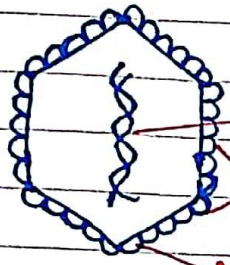
PHARMACEUTICAL MICROBIOLOGY

UNIT => 3
PART => 1

Viruses :

- Viruses is the smallest injective agent and seen by the ultra microscope.

-> structure of virus :

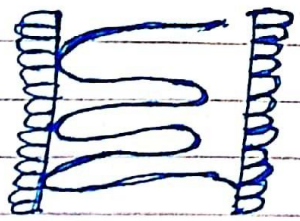


-> Nucleic acid
capsomere (made up of protein)
-> capsid (single capsomere)

-> classification based on the Morphology :

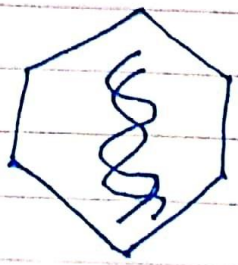
i) Helical symmetry
ii) Icosahedral symmetry
iii) complex symmetry

* Helical symmetry : -> Tobacco mosaic virus, Rabies virus

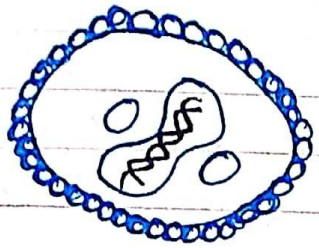


* Icosahedral symmetry :

↓
Poliovirus,
Herpes simplex virus



* Complex Symmetry :- → Bacteriophages.



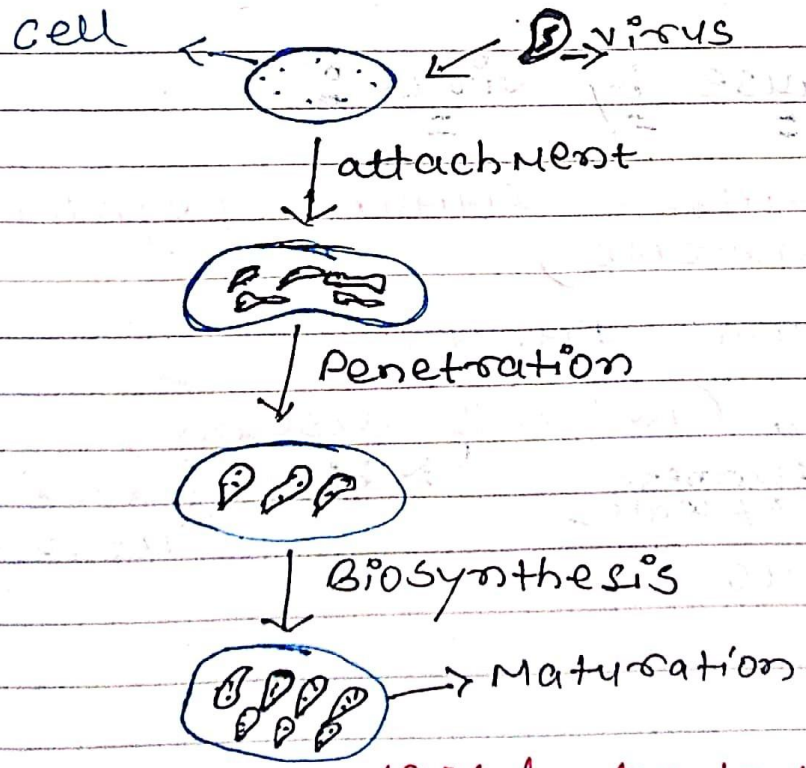
→ classification based on Host cell :-

i) Animal (viruses) → RNA/DNA

ii) Plant (viruses) → RNA

iii) Bacteria (viruses) → DNA

* Replication of virus :-



after divided, due to less space it burst & travel in our body.

- virus is a latin word its mean 'poison'
- study of virus called 'virology'.
- characterstics : ultra micro, belong from parasites family, non-cellular.
- when virus contact with living cell, then virus is activate another they are deadly form
- when virus is activated they can replicate easily due to the RNA / DNA is present in virus.
- If other body touch that virus body then they will get infect also.

★ Disease cause by virus :

i) AIDS (Acquired Immune Deficiency Syndrome)
↓
HIV (Human Immuno virus)

ii) Hydrophobia (Rabbies disease)
↳ fearness of water
↳ bite by dog or monkey.
↓
Phepdovirus

iii) Polio
↓
Polio virus.

