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Handbook of Practical Examination in Microbiology

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Foreword
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CHAPTER

4

Culture Media and Important Biochemical Reactions

To identify the cause of infection, it is necessary to grow the organism from infected material. For the identification of the organism, its growth as well as its isolation in the pure form is essential. For studying their characteristics their culture is essential.

Hence, to obtain suitable growth of the bacteria, the culture media should approximate to the composition and reaction of tissues and body fluids in which these bacteria grow. The food requirements of bacteria vary with their natural environment. They derive their energy by oxidation and by decomposition of food materials, such as proteins and carbohydrates. At the same time, simple organic substances like amino acids are utilized in the synthetic metabolism of these organisms.

The basic requirements of culture media are:

- Energy source
- Carbon source
- Nitrogen source

Salts such a sulfates, phosphates, chlorides, carbonates of K, Na, Mg, Fe, Ca and traces of Mn, Cu, etc.
Satisfactory pH 7.2 to 7.6.

Adequate oxidation—reduction potential (Eh).

Other growth factors:

- Tryptophan—for *S. typhi*
- Glutathione—For gonococci
- X, V factor for *Haemophilus*.

Ideal culture medium

- Must give satisfactory growth from small inoculum
- Growth should be rapid
- Easy to prepare
- Should be cheap.

LOUIS PASTEUR

He first used liquid media like urine, chicken broth, meat broth, etc. for culture.

ROBERT KOCH

He used solid media and started the use of potato in media. Then he used gelatin for solid media. But, this was found unsatisfactory as it melts at 24°C and gets liquefied by growing organisms.

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Hence, Frau Hesse introduced agar agar as a base for solid media. Agar is prepared from variety of seaweeds. The chief constituent of agar is long chain polysaccharide. It also contains inorganic salts and protein like material.

Agar gets melted at 98°C and usually sets at 42°C depending upon agar concentration.

Different types of agar are available, e.g. Japanese agar, New Zealand agar, etc. It is found useful and satisfactory for preparation of solid media and it is used in the powder form.

The other important ingredient of media is peptone. It is a complex mixture of partially digested proteins. It consists of proteoses, polypeptides and amino acids and variety of inorganic salts including phosphates, potassium, magnesium and other factors like riboflavin.

Special brands of peptone are also available, e.g. neopeptone, proteose peptone, etc.

Meat extract is also available and is known as Lab-Lemco. Other ingredients are blood, serum and yeast extract.

CLASSIFICATION OF MEDIA

A. Simple Media/Basal Media

- Liquid
  - Peptone water
  - Nutrient broth
- Solid
  - Nutrient agar.

B. Enriched Media

When we add blood, serum or egg in the basal medium, then it is called as an enriched medium.

- Blood agar
- Chocolate agar
- Loeffler's serum slope
- Brain heart infusion broth/agar (BHI)
- Dorset egg medium.

Dorset egg medium

Some organisms are more exacting in their nutritional needs. Hence, they do not grow in simple or ordinary media. They are called as fastidious organisms. They require blood, serum or body fluid in the basal medium for their growth. Thus, they require enriched medium for their growth, e.g. streptococci, pneumococci, meningococci, gonococci, etc.

C. Enrichment Media

These media are liquid.

- Bile broth
- Selenite F broth
- Tetrathionate broth
- Alkaline peptone water (APW)
- Monsur's taurocholate tellurite peptone water.

In mixed cultures or in materials containing more than one bacterium, the bacterium to be isolated is often overgrown by the unwanted bacteria.

Usually, the nonpathogenic or commensal bacteria tend to overgrow pathogenic ones, e.g. *S. typhi* being overgrown by *E. coli* in cultures from feces. In such situations, substances are incorporated in the medium which have a stimulating effect on the bacteria to be grown or inhibitory effect on those to be suppressed.

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Due to addition of number of such substances in the liquid medium, absolute increase in the number of wanted bacterium relative to other (unwanted) bacteria occurs. Such media are called as enrichment medium.

These are specially used for *Salmonellae* and *V. cholerae* organisms, when they have to be isolated, e.g. Bile broth, Selenite F broth, Tetrathionate broth, etc. for *Salmonella* and APW, Monsur's taurocholate tellurite peptone water for *V. cholera*.

