# Evaluation of Disinfectants


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# Introduction

- Disinfectants used in hospitals and laboratories must be tested periodically to ascertain its potency and efficacy.

- Evaluation is **not for conc.** But it is for the activity of the agent under certain conditions and uses

# Disinfection process validation

Defined as:

"establishing documented evidence that a disinfection process will consistently remove or inactivate known or possible pathogens from inanimate objects."

# Factors affecting the disinfection process:

**Effect of temperature;**

- Bacteriologist Koch had noted that anthrax spore were more readily killed by the same concentrations of phenol if the temperature was elevated.

**Effect of pH;**

Any change in PH will affect:

- Rate of growth of bacteria >> growth is optimal at pH (6-8).
- Potency of antimicrobial agent >> due to ionization at pH.
- Ability of drugs to combine with cell surface sites of bacteria.

**Effect of surface activity;**

- The addition of low concentrations of surface active compounds may potentiate the biological effect of an antibacterial agent.

**Presence of interfering substances;**

- The presence of other material may reduce the effect of such an agent by adsorbing or inactivating it and thus reducing the amount available for combining with the cells it is desired to kill.

# Disinfectant tests

- Carrier test
- Suspension test
- Capacity test
- Practical test
- In-use test

# Carrier test

***Principle:***

- A carrier such as a silk or catgut thread is contaminated by submersion in a liquid culture of the test organism.
- The carrier is then dried and brought in contact with the disinfectant for a given exposure time.
- cultured in a nutrient broth;
- **No growth** indicates activity of the disinfectant tested whereas **growth** indicates a failing.

- By multiplying the test concentrations of the disinfectant and the contact times, a potentially active concentration-time relationships of the disinfectant is obtained.

***Limitations***

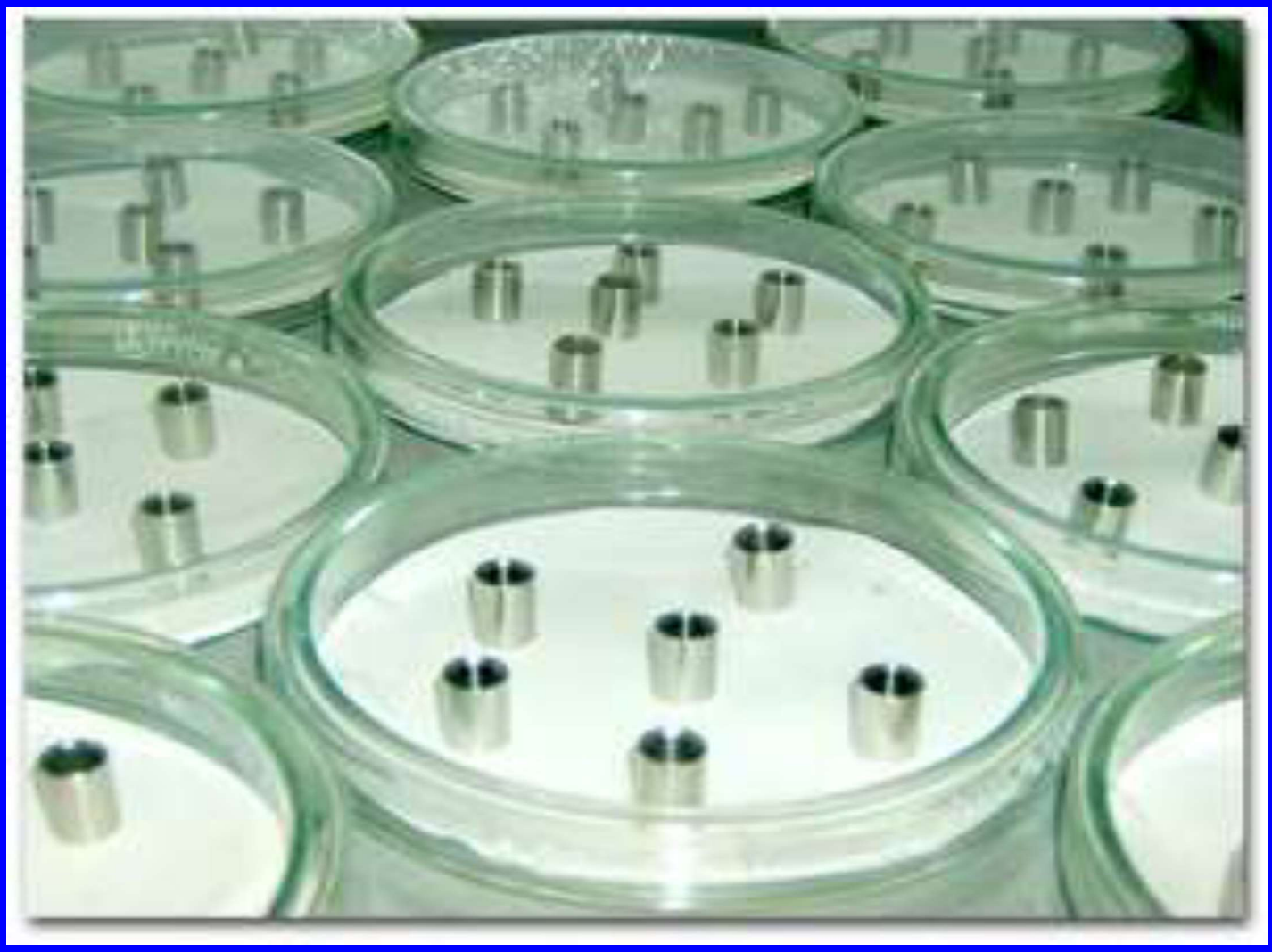
a) The number of bacteria dried on a carrier is hard to standardize