★ Virus cultivation :

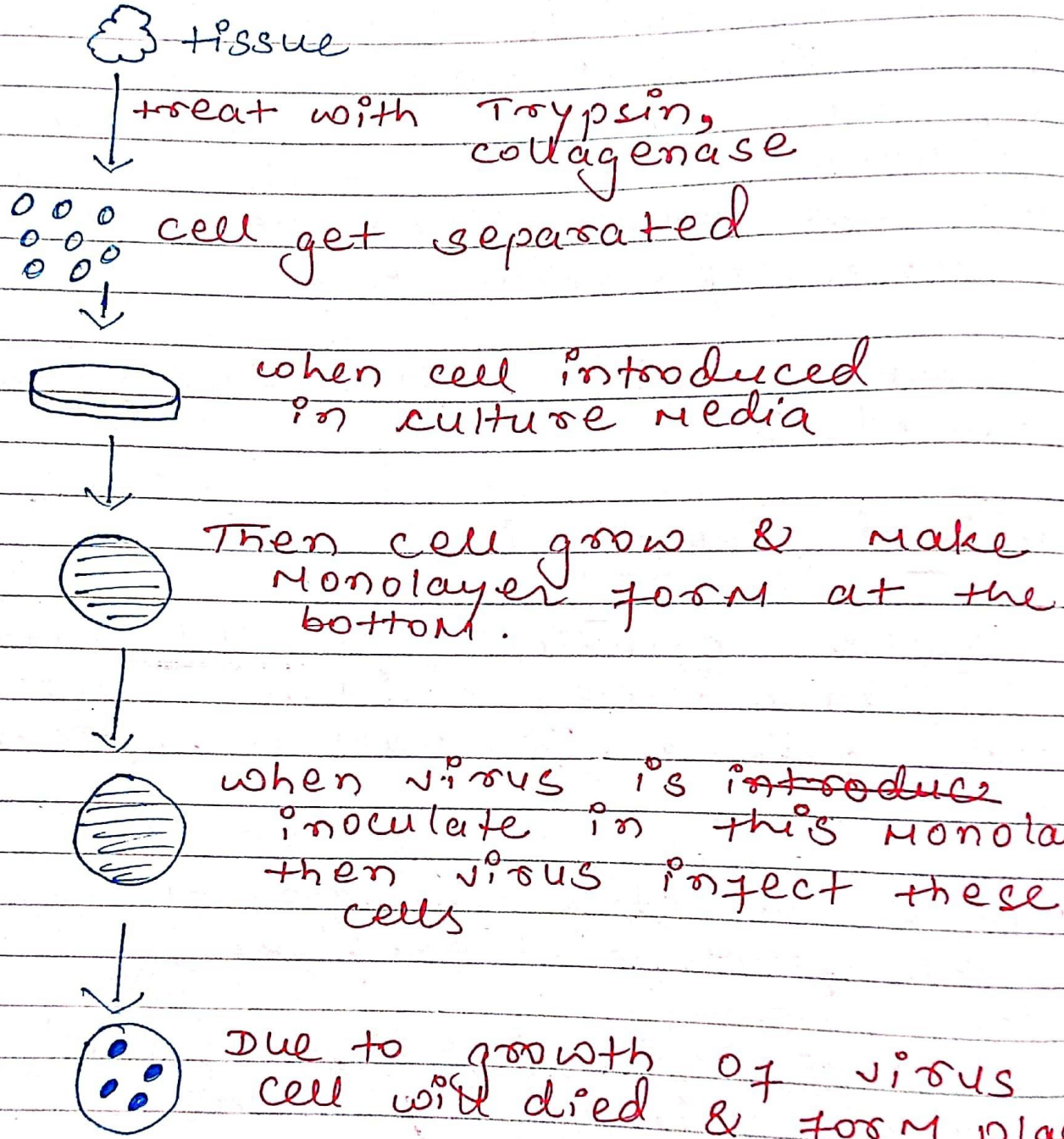
- viruses are obligate intracellular parasite so they depend on host for their survival. They cannot be grown in non-living culture media or on agar plate, they must require living cells to support their replication

→ viruses can be cultured these methods :

i) Animal Inoculation

- viruses sometime are cultivated in laboratory animals such as mice, guinea pig, hamster, rabbits etc.
- These selected animals should be free from disease then,
- viruses can inoculated to these animals by intranasal route or intracerebral.
- After inoculation, virus multiply in host & develop disease. The animals are observed symptoms of disease & death. then the virus is isolated & purified from the tissue of these animals.

ii) Cell culture



Fungi

- These are the member of Eukaryotic organism that includes microorganism such as yeast, mold, mushroom, etc.
- Generally they are aerobic in nature.
- They were first identified by 'Agostino Bassi' (bacteriologist) in 1835.
- The study of fungi is called as Mycology.

