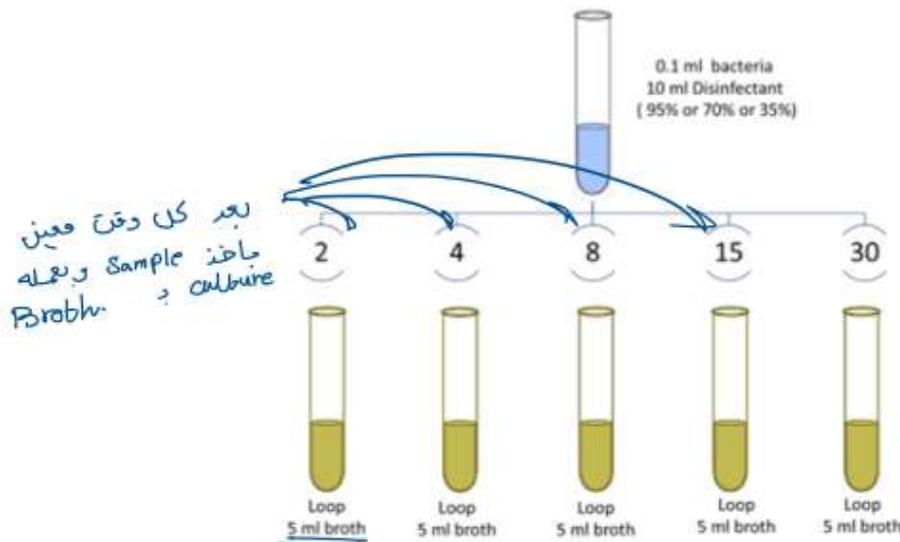
بناخذ بعين الاعتبار عدد M.O
 مثل ما اعرصتها لا disinf.
 كيف حسنها 9
 $GE = \log NC - \log ND$
 عدد M.O قبل ما اعرصتها - عدد M.O بعد ما اعرصتها disinf
 اذا طلع $GE = 1$
 معناها صار عندي Killing 90% ليكتير جا يعني من عايش 10%
 $GE = 2$ يعني صار عندي Killing 99%
 * حتر اعينر disinfectant جيد (acceptable)
 لازم تكون GE له تساوي 5 او اكثر
 يعني قادر انه يقتل 99.999% من M.O

Practical Part A (Qualitative Suspension Test)

This test is followed only by the DGHM guidelines.

Procedure (under aseptic conditions):

1. 0.1 mL of a bacterial suspension of 1×10^8 cells/mL is exposed to 10 mL of disinfectant with various dilutions (95%, 70% or 35% Ethanol).
↖ *Concen.*
2. After certain cumulative exposure times (2, 4, 8, 15 and 30 minutes), take a 0.001 mL sample (using inoculating loop: flamed and cooled) from the reaction mixture and add it to 5 mL NB. This step is both neutralization and subculture.
3. Incubate test tubes overnight at 37°C.



Interpretation of Results:

After incubation; each test tube (each subculture) should be examined whether it is turbid (+) or clear (-).

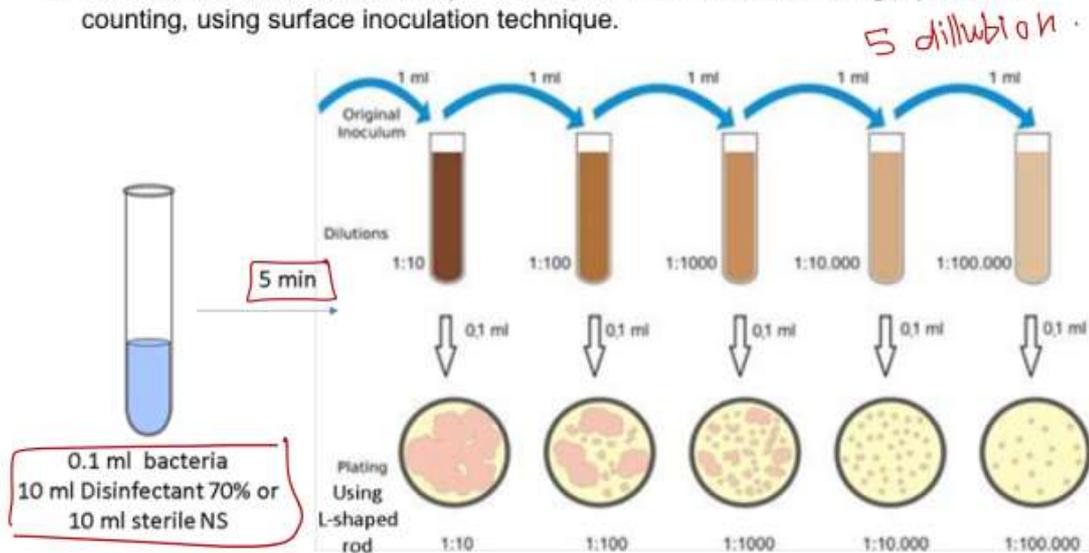
turbid → G (+)
clear → NG (-)

مثلاً 35% ethanol بعد 15min
قادرة تقتل 10^8
لما 95% قادر يقتل
بعد 2 min

Practical Part B (Quantitative Suspension Test):

Procedure: (under aseptic conditions) 70% ethanol.

1. Expose 0.1 mL of bacterial suspension to 10 mL of disinfectant (70% ethanol).
Add another 0.1 mL to the control (sterile NS or sterile DS)
2. After 5 minutes, neutralize 1 mL of reaction mixture using 9 mL NB.
3. Serially dilute the neutralized sample 4 times (by 10x dilution factors).
4. Subculture the neutralized sample and the followed 4 dilutions on agar plates for counting, using surface inoculation technique.



Interpretation of Results:

Counts are selected to be from 25 - 250 colonies. For results having more than one accepted count; the number closer to 250 (in control series), and the number closer to 25 (in disinfectant series) are used in the ME equation.

- Note: Do not forget to multiply the selected number of colonies by the corresponding dilution factor.

* In control series → M.O الأقرب من 300

* In disinfectant → M.O الأقرب من 30

* more than 300 → TNTC

* less than 30 → Statistically Invalid too few.

Example:

Calculate the ME for 70% ethanol which was used during a quantitative suspension test, knowing that the subculture step was performed using surface inoculation technique: !

زى ما كينا
العدد المقبول
من [30 - 300]

Dilution Factor	# of CFUs	
	Control Series	Disinfectant Series
10^1	TNTC	TNTC
10^2	TNTC	95
10^3	310 <i>more than 300 X</i>	33 ✓
10^4	240 ✓	28 <i>less than 30 too few X</i>
10^5	120	0

ME = Log NC – Log ND

ME = Log (240 * 10^4) – Log (33 * 10^3)

ME = 1.86

means ≈ 99% Killing.

* ما تنسوا
تضربوا العدد
بار
dillution factor