b) The survival of the bacteria on the carrier during drying is not constant.

# The AOAC Use-dilution test

- AOAC (American Association of Official Analytical Chemists)
- A carrier-based test.
- Organisms: *Salmonella cholerasuis, S. aureus* and *P. aeruginosa*.
- Carriers (stainless steel cylinders) are meticulously cleaned, sterilized, cooled and inoculated with a test organism by immersing in one of the culture suspensions.
- The cylinders are drained on filter paper, dried at 37°C for 40 minutes, exposed to the use-dilution of the disinfectant for 10 minutes.

- After transfer from the disinfectant, the treated test surfaces are incubated in the neutralizing growth medium for 48 hours.
- The number of tubes showing growth of the target microorganism is recorded.
- **To "pass" a 60 carrier test**, at least 59 of the 60 surfaces tested must demonstrate complete disinfection (no detectable growth of the target microorganism in the test tubes containing neutralizing growth medium).
- **To "pass" a 10 carrier test**, complete disinfection must take place on all test surfaces.



# Suspension tests

***Principle:***

- A sample of the bacterial culture is **suspended** into the disinfectant solution
- After exposure it is verified by subculture whether this inoculum is killed or not.
- Suspension tests are preferred to carrier tests as the bacteria are uniformly exposed to the disinfectant.

## *Types*

**a) Qualitative suspension tests:**

- A loopful of bacterial suspension brought into contact with the disinfectant
- A loopful of this mixture cultured for surviving organisms.
- Results expressed as **'growth' or 'no growth'.**

## B) Quantitative suspension tests.

- The number of surviving organisms (B) is counted and compared to the original inoculum size (A).

**microbicidal effect (ME) = Log (A) - Log (B)**

ME = 1 → killing of 90% of the initial number
ME = 2 → 99% killed.

- A generally accepted requirement is:

ME ≥ 5 →99.999% of the germs are killed.

## c) Phenol coefficient:

- Phenol coefficient of a disinfectant is calculated by dividing the dilution of test disinfectant by the dilution of phenol that disinfects under predetermined conditions.

# Determination of phenol coefficient

**1- Rideal Walker method:**

- Organism: Salmonella typhi suspension.
- Temp: 20°C
- 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.
- Growth or no growth
- R.W phenol coefficient = That dilution of disinfectant which disinfects the suspension in a given time is divided by that dilution of phenol which disinfects the suspension in same time

| Disinfectant | Dilution | Growth of test organism in subculture after exposure for: | | | |
|---|---|---|---|---|---|
| | | 2.5 mins | 5 mins | 7.5 mins | 10 mins |
| Test disinfectant | 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 | 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 |

**Disadvantages of the Rideal-Walker test:**

1- No organic matter is included.