→ Comparision of fungi & Bacteria :

Characteristic	fungi	Bacteria
i) cell type	Eukaryotic	Prokaryotic
ii) optimum ~~ph~~	4-6	6.5-7.5
iii) cell membrane	sterol present	sterol absent except Mycoplasma
iv) oxygen requirement	Aerobic	Aerobic & Anaerobic
v) cell wall component	chitin, Polysaccharide	Peptidoglycan & polysaccharide

Classification of fungi

- Morphological
  - → yeast
  - → yeast like fungi
  - → Moulds
  - → Dimorphic fungi
- Taxonomical
  - → zygomycetes
  - → Ascomycetes
  - → Basidiomycetes
  - → ~~Dutromycetes.~~ Deuteromycetes
  - → Deuteromycetes

* Morphological classification :

i) Yeast :

- They are round, oval, unicellular fungi
- It contains only single cell.
- Generally these are aerobic.
- They generally survive in 32 - 37°C.

eg => saccharomyces, cerevisae, Cryptococcus neoformans.

ii) yeast like fungi :

- candida albicans is the good example of yeast like, In this, the bud remains attached to mother cell & elongateds, followed by repeated budding & forms • chain of elongated cell of fungi form pseudohyphae.

iii) Molds :-

- It contains multiple identical nuclei & grows in the form of mycelium or hyphae of filaments.
- It gives fuzzy appearance on the surface of media & forms black, green, white or pink colour.
- They are strictly aerobic.
- They generally grow under 22-28°c.
- They can reproduce either by sexual or asexual reproduction.

eg => Aspergillus Niger, Aspergillus fumigatus.

iv) Dimorphic fungi :-

- They are exist in two forms whereas yeast or molds.
- The molds like forms produce vegetative & yeast like forms reproduce by budding.

eg => Mucor rouxii, Histoplasma capsulatum.

* Taxonomical classification :

i) Zygomycetes :

- They form asexual spores i.e, sporangiospores & sexual spores i.e, oospores, zygospores.

ii) Ascomyces :

- They forms ascospores in a sac called ascus by sexual process.

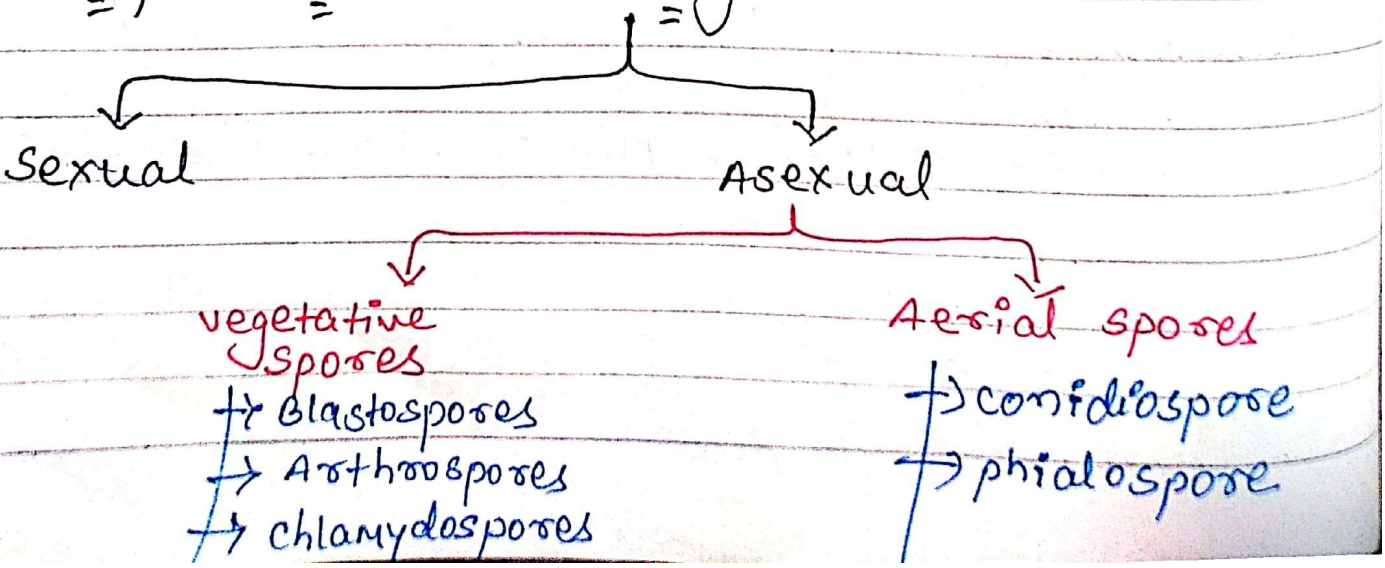
iii) Basidiomycetes :

