of Cellulose acetate and Cellulose nitrate.

## Disinfectant

It is the process of destruction or removal of microbes and reducing them to a level not harmful to health.

If the object is inanimate (Non living) such as Benches, chairs then the chemical agent is K/a disinfectant.

If the object is animate (living) such as human body how a chemical agent is used as Antiseptic

Disinfectant may be bactericidal and bacteriostatic

Classification of Disinfectants — classification, Acid, Base

1) Acid Alkali :- Generally strong acid and alkali kill the bacteria but weak acid weak base inhibit their growth

2) Halogen :- Cl, Br, I, F are used as Germicidal

3) Heavy Metal :- Hg, Ag, Cu are most widely used heavy metal they combine with cellular protein of Bacteria and acts as antimicrobial

4) Alcohol :- They facilitate protein denaturation 70% ethanol is used for disinfection ethylalcohol and propyl alcohol are frequently used as chemical reagent for disinfection

Aldehyde :- 10% formalin sol$^{n}$ is a standard chemical disinfectant Aldehyde produces Bactericidal, sporicidal and Viricidal action.

Dyes :→ It has activity against Mycobacterium Tuberculosis, fungi and Viruses
It is used as 2% Buffer sol^n
It can
- Dic
- Bld
- Dyes

} from chemical method

Quaternary Ammonium compound :→ widely used for control of microbes growth on floor, wall, nursing home and other public places.

Detergent and soap :→ These are widely used as surfactant wetting agent and emulsified
These are classified into :-
- Anionic
- Cationic
- Non Ionic
- Amphoteric

Most important antibacterial reagent are Cationic Surfactant
Acterimide, Benzalkonium chloride

Mechanism of Action :-
Alteration of membrane permeability
Damage to protein
Rupturing of cell membrane
Damage to nucleic acid
Interfere with metabolic pathway

Factor Influencing Disinfection

1) Conc$^{n}$ of Disinfectant :- Conc$^{n}$ of disinfectant and disinfection effective is related to each other exponentially but not linearly

2) Temperature :→ On increasing temp$^{r}$ the lethal effect of bacteria will increase

3) Time of contact :- Sufficient time of contact is required for disinfectant to exert its action

4) pH of environment :→ pH at 6-8 is optimum for growth of bacteria and decline on either side will effect bacteria growth

5) Chemical structure of Disinfectant :-
Substitution of alkyl chain upto 6-C inland is para position to phenoles ↑ OH group ↑ the activity but greater than 6-C inland ↓ es the activity of phenol
Halogination ↑ se the activity of phenol
Nitration ↓ se the activity of phenol

6) Type and number of microorganism present :→ Number and type of microorganism also effect disinfaction
eg:→ Vagitative bacteria are rapidly kill while bacterial spores are difficult to distroy

7) Interfairing substance in the environment :→ Material such as blood, milk, food residue if present is small amount may decrease effictiveres of disinfactant

8) Synergism and antagonism :→ Synergistic effect if shown by to anti microbial agent will increase the disinfactant activity, while antagonistic effect will decrease the activity of disinfactant.

## Evaluation test of disinfactant :—

This is the process of stablising documented evidences that a disinfectant will remove or inactivate known or possible pathogen from sample.

### Method for evaluation

Carrier test :→ A carrier such as thread is contaminated by submergen in a liquid culture of test organism then carrier is dried and broad in contact with

disinfectant for a given exposure time
If no growth is seing that disinfectant is more effective, If growth of microorganism is observed this intigates disinfectant less effective

No growth → more effective
Growth → less effective

**Capacity test :→** This test determind the capacity of disinfectant to retain its activity in the presence of increase contamination level.
Bacterial suspension is added untill disinfectant capacity to kill is adsosted
Best known capacity test is
**Kelsey - sykes test :—**

organism — ① S - aureus ② E coli
③ Proteus Vulgaris
④ Pseudomonas aruginosa

Three successive addition of bacterial suspension are made to determine Concentration of disinfectant that will be active / effictive in dirty Condition

If all Microorganis. are Killed → effective test
" " Not Killed → less effective.

Suspension test :→ In this test sample of bacterial culture is suspended in to disinfectent solution.

- These are classified into three types.
  - I- Qualitative test
  - II- Quantitative test
  - III- Phenol coefficient test

Qualitative test :→ Loopful of bacterial suspension is broth in the contact with the disinfectant.