2- Microorganism *Salmonella typhi* may not be appropriate.

3- Time allowed for disinfection is short.

4- Used to evaluate phenolic type disinfectants only.

## 2- Chick Martin test:

- Organism: mixture of *S. typhi* or S.aureus + dried yeast **(organic load)**
- Temp: 30°C
- Recovery media after contact time 30 min (loopful)---incubate at 37°C for 48hr
- Growth or no growth
- C.M phenol coefficient = mean of lowest conc. of phenol prevent growth in subculture/ unknown disinfectant

(The phenol coefficient is lower than that given by Rideal Walker method)

| | Rideal -Walker | Chick-Martin |
|---|---|---|
| Volume medium | 5.0 ml | 10.0 ml |
| Diluent for test disinfectant | Distilled water | Water with yeast suspension or feces |
| Reaction temperature | 17.5±0.5°C | 30°C |
| Organism | *Salmonella typhi* | *Salmonella typhi*, *Staphylococcus aureus* |
| Sampling times | 2.5, 5.0, 7.5, 10.0 min. | 30.0 min. |
| Calculation of coefficient | Dilution test killing in 7.5 mins divided by same for phenol | Mean concentration of phenol showing no growth after 30 min. divided by same for test |

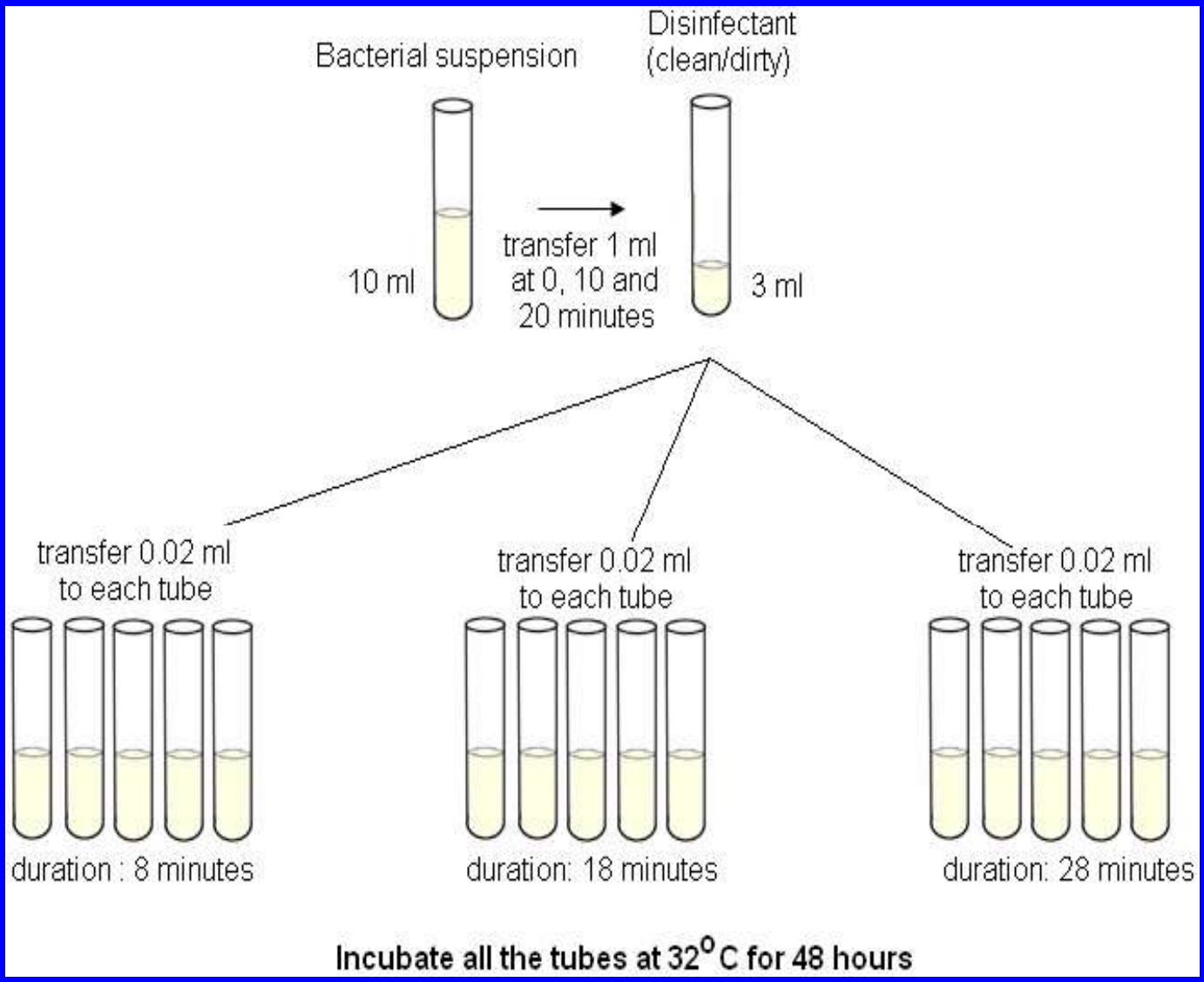
# Capacity tests

***Principle:***

- The ability to retain activity in the presence of an increasing load is the capacity of the disinfectant.
- In a capacity test, the disinfectant is challenged repeatedly by successive additions of bacterial suspension until its capacity to kill has been exhausted.
- Capacity tests simulate the practical situations of housekeeping and instrument disinfection.
- Best known capacity test is the **Kelsey-Sykes test *(Kelsey and Sykes, 1969).***

# Kelsey-Sykes test

- A triple challenge test, designed to determine concentrations of disinfectant that will be effective in clean and dirty conditions.
- Organisms: 4 organisms (*S. aureus, E.coli, P. aeruginosa* and *Proteus vulgaris*)
- Three successive loads of bacteria (additions) (0, 10, and 20 min)