- They form sexual spores called basidiospor -es on the tip or surface of basidium.

iv) Deuteromycetes :

- They donot produce sexual spores.
- Most of fungi of medical importance belong to this class.

Reproduction in fungi



→ Sexual reproduction in fungi :

- In sexual reproduction they form two different cell (male & female cell) called Plasmogamy followed by fusion of two nuclei called karyogamy as a result zygote is formed with formatn of sexual spores.

→ Asexual reporeduction in fungi :

- fungi which are medically importance, mustly produce by this method. They produce different asexual spores by mitosis.

=> vegetative spores :

i) Blastospores : formed by budding process from parent cell as in the yeast.

ii) Arthrospores : This is the rectangular or cuboidal thick wall spores which is formed by fragmentation of the end of hyphae.

iii) chlamydospores : These are thick walled resting spores which develop from hyphae after long dormacy.

=> Aerial spores :

i) conidiospores or conidia :

- Microconida -> spores is small & singly.
- Macroconida -> large in size, multicellular

ii) phialospores : modified conidia, borne at tip of flask shaped specialized ~~conidiospores~~ candiophore called phialide.

II Cultivation of fungi :

- Sabouraud agar is a type of agar growth media containing peptone used to cultivate fungi.
- It was develop by raymond Sabouraud in year 1892.
- The standard temp. for incubation of fungi is 30°C in humidified environment for 21 days.
- After this incubation for 21 days the colonies of fungi form in culture media.

MICROBIOLOGY

UNIT → 3
Part → 3

classification and mode of action of disinfectants

- Disinfectant is a process to remove the infection by reducing the number of microorganism present on any site or article.
- Disinfectant not necessarily eliminates all microorganisms, but it can reduce the number of microorganism to such a limit, that they can not cause any infection.
- It is not complete or 100% removal of microbial cells.
- It has very less effect on spores.
- Disinfectant : These are chemical which are used for the purpose of disinfection
- Antiseptic : Antiseptic is the disinfectants, which are applied on skin or any living tissue, & this process is called Antisepsis.

classification of disinfectants :

Based on consistency

Liquids
- Alcohol
- phenol
- Aldehydes
- Halogens
- Acids & Ester

Gases
- formaldehyde vapours
- Ethylene di-oxide
- propylene oxide
- β- propiolactone

a/c to effectiveness on bacterial cell, viruses & spores.

* Based on Spectrum of activity

Low level	Intermediate level	High level
• Quat. Ammonium compounds • Idophores • Phenols	• Alcohols • chlorine • Phenols	• Hydrogen peroxide • Aldehydes • Glutaraldehydes.
These chemicals kill - vegetative microbial cell - some fungi - few viruses - no effect on spore	• kill vegetative cell • all fungi • some viruses • Not effect on spores	• kill vegetative microorganism • all fungi • all viruses (inactivate) • Not effect against spores.

* Based on mode of action

- The classification divides the disinfectants on their mechanism of action and site of action to destroy the microbial cells.

→ Act on cytoplasmic membrane → Quat. Ammonium compounds, Phenols, alcohol.

→ Denaturation of cellular protein → Aldehydes, Halogens.

→ Oxidation of cellular contents → Hydrogen peroxide, Ethylene Di-oxide.

→ Alkylation of cellular content → formaldehyde, Beta propiolactone, Glutaraldehyde.

Disinfectants	Mode of Action	Example
i) Alcohols :	Membrane damage, protein denaturation, cell lysis	: Ethanol, propanol, Benzyl alcohol, chlorobutanol.
ii) Aldehydes :	React with amino acids (inhibit transln, transcptn)	: formaldehydes, Glutaraldehydes.
iii) Biguanides :	rupture cell membrane & change the cell permiability	: chlorhexidine
iv) Halogens :	Interation with thiol & amino groups lead to protein denaturation	: Hypochlorite, Iodine, Idophores.
v) oxidizing agents :	oxidization of cellular component	: Hydrogen peroxide.
vi) Quaternary Ammonium compound :	cell membrane damage, loss of cytoplasm, at high conc. cause cell coagulation	: Benzethonium chloride, cetrimide, Benzylconium chloride.
vii) organic Acid esters :	cause cell lyses, membrane damage, cytoplasmic leakage	: Methyl, Ethyl, propyl, butyl parabens.

