Culture: Culture medias

↳ provides approp. "Nutrients & pH." to the bacteria so that they can grow & multiply.

Classificatn of Culture media

I) (Based on Constituents). – (2 types).

(i) simple media (basal)

(ii) Special media (⑤types).
- enriched
- enrichment
- Differential / indicators
- Transport
- selective.

(i) simple media: (basal)

- peptone water + NaCl + Meat extract

Nutrient broth.
↓ means
liquid.

⊕

Agar (2%)

↓

Nutrient agar.
(solid media).

(ii) Special media: (⑤types).

a) Enriched media: (always solid). promote → growth of pathogens

Nutrient Agar (NA) ⊕ Blood / Egg / Serum.

Egg → LJ media.

Blood → Blood Agar (5% sheep blood).

→ Chocolate agar (RBC lysed & Hb released)

* Blood Agar	Chocolate Agar
* Blood is added when NA is cool/low T.	* Blood is added when N. Broth is not yet cooled & at High T.
* RBCs are intact	* RBCs lysed & Hb released & chocolate colour is imparted

* Egg Enriched media is aka LJ media which is the selective media for Mycobacterium TB (MTB). Thus egg media is an example for: enriched (LJ) media as well as selective media.
(LJ - Lowenstein Jensen)

* Blood enriched media is NON selective media where all orgs. grows. unlike LJ media.

* LSS media - Loeffler's Serum slope is also a selective media for Corynebacterium Diphtheria.

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b) Enrichment Media: (always liquid) → broth.
↓ inhibit
Commensals.
↓
only pathogens can grow.

eg: i) Selenite F broth (SLF).
ii) Tetra Thionate broth (TTB). } for Salmonella & Shigella.
iii) Alkaline peptone water } for Vibrio

Differential / Indicator Media :

i) McConkey Agar (MA). → Lactose → red/pink → Lactose Fermenter (LF).
→ pale/colorless (alkaline) → NLF

Note:

* Bacteria ferment the lactose into acid & sometimes gas is also produced.
* Acid is used to detect fermentat°. by pH indicator (Neutral Red).

if ā is present → pink/Red (neutral red is red in colour in acidic medium)
No ā → pale/Colorless

③ Consistuents of MA:

- Lactose → differentiate b/n LF & NLF
- NaCl → electrolytes → favours swarming → overlapping of colonies.
- Bile salt → inhibits Gram -ve bacteria

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ii) CLED. ~~Agar~~

C - cysteine → Growth factor

L - Lactose → LF & NLF

ED - Electrolyte Deficient → No NaCl → No swarming

No Bile salt → so G+ve & G-ve fungi. can grow *

↓

although all these grow, we can also differentiate b/n them.

That's why CLED med is Non selective & Differential

Blood / Chocolate Agar → Non selective & Non differential

CLED media → Non selective & Differential

MA → Selective & Differential

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② advantages of CLED over MA:

i) It is a non selective & differential media,

ii) All G+ve, G-ve & fungi can grow & we can easily differentiate them

(ii) ~~It contains~~ There is No swarming in CLED media

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d) Transport media:

↳ some special subs. are added to keep the org. alive so as to TRANSPORT the media to long distances

eg: (i) PIKES media → Streptococcus.

(ii) Amies / Stuart media → Neisseria.

(iii) Clairy Blair. media → pathogens in Stool sample. (Vibrio).

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* only certain microbes can grow, others can't.

eg: * TCBS Agar (Thiosulphate Citrate Bile Salt Sucrose) } → only Vibrio.

* Wilson Blair Agar → Salmonella only.

* XLD agar, DCA, SSA → both Salmonella & Shigella

II) CLASSIFICN BASED ON O_2 requirement

i) Aerobic

They don't have the capacity to remove O_2 from the culture media

So O_2 will be present.

eg: Most of the Agar.

Note: How can we grow Anaerobes in Aerobic media?

Simple use, Anaerobic culture methods (5).

i) Mc Intosh Fiddes Jar
ii) Gas pack.
iii) Glove box.
iv) Pre Reduced Anaerobic System. (PRAS)
v) Anoxomat (automated).

ii) Anaerobic

capability to remove O_2 inside the Agar. bcz of some Reducing agent

Anaerobic. (so No O_2).

eg: Only ②:

i) Thioglycollate broth (Actinomycetes) } both are liquid
ii) RCM broth
(Robertson. Cooked Meat)

Selective med. for Clostridium

(i) (Biochem. methods) (ii) Molecular (iii) Immunological (Refer Immunology)

⑨ Imp. Rxns:

(i) Catalase Test:

* Detect Catalase in bacteria
* Most of the bacteria are Catalase ⊖ve.
* Catalase ⊖ve bacteria are.
  - Streptococcus
  - Pneumococcus
  - Enterococcus

  GPC. (only GPC which is Catalase ⊕ve is Staphylococcus).