D. Indicator Media

These media contain an indicator which changes color when a bacterium grows in them, e.g.

- *Wilson and Blair medium:* It is a selective medium for *Salmonellae*. This consists of sulfite. *S. typhi* reduces sulfite to sulfide in the presence of glucose and the colonies of *S. typhi* get black metallic sheen, hence, it may be called as indicator medium.
- *MacConkey medium:* This contains peptone, lactose, agar, neutral red and taurocholate. In this medium, lactose fermenting colonies appear pink in color whereas non-lactose fermenting colonies appear pale or colorless, so this may also be termed as an indicator medium.
- *Blood potassium tellurite (BPT) agar:* Though it is a selective medium for *C. diphtheriae*. Potassium tellurite in this medium gets reduced to metallic tellurium by *C. diphtheriae* and produce black colonies, hence, it may be called as indicator medium.
  On the other hand, it differentiates alpha hemolytic organisms from beta hemolytic organisms. Hence, it may be called as differential media.
- *Thiosulfate-citrate-bile salts-sucrose (TCBS) agar medium:* Although it is a selective medium for *V. cholera* and colonies of *V. cholerae* in this medium have yellow color in the beginning and later on become green in color. Hence, it may be called as indicator medium.

E. Differential Media

A medium which has substances incorporated in it enabling it to bring out differing characteristics of bacteria and thus, help in differentiation, e.g. MacConkey's medium. It contains peptone, lactose, agar, neutral red and taurocholate. Lactose fermenting organisms show pink color colonies, e.g. *E. coli*, *Klebsiella*. Non-lactose fermenting organisms produce colorless colonies, e.g. *Salmonellae*, *Shigella*, *Proteus,* etc.

Thus, it differentiates lactose fermenting and non-lactose fermenting organisms.

Blood agar is an enriched medium. But, it may be called as differential medium as it differentiates between alpha hemolytic and beta hemolytic organisms and non-hemolytic organisms. For example, alpha hemolysis is produced by *Streptococcus viridans* and *Streptococcus pneumoniae*, and beta hemolysis is produced by *Staphylococcus aureus* and beta hemolytic streptococci.

Note: Some of these terms for media may be interchangeable. For example, Blood agar medium is an enriched medium, but bacteria lysing red cells show a clear zone around the colonies, therefore this is an indicator medium as well. So also, MacConkey is an Differential medium as well as an Indicator medium.

F. Selective Media

If inhibiting substances are added to the solid medium to suppress the growth of unwanted organism from the mixed material or samples like stool, then it enables the growth of the wanted organism in large numbers. Such media are called as selective media, e.g.

- For *Salmonella*
  - Deoxycholate Citrate Agar (DCA)
  - Wilson and Blair
  - XLD Agar
  - SS Agar

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- For *Shigella*
  - DCA
  - XLD Agar (Xylose Lysine Deoxycholate Agar)
  - SS Agar (*Salmonella-Shigella* Agar)
- For *Vibrio cholera*
  - Bile Salt Agar
  - Monsur's GTTA (Gelatin Taurocholate Trypticase Tellurite Agar)
  - TCBS (Thiosulphate Citrate Bile salt Sucrose agar)
- For *C. diptheriae*
  BPT (Blood Potassium tellurite agar)
- For *S. aureus*
  - Ludlam's Medium (lithium chloride and tellurite)
  - Salt Milk Agar (8–10% NaCl)
  - Media containing polymixin
- For *Pseudomonas*
  Cetramide Agar.
- For *B. anthracis*
  Media containing PLET (Polymixin Lysozyme, EDTA and Thallous acetate).
- For *M. tuberculosis*
  LJ Medium
- For fungus
  Sabouraud's medium.

G. Transport Media

In case of delicate organisms, which may not survive in the time taken for transporting the specimen to the laboratory (or may be overgrown by non-pathogens, e.g. dysentery and cholera organisms in feces), then to avoid this, special media are devised for transporting these specimens. Such media are called as transport media.