MICROBIOLOGY

UNIT ⇒ 3

PART ⇒ 4

factors influencing disinfection :

i) Concentration :

- concentration of disinfectant : $\propto$ Rate of disinfection
- concentration of disinfection should not be too less & also should too more.
- It should be 'optimum'.

ex → The optimum conc. of phenol is about 1%

ii) Temperature :

- As the Temperature of disinfectant ↑se $\Longrightarrow$ Rate of disinfection ↑se
- Rate of disinfection normally ↑se with temp.

iii) Time of contact :

- sufficient time of contact must be allowed for the disinfectant to exert its action.

iv) pH :

- A change in pH during the disinfectant process can affect the growth of microbes.

- A pH of 6-8 is optimum for the growth of many bacteria and the rate of growth declines on either side of this range.

- Therefore disinfactant should have pH range other than 6-8.

v) Surface Tension :

- The contact b/w aqueous soln of disinfectants is increased if they have surfectant property.

vi) formulation of the disinfectant :

- Effectiveness of chlorhexidine and quaternary ammonium compounds may be greater in 70% alcohol than in aqueous soln.

* Evaluation of Disinfectants :

- Evaluation of Disinfectants referred to check the ability or efficacy of any disinfectant against specific microorganisms to establish its effectiveness.

- There are many methods for evaluation of disinfectants.

- Some major methods of evaluation are :

→ Phenol coefficient test (Rideal - walker Test)
→ Chick Martin Test.
→ Kelsey Sykes Test.

i) Phenol Coefficient Test :

- In Phenol coefficient Test, any test chemical (disinfectant) is compared with phenol for its anti-microbial activity.

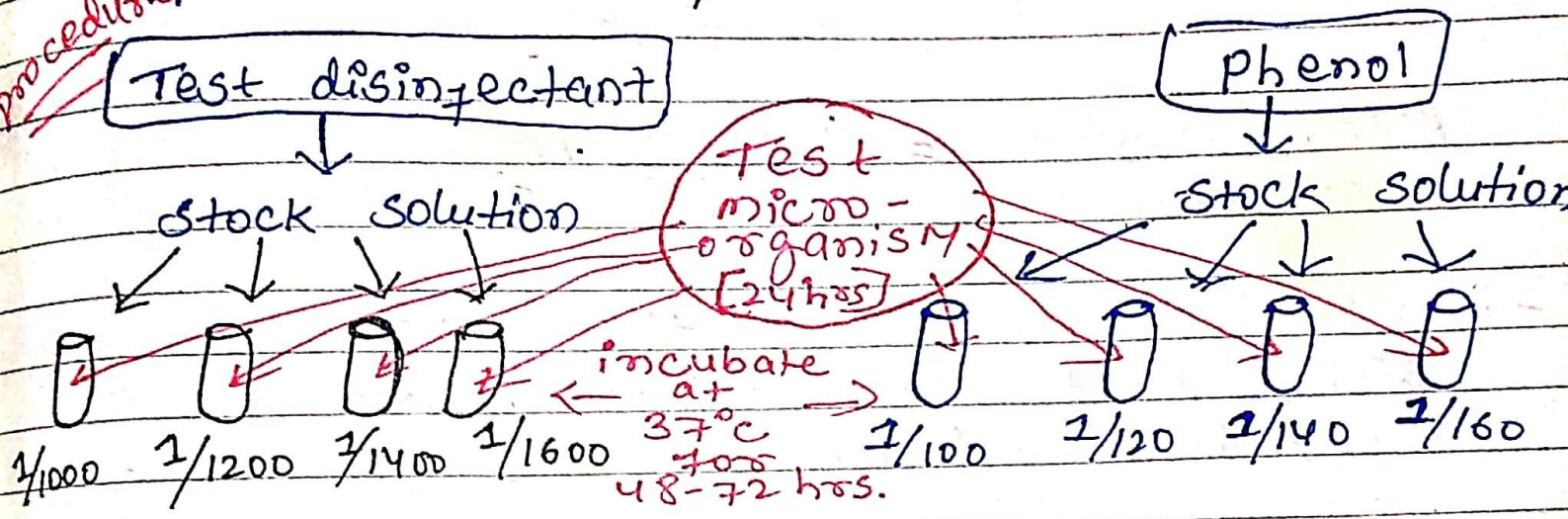
- In this test, we use any of these three microorganism :
  - Salmonella typhi
  - Staphylococcus aureus
  - Pseudomonas aeruginosa.