(ii) Oxidase Test:

* detect Cyt. oxid.
* Most of bacteria are Oxid ⊖ve.
* only ⑨ bacteria are Oxid ⊕ve & all the ⑨ are G-ve

1) Meningococci.
2) Gonococci.

G-ve cocci. (GNC).

3) Vibrio
4) Pseudomonas.
5) Campylobacter
6) Helicobacter.
7) Bordetella.
8) Brucella.
9) Hemophilus.

G-ve bacilli. (GNB)

* To detect whether the bacteria is able to produce STRONG acids or not.

* Most of the org. are MR ⊕ve

(iv) Voges Proskauer (VP). Test:

* Whether the bacteria is able to produce WEAK acids or not.

* Most of VP ⊖ve.

* Only few are VP ⊕ve.

- → Klebsiella (⊛ differentiating factors from other G-ve Enterobacteriaceae family)
- → Vibrio Cholera. ELTOR (To differentiate b/n V. Chol. Classic vs ELTOR)
- → Yersinia enterocolitica. (To diff. from other sps. of Yersinia).

(v) Indole Test:

* To detect "Tryptophinase" enzyme.

Tryptophan → Indole

(vi) Mannitol Motility Test:

* To detect Mannitol. fermentation / not. & Also. Motility

* How to detect ferment? → Change in color. of pH indicator (Ferment → ā produced).

* How Motility? → Here we use. Semisolid media → To Test motility.

Solid media → org. can grow only on surface.

Semisolid " → " " surface + inside

↓

Hazy. → To detect motility

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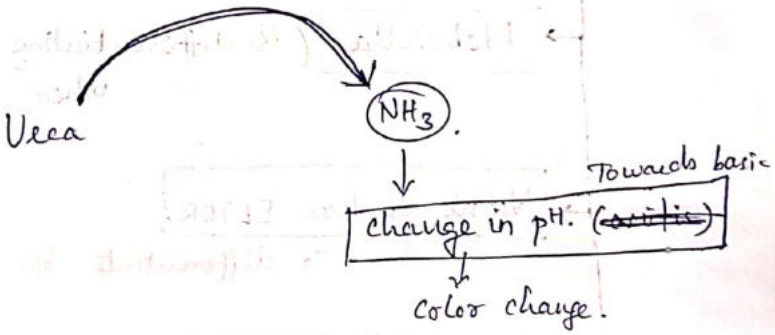
vii) Citrate Test:

* Whether org utilize citrate / not.

viii) Urease Test:

To detect. Urease



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ix) TSI - Triple Sugar Iron Test:

* Triple Sugar is present.
* To detect whether sugar is fermented / not.
  (i) ↓ change in color (pH change due to).
* Also sometimes Gas is produced during fermentat"
  (ii) ∵ ③ sugars are present, large amt. of gas is produced if more fermentat".

↓

This will cause BREAK IN SOLID MEDIA due to gas accumulatn

* (iii) To detect H_2S gas prodn ($H_2S + Fe \rightarrow$ Black).

TSI (Triple Sugar Iron) Test

used to detect

fermentatn of sugar. → by → change in color

Gas prodn → by → break in media.

Some produce H_2S gas.

H_2S + Iron (Fe)

BLACK COLOUR IS PRODUCED

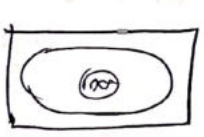
(ii) IDENTIFICn by MOLECULAR METHODS.

m/c used is (PCR).

PCR – ③ steps

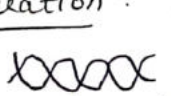
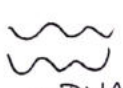
→ Step ① : Nucleic ā extracn

* by Enzyme (lysozyme) method.

 + lysozyme → Bacterio – lysis + Release of Nu. ā

→ Step ② Amplification (③ steps).

(i) Denaturation :

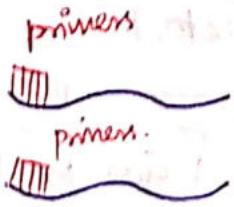
 95°C → 

dsDNA ssDNA.

Materials to be added

NA ⊕ primers ⊕ polymerase ⊕ dNTP's → raw materials

(ii) Annealing (55°C)



→ ssDNA ⊕ primers.

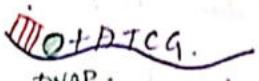
↓

This is happen only if its complimetary to each other

↓

ie) The pt. really has the disease we are looking for

(iii) Extension (72°C).



→ ds DNA.

* After Amplificatn, we have to identify whether the amplifn has occured / not.

* Bcoz if the pt. is not suffering from COVID & we for doing PCR for COVID-19 then amplifn won't happen

Step ③: Detection of Amplified segments

↓ done by

GEL electrophoresis. ⇒ in case of Conventional PCR

↓

. used for research purposes.

(OR)

FLUORESCENT METHOD

→ in case of Real time PCR (RTPCR)

How different Temp. (95° → 55°-
maintained correctly at ③ diff

→ Using Thermocycler