- Temp: 20$^{o}$C
- Calibrated pipette for subculture rather than loop
- Clean and dirty conditions
- Assessment (kill or not) (no phenol coefficient)


Bacterial suspension
Disinfectant
(clean/dirty)
10 ml
transfer 1 ml
at 0, 10 and
20 minutes
3 ml
transfer 0.02 ml
to each tube
transfer 0.02 ml
to each tube
transfer 0.02 ml
to each tube
duration : 8 minutes
duration: 18 minutes
duration: 28 minutes
Incubate all the tubes at 32°C for 48 hours

- Sets that contain **two or more negative cultures** are recorded as a **negative** result.
- The disinfectant passes at the dilution tested if negative results are obtained after the first and second challenges.
- The third challenge is not included in the pass/fail criterion but positive cultures serve as inbuilt controls.
- If there are no positive cultures after the third challenge, a lower concentration of the disinfectant may be tested.

| Concentration | Inoculum count | Challenge number | | | Result |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| 1.0 | $2 \times 10^9$ | +++++ | +++++ | +++++ | Fail |
| 1.5 | $2 \times 10^9$ | ----+ | --+++ | +++++ | Pass |
| 2.0 | $2 \times 10^9$ | ----- | ----- | ----+ | Pass |

- Good guideline for the **dilution of the preparation** to be used.
- Disadvantage of this test: **complicated.**
- Modified Kelsey-Sykes method.

# Test for stability and long-term effectiveness

- Recommended concentrations based on Kelsey Sykes test apply only to freshly prepared solutions
- but if the solutions are likely to be kept for more than 24 hours, the effectiveness of these concentrations must be confirmed by:
- **Supplementary test** for stability of unused solution and for the ability of freshly prepared and stale solutions to prevent multiplication of a small number of bacteria that may have survived the short term exposure.
- *P. aeruginosa* is used as test organism.