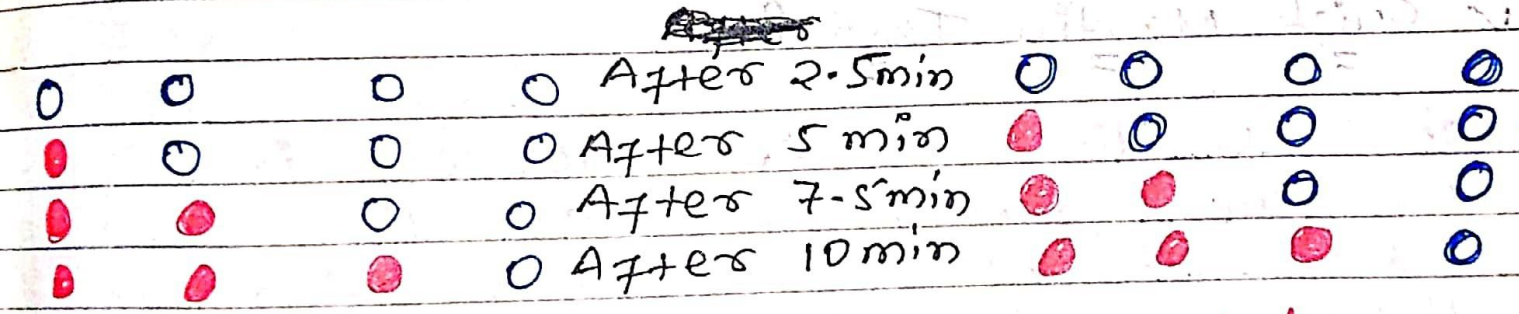
- The result is shown in the form of phenol coefficient.

dilution → 5 ml.

- If the phenol coefficient if less than 1 then test disinfectant is less effective than phenol.
- If phenol coefficient if greater than 1 then test disinfectant is more effective than phenol.

procedure





[Note: By help of inoculating loop take sample & place in recovery culture media & check which dilution disinfectant kill microorganism & how much they take time]

calculate

Rideal walker coefficient = Higher conc. of test disinfect. that kill microorganism in 7.5 min but not kill in 5 min / High conc. of phenol that kill microorganism in 7.5 min but not kill in 5 min.

Rideal walker coefficient = $\frac{1200}{120} = 10$.

→ This indicates that the test disinfectant is 10 times more efficient than phenol.

ii) Chick Martin Test :

- Chick Martin Test is performed in the much similar way as the Rideal - walker Test, but with a little variation.

- In this evaluation, the disinfectant are evaluates in the medium containg 3% organic matter (dried human faeces or dried yeast).

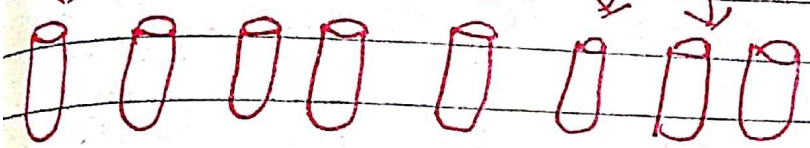
chick-Martin coefficient = conc. of test disinfectant that kills microorganism / conc. of the phenol that kills microorganism.

3gm organic Matter + 100ml water

↓

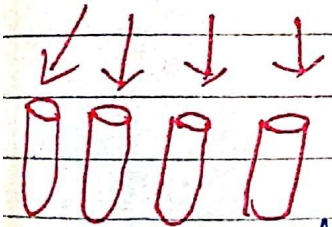
3% suspension of organic matter

Microorganism (2ml) + 3% organic Matter (48ml) ← Microorganism 24hrs old culture

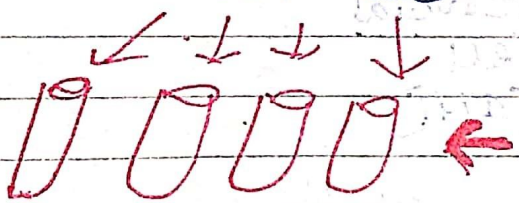


2.5 ml in each test tube

Different Dilutions phenol 2.5ml each test tube



D/t Dilution Test disinfectant 2.5 ml each test tube



Contain 2.5 ml microbial suspension + 3% organic Material.

contact allow for 30 min.

