# Culture Media

## $O_2$ concentration

- Aerobic
- Anaerobic (reducing substances)
  - RCM (Robertson Cooked Meat)
  - Thioglycollate Broth

## Consistency

- Solid (2% agar)
- Semisolid (0.5–1% agar)
- liquid

## Constituents

- Simple/Basal media
  - Nutrient broth
  - Nutrient agar
- Special media (Special constituents)

- All

# Special Media

## Enriched
(help in growth of pathogens)

always solid

1) Blood → Blood agar, Chocolate agar
2) Serum - loeffler's serum slope
3) Egg - lowenstein Jensen

## Enrichment
(inhibit the commensals)

always liquid (stool sample - 4hrs)

1) Vibrio → Alkaline peptone water, Monsuri's peptone water
2/others - Selenite F broth (Salmonella Shigella) Tetrathionate Broth

## Differential
(differentiate organism)

1) MacConkey
2) CLED

## Transport
(keep organism alive)

1) Streptococcus - Pike's media
2) Gonococcus - Amie's Stuart
3) Stool - Sachi buffered glycerol saline (used earlier)

(except Vibrio & Campylobacter)

Now - Cary Blair Medium (all organisms)

Vibrio → Cary blair medium, VR media (Venkataraman Ramakrishnan medium), Autoclaved sea water

## Selective
(selective to pathogen)

Note:

acidic - red

inhibit gram +ve organisms

MacConkey agar - Nutrient agar + lactose + Neutral red + bile salts (Sodium taurocholate / Sodium deoxycholate) + NaCl

basic - colorless

swarming of proteus

CLED (Cysteine lactose Electrolyte Deficient agar) - Nutrient agar + Cysteine + lactose + Bromothymol blue

Cysteine → growth factor

acidic - yellow - LF

basic - blue - NLF

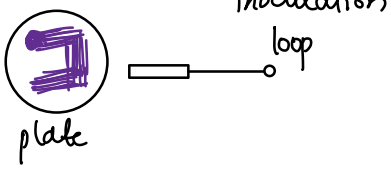
No Bile salt - gram +ve organisms grow, even fungi can grow

No NaCl - swarming of proteus inhibited

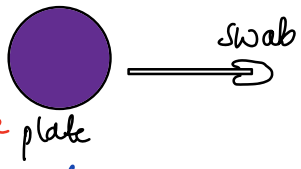
# Culture methods

## Routine

1) Streak




2) lawn

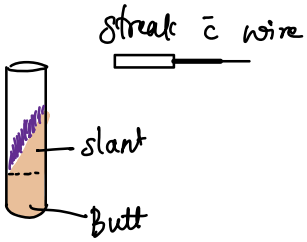



carpet culture

∴ used for AST

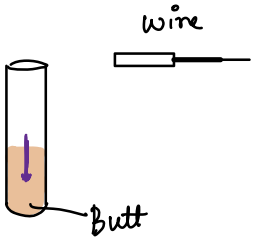
3) Stroke

slant tube media




4) Stab

Butt tube media




## Anaerobic

(remove $O_2$)

1) McIntosh Field jar
2) Gas pak
3) Glove box
4) PRAS (Pre-reduced Anaerobically sterilized)
5) Anoxomat (automated)

# IDENTIFICATION (Sample)

- Culture & Biochemical (Gold standard) → AST
- Molecular (Gene) → AST ↓ for MTB
- Immunology (Ag/Ab)

## Culture & Biochemical

- Catalase
- Oxidase
- MR
- VP
- Indole
- Mannitol motility
- TSI (Triple sugar iron)
- Citrate
- Urease

## Molecular

- NAAT (Best)
  - PCR
  - LCR
- Hybridization
  - FISH
  - RFLP (Restriction Fragment length polymorphism)

## Immunology

- Ag
  - ELISA
  - D-IFA
  - Rapid tests
- Ab
  - ELISA
  - I-IFA
  - Rapid tests

Note:

Most Bacteria

catalase ⊕ oxidase ⊖

MR ⊕ VP ⊖

- MR → Methyl Red
- VP → Voges Proskauer

## Triple Sugar Iron

3 sugars — GLS  Glucose, lactose, Sucrose

- fermentation (indicator color changes ∵ acid produced)
- $H_2S$ → Black colour
- Gas → Break the media or push the media above

Indicator : phenol red

yellow - acidic

red - basic

| | Salmonella typhi | S. paratyphi A | S. paratyphi B |
|---|---|---|---|
| Sugar fermentation | + | + | + |
| $H_2S$ | Speck of $H_2S$ | No $H_2S$ | full of $H_2S$ |
| Gas | No gas (anaerogenic) | Gas | Gas |