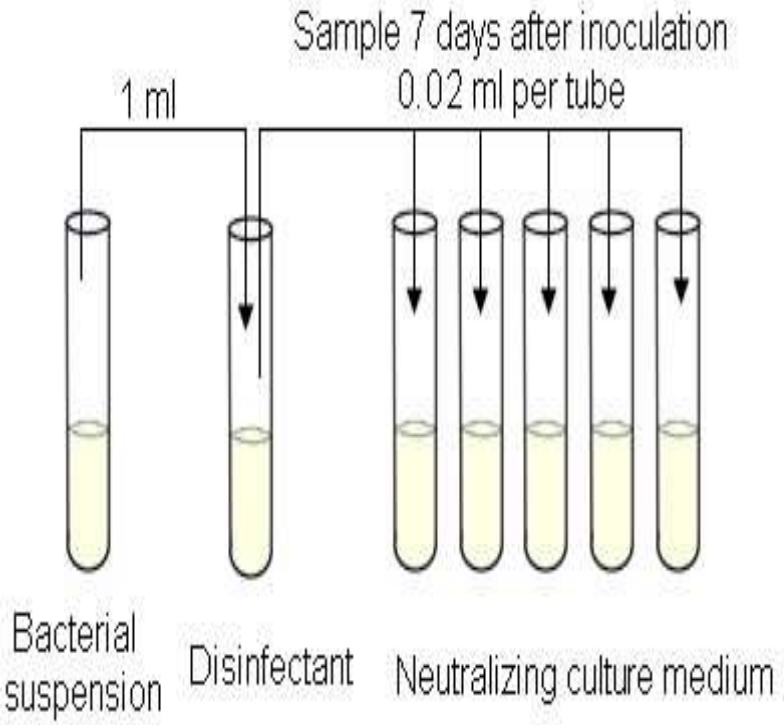
Sufficient disinfectant solution is prepared for two tests:

1- One portion is inoculated immediately and tested for growth after holding for seven days at room temperature.

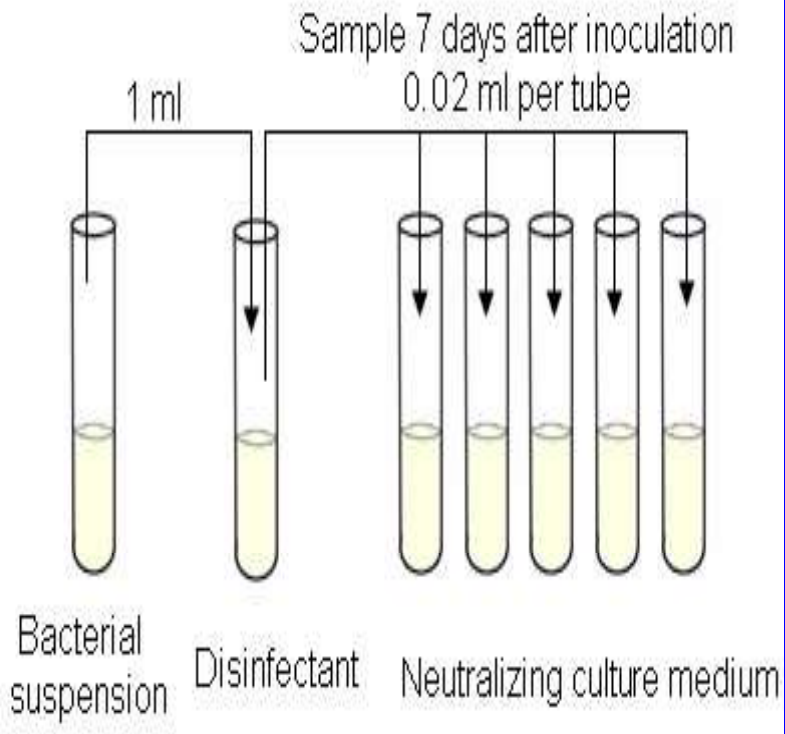
2- The other portion is kept at room temperature for seven days and then inoculated with a freshly prepared suspension of test organism.

If growth is detected, a higher concentration of disinfectant must be tested in the same way.