Phenol & Microorganism

Test Disinfectant & Microorganism

↓

Transfer one loopful of reactn mixture to 10ml. recovery culture media & incubate on 37°C for 48 hrs.

↓

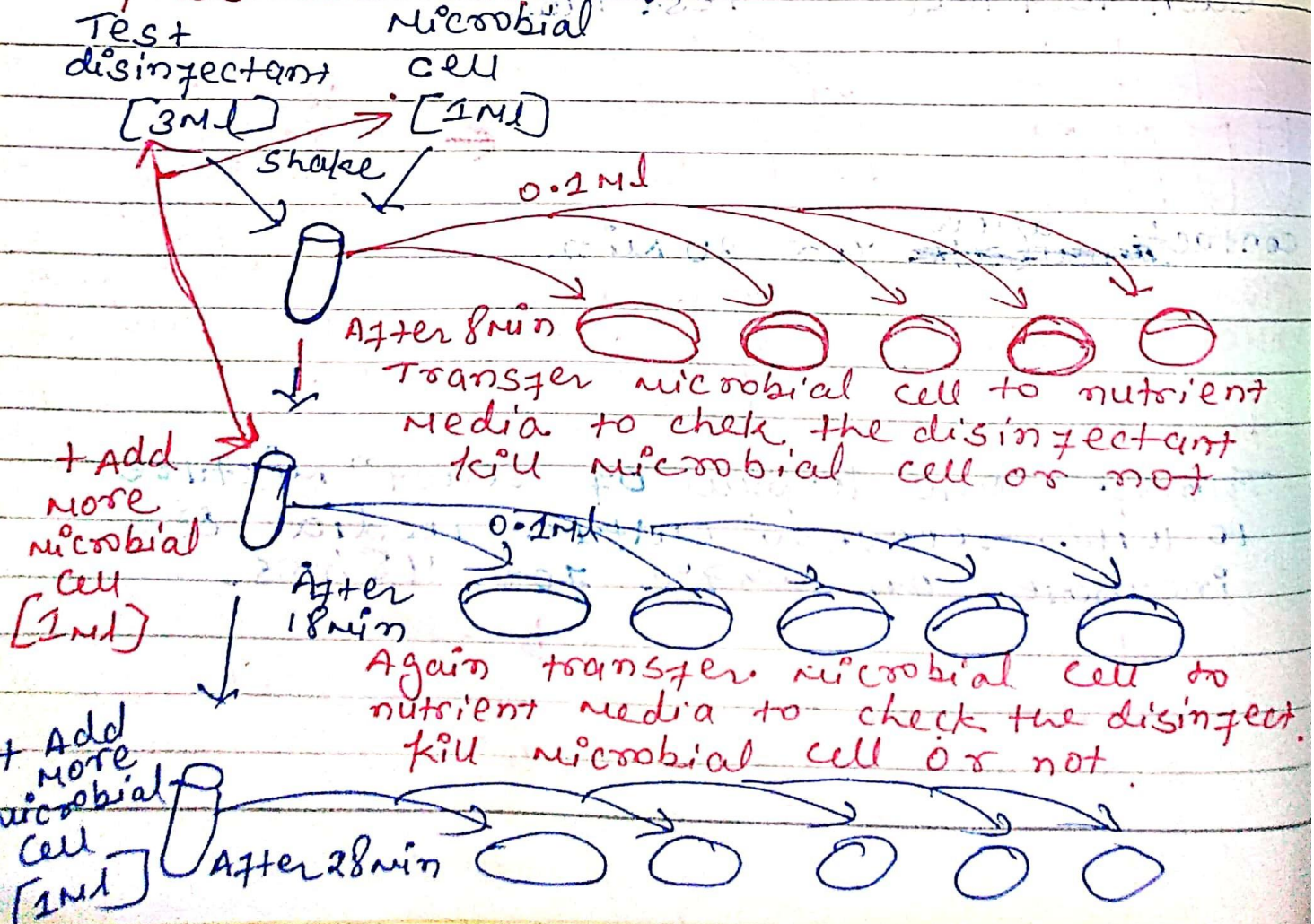
Observation that which conc. of phenol & test disinfectant kill microorganism

iii) Kelsey - Sykes Test

- Kelsey - sykes test is a triple challange test.

[Test disinfectant checked three times with successive addition of microbial suspension with the same conc. of disinfectant].