# AST & AMR

## Antibiotic Susceptibility testing

Methods of AST

- **Phenotypic**
  - Disc diffusion (mc) method
  - Broth dilution method (Gold std method)
- **Genotypic**
  - PCR
    - Gene
- **Automated**
  - Vitek uses broth dilution method

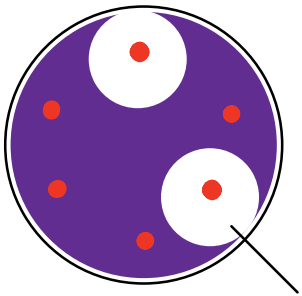
## Disc Diffusion method

**Kirby Baeur Method**

**Mueller Hinton Agar**

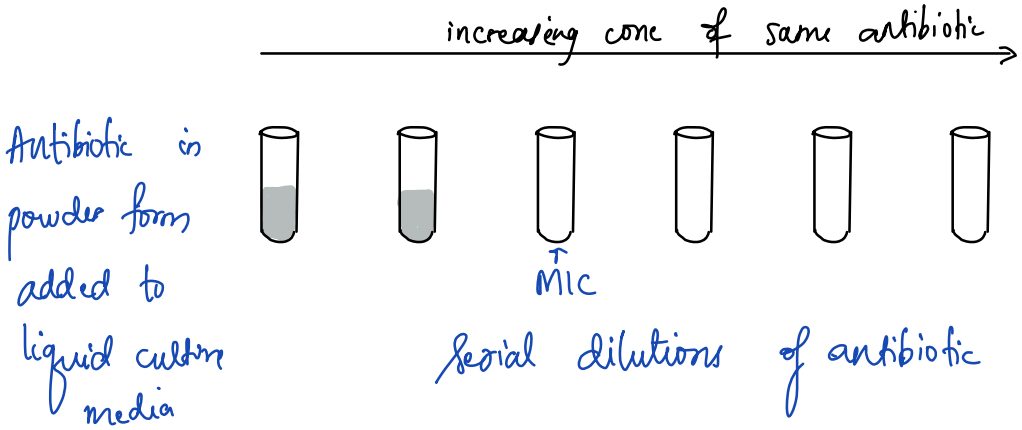
lawn culture ≅ swab

6 Antibiotic discs can be placed in plate (Diameter - 90mm)



Zone of inhibition (sensitive)

## Broth dilution




Add organism & incubate

if clear → sensitive to that dose

also tells minimum inhibitory concentration

gold standard - ∵ sensitivity & dose (MIC)

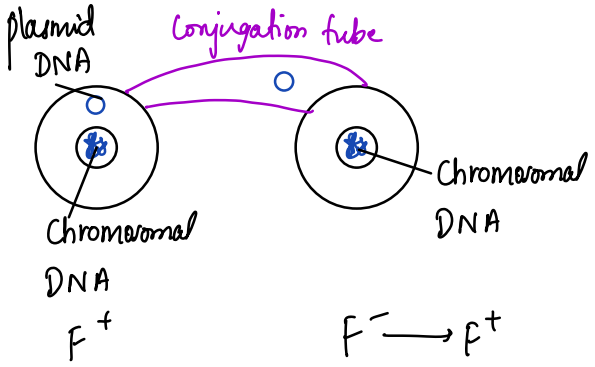
# Antimicrobial resistance

Gene transfer — all organisms except 1 → Mutation — MTB

Conjugation | Transduction | Transformation | Transposons

(mc method in all bacteria except Staphylococcus where transduction is mc)

## Conjugation




bacteria → $F^+$

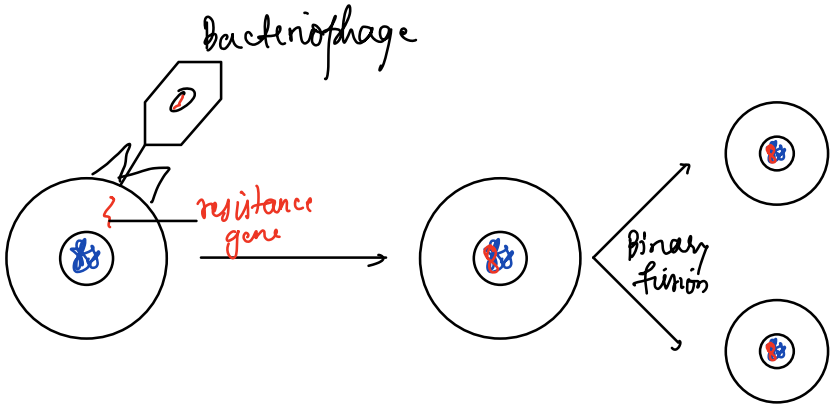
$\bar{c}$ plasmid (∵ fertility factor)