**Stage 1: Disinfectant freshly diluted**




**Stage 2: Disinfectant dilution stored 7 days before inoculation**



# Practical tests

## *Principle:*

- The practical tests **under real-life conditions** are performed after measuring the time-concentration relationship of the disinfectant in a quantitative suspension test.
- The objective is to verify whether the proposed use dilution is still adequate in the conditions under which it would be used.
- The best known practical tests are the **surface disinfection tests.**

# Surface disinfection tests

- Assess the effectiveness of the disinfectant against surface-adhered microorganisms.
- The test surface (e.g. a microscopic slide) is contaminated with a standardized inoculum of the test bacteria and dried.
- Then a definite volume of the disinfectant solution is distributed over the carrier
- After the given exposure time the number of survivors is determined by impression on a contact plate or by a rinsing technique, in which the carrier is rinsed in a diluent, and the number of bacteria is determined in the rinsing fluid.

## Difference between a carrier test and a surface disinfectant test

**Carrier test:**

- The carrier is submerged in the disinfectant solution during the whole exposure time

**Surface disinfectant test:**

- the disinfectant is applied on the carrier for the application time and thereafter the carrier continues to dry during the exposure.
- Surface tests can reflect in-use conditions like contact times, temperatures, use-dilutions, and surface properties.

# Surface Time kill Test

- A 24 hour culture in nutrient broth culture is prepared. A volume of microbial culture is placed onto the center of each of a number of sterile test surfaces.
- This inoculum can be spread over the sterile test surface in a circular pattern to achieve a thin, uniform coverage with the test microorganism.
- To measure initial microbial concentrations, one or more untreated, inoculated test surfaces are harvested.
- The remaining inoculated test surfaces are treated with the disinfectant, each for a different length of time.
- Immediately after the treatment times have elapsed, the test surfaces are placed into a solution that neutralizes the disinfecting action of the product,
- microorganisms surviving treatment with the disinfectant are cultured and enumerated.

# In-use test

***Principle:***

- A simple to use test was described by **Maurer in 1985** that can be used in hospitals and laboratories to detect **contamination of disinfectants**.
- A 1 ml sample of the disinfectant is added to 9 ml diluent which also contains an inactivator.
- Ten drops, each of 0.02 ml volume of the diluted sample are placed on each of two nutrient agar plates.
- One is incubated at 37ºC for three days and the other at room temperature for seven days.
- Five or more colonies on either plate indicate contamination.

# Testing schemes

**1- The first phase**

- concerns laboratory tests in which it is verified whether a chemical compound or a preparation possesses antimicrobial activity:
- For these preliminary screening tests essentially **quantitative suspension tests** are considered.

**2- The second stage**

- Still carried out in the laboratory but in conditions simulating real-life conditions. Not disinfectants, but disinfection procedures are examined.
- It is determined in the **practical tests** in which conditions and at which use-dilution after a given contact time the preparation is active.

**3- The third phase**

- Comprises the **in-use tests**.
- In these tests it is verified whether, after a normal period of use, germs in the disinfectant solution are still killed.

# Bactericidal tests

A bactericidal test must include the following sequence of steps:

1. The test organism is exposed to a suitable concentration of the disinfectant

2. Samples are taken at specified times and added immediately to a culture medium containing the appropriate disinfectant inactivator

3. The treated samples are cultured for surviving microorganisms.

# Test organisms

- Specified strains of *S. aureus, P. aeruginosa, P. vulgaris* and *E. coli* are usually recommended.
- A synthetic broth is recommended for preparing a series of subcultures to be used in the tests.
- The 24-hour broth culture may be used without further treatment; however, it is usually filtered (to remove slime) and centrifuged. The washed bacteria are resuspended in hard water to which autoclaved yeast or serum may be added to simulate dirty conditions.
- Finally, the suspension is shaken with glass beads on a vortex mixer and a viable count is set up immediately before performing the test.

# The disinfectant

- The concentration or dilution of the disinfectant to be tested may be based on manufacturer's recommendations.
- The solutions should be prepared on the day of test.
- Distilled water or standard hard water is used to make dilutions. Tap water is unsuitable because it may contain chemicals that may precipitate with some disinfectants.

Thank you .....