- The duration of test is 30 min.
- Any one microorganism is used for this test P. aeruginosa, P. vulgaris & E. coli.
- The result are reported as pass or fail :



STERILITY TEST

* principle : sterility test is performed for detection of micro-organism in the parenteral product.

- When a parenteral product is placed in a culture media. It provides temp., pH and nutrition to microorganism for their growth.

* culture media : Two culture media are used for sterility test.

A) Thioglycollate culture media :

- This culture medium is used for identification of aerobic and anaerobic bacteria.

B) Soyabean casein culture media :

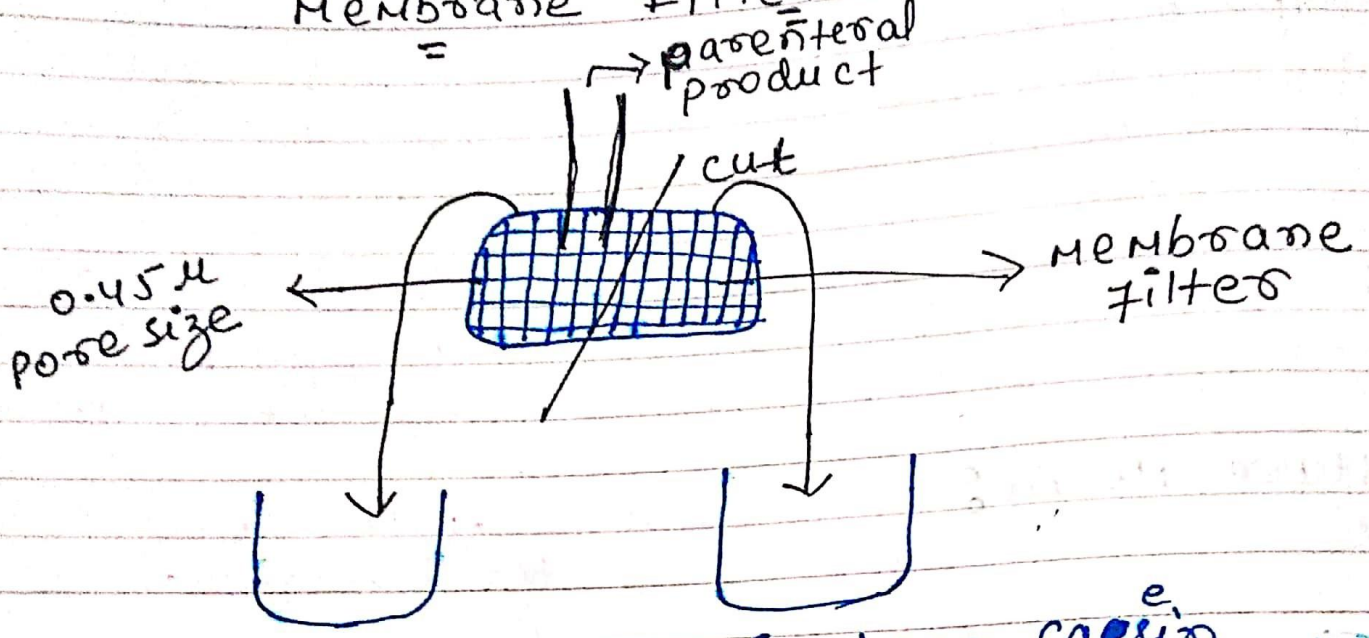
- This culture media is used for identification of fungi.

* Method => sterility is performed by two methods

A) Direct filteration method.

B) Direct Innoculation method.

Membrane filteration method



Thioglycollate: Soyabean casein

↓ ↓

30-35°C 20-26°C

↓ ↓

7 days 7 days.

Result =):

- filteration of sample under test through a membrane having normal pore size 0.45 μ.
- After filteration, membrane is removed from metallic holder & divided into two halves.
- The first half is transferred into thioglycollate filter media which is used for identification of aerobic & anaerobic bacteria

↓

- stand at 30-35°C for 7 days.

- second half transfer into soyabean casein filter media which is used for identification of fungi.
  ↓
  stand at 20-25°C for 7 days.
- observe the growth in media.

→ observation & Result : turbidity observe → fails.
start growth

- If there is no growth of micro-organism the parenteral product pass test of sterility.
- If there is growth of microorganism the parenteral product fails test of sterility.

if turbidity → fails observe
if turbidity → pass not observe

Direct inoculation

- Test tube ~~of~~ contain culture media
- Mix the sample which are test in the culture media
- Soyabean casein → incubate 20-25°C 14 days at
- thioglycollate medium → incubate 30-35°C 7 days atleast.