No plasmid → $F^-$

creates a copy of plasmid DNA & transfer it

resistance gene is usually present in plasmid DNA

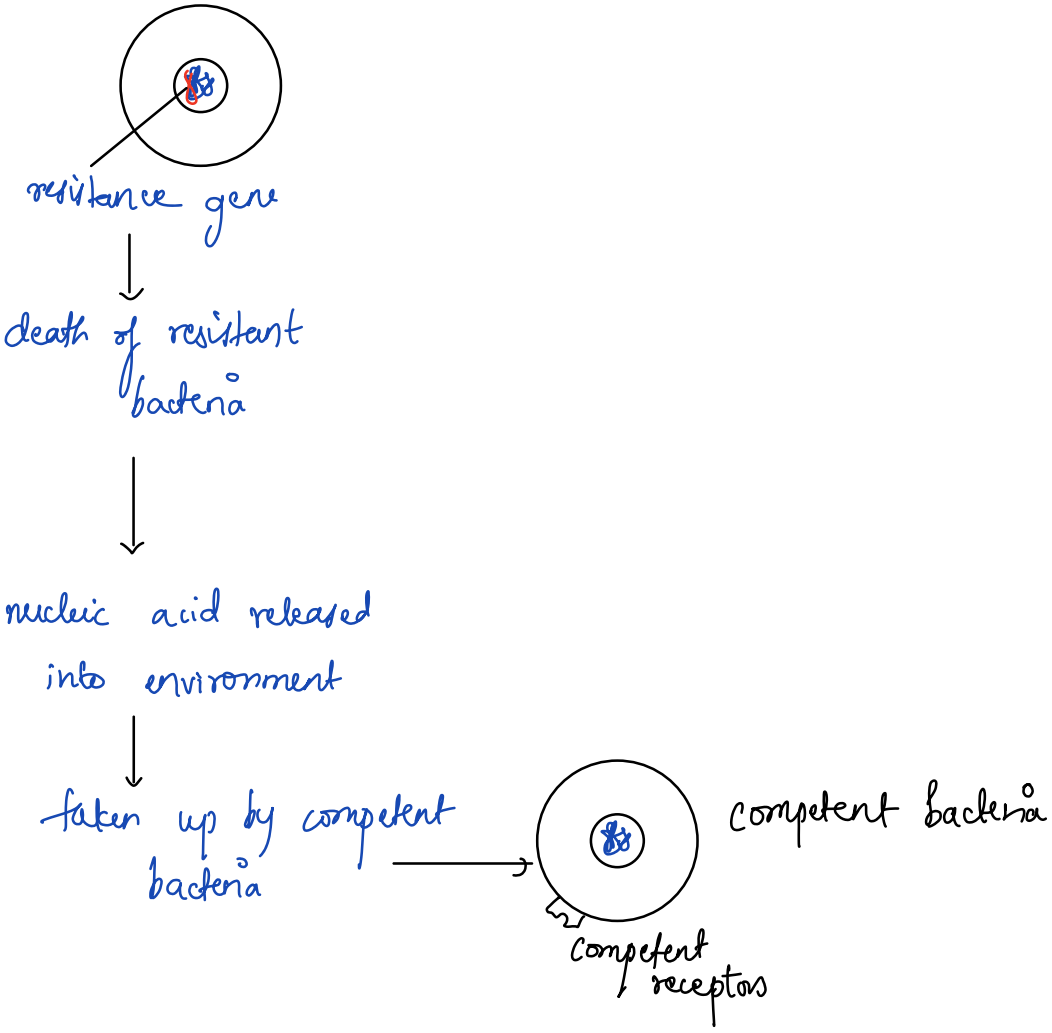
# Transduction




Transduction

| Lytic cycle | Lysogenic cycle / Temperate cycle |
| --- | --- |
| nucleic acid does not get incorporated | nucleic acid of bacteriophage gets incorporated |
| ↓ | |
| viral components synthesized | |
| ↓ | |
| lysis of bacteria | transfer of drug resistance |
| viral replication | |

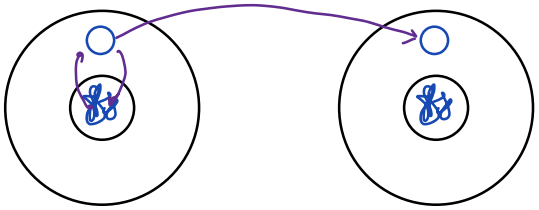
# Transformation



## Transposons

Small piece of nucleic acid

jumping genes



- can jump from plasmid to chromosomal DNA of same bacteria or b/w bacteria
- can transfer resistance gene