Result :-

Growth :- Disinfectant is ineffective

No growth :- Disinfectant is Effective

Quantitative test :- In this test number of serviving Organism(B) is counted and compair to original inoculum size (A)

- Microbial effect ⟶ $\log A - \log B$
  - ME > 5 ⟶ 99% germs are killed

Phenol Coefficient test :→ This test is used for evaluating effectiveness of disinfectent phenol

- Staphylococcus aureus and Salmonella typhi are used in this test
- Dilution of phenol and experimental disinfectant are inoculated with Staphylococcus aureus and incubated with 20-37°C for 2-3 days and compaired with each other
- Two methods are used
  - I) Rideal Walker method
  - II) Chick Martin test

Practical test :-> This test uses real life condition

- The test surface example microscopic slide is contaminated with standradized inocula the test bacteria and dried
- Definate volume of disinfectant of sol$^n$ is distributed over the sol$^n$
- After the given exposure time the number of survival microbes the determined
- This test is carried by to verify if the selective diluted still evalute under the condition in which is must be used
  1. Lab
  2. real life
  3. field

Sterility testing :→ Are performed to check wheater are Sterilize pharmaceutical product is free from contaminating micro-organism

steps involves in sterility testing :→

I) Selection of the sample size
II) Selection of the Quantity of product
III) Media requirements
IV) Test microbes
V) Method of testing
VI) Result and observation

Selection of the Sample Size :→

No of items in a batch | Bulk solid | Min no of items recomended to be listed

< 4 containers — Each container will be evaluating

greatter than 4 and < 50 → 20% or 4 containers which ever is greater

> 50 → 2% or 10 containers whichever is greater

For Parenterals :→

If No of

< 100 containers → 10% or 4 will whichever is greater

> 100 but less than < 500 → 10 containeres

> 500 — 2% or 20 containers whichever is greater

opthalmic and ~~non~~ other non injectable preparation :-

< 200 container — 5% or 2 containers whichever is greater

> 200 container → 10 containers will be evaluated

(2) Selection of Quantity of product :→

Quantity per container Qty to be used for testing

In case of solid :→

< 50 mg → whole container tested

> 50 but less < 200 mg — half content but not > < 50 mg <

> 200 mg → 100 mg will be testing

In case of liquid :→

< 1 ml → whole content are checked

1 – 4 ml → half content

> 4 but < 20 ml → 2 ml

> 20 but < 50 — 5 ml

> 50 ml — 10 ml

Culture media requirement :→

FTM ( Fluid thioglycolate medium )

L – 16

L – cystein, then agar sodium chloride glycose mono hydrate ( anhydrous ), yeast extract Pericreatic digest of caesin, sodium thioglycolate, resazurin, sodium solution, water.

Alternative thioglycolate medium ( ATM ) :→

composition :–

Lcystein, sodium chloride, glucose monohydrate ( anhydrous ), yeast extract, pericreatic digest of caesin, sodium thioglycolate, water.

Soyabean Caesin digest medium ( SCDM )

OR

triptosoyabroth

composition

Pericreatic digest of caesin, papic digest of Soyabean meal, sodium chloride, dipotassium hydrogen phosphate, glucose mono hydrate ( anhydrous ), water.

i) FTM is primarily used for culture of anerobic bacteria and is used with clear fluid products.

ii) ATM is used for turbid of viscial product and is used for culture of anerobic bacteria.

iii) SCDN : is used for turbid product and for culture of both fungi and aerobic bacteria.

Test microbes :→

FTM :→ Styphylococcus aureus, Clostridium sporogenes, Pseudomonous aeruginosa

Temp$^r$ - 30-35°C for 3 days

ATM :→ Clostridium sporogenes, Bacillus subtilis

Temp$^r$ :- 30-35°C for 3 days

SCDM :- Candida albicans, Aspergillus, Brasilienses

Temp$^r$ :- 20-25°C for 3 days

* If test sample is bacteriostatic or fungistatic a suitable neutralizing agent are used.

| Antimicrobial agent | Inactivating agent |
|---|---|
| Penicillins | Penicillinase |
| Cephalosporin | Cephalosporinase |
| Sulphonamide | Paraaminobenzoic acid |
| chloramphenicol | Acetyl transferase |

Test Method :→

Method A

Membrane filtration method :→

Membrane has nominal pore size not greater than 0.45 microns micron and diameter of approx 50 nm

→ Cellulose nitrate filter are generally used.

→ This method involves filtration of sample through membrane filter.

- The filtration is carried out in vacuum and after filtration membrane is cut into two half and is placed in test tube containing media.
→ Incubate for not less than 14 days
→ This method is used for oily preparations, ointment that can be put into water, liquid product where Vol$^m$ in a container is 100 ml or more.

Method B :-

Direct Inoculation Method :→

This method involves inoculation of required volume of a sample in two test tubes containing culture medium FTM and SCDM

Volume of the preparation under examination is not more than 10% of the medium volume.

Incubate the media for more than 14 days.

# Result Interpretation after Incubation

After Incubation

If growth Observe

Containers are reserved and retest is performed

Growth observe

If distinguisable from reserved that growth in first containers

retest

No growth

Sample pass

Growth

Sample fail

No growth observe

If not distinguishable

Sample fail test.

If growth is not observe

Sample passes sterility test