- For Gonococci — Stuart's Medium
- For *Shigella* — Cary Blair Medium, Sach's buffered glycerol saline
- For *V. cholerae* — Venkatraman Ramkrishnan (VR) Medium
  Cary Blair Medium
- For *Salmonella* — Cary Blair Medium

H. Sugar Media

It is a fermentable substance. Routinely four sugars are used in the bacteriology laboratory, e.g. Lactose, Glucose, Mannitol, Sucrose (LGMS). Other sugars are:

- Arabinose
- Xylose
- Mannose
- Maltose
- Starch
- Inulin
- Raffinose
- Glycerol
- Sorbitol
- Salicin
- Aesculin
- Inositol.

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The usual sugar media consists of 1% of sugar in peptone water along with an indicator.

A small tube (Durham's) is kept inverted in the sugar tube to detect gas production.

Usually Andrade's indicator is used. If color of the sugar is changed from colorless to pink, then it indicates production of acid and if gas is produced, then it is collected in the Durham's tube.

Some organisms require serum for their growth. Then serum (usually 3% serum is added) is added to the sugar media. Such sugars are called as Hiss serum sugars, e.g. for *C. diptheriae*, *Str. pneumoniae*.

I. Anaerobic Media

These media are used to grow anaerobic organisms', e.g.

- RCM (Robertson's cooked meat medium)
- PRAS (Pre-reduced anaerobically sterilized)
- Thioglycollate broth.

IMPORTANT MEDIA

A. Simple Media

- Liquid
  a. Peptone water
  b. Nutrient broth
- Solid Nutrient Agar

a. *Peptone water*

It is a liquid medium and colorless. It contains 1% peptone and 0.5% sodium chloride in distilled water. It is a complex mixture of partially digested proteins.

Contents: Proteases, polypeptides, amino acids, various amount of inorganic salts including phosphates, potassium, magnesium and growth factors like riboflavin.

Uses :

- For study of proteolytic activity of the organisms, e.g. formation of Indole, H_2S
- To see the motility
- Base for sugar media.

Sterilization—By autoclave.

b. *Nutrient broth:*

It is liquid in form. It is pale yellow in color.

Contents:

- Meat extract
- Peptone
- Sodium chloride
- Distilled water.
- Sterilization—By autoclave.

Uses:

- Common liquid medium for nonfastidious organisms
- Base for preparation of solid media.

c. *Nutrient agar:*

- It is prepared by adding agar to the nutrient broth (usually 2%)
- When concentration of agar is reduced to 0.2–0.5% then semisolid or sloppy agar is formed which facilitates the motile organisms to grow and spread.

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- When concentration of agar is increased to 6% then it prevents spreading or swarming of the organisms like *Proteus, Cl. tetani.*
- Broth with agar is put in the steamer to dissolve the agar. Then this broth–agar fluid is filtered while it is hot. It becomes solid when cooled.
- Usually, it is transparent.

Uses:

- For growth of nonfastidious organisms
- Base for enriched media and differentiating media.

B. Enriched Media

- Blood agar
- Chocolate agar
- Loeffler's serum slope.

a. *Blood agar:*

The medium is prepared by adding sterile blood into sterile nutrient agar, which has been melted and cooled to 50°C. Concentration of blood varies from 5–10%. But, usually 10% is used. Sheep blood is commonly used. Horse blood is also used.

Human blood is not recommended as it contains antibacterial substances. The medium is always opaque.

Use:

- For the growth of fastidious organisms, which fail to grow on Nutrient agar, e.g. Streptococci, meningococci, *Str. pneumoniae, H. influenzae,* gonococci.
- It is suitable for gonococci
- It also shows hemolytic activity of bacteria. e.g.:

Beta hemolysis—Staph. aureus and beta hemolytic streptococci
Alpha hemolysis—Str. viridans and *Str. pneumoniae.*

b. *Chocolate agar:*

It is prepared by adding 10% of sterile blood in sterile nutrient agar. Melt the agar then cool it in water bath at 75°C. Then add blood (10 %) and allow the medium to remain at 75°C. Mix gently until blood becomes chocolate brown in color within 10 minutes. Then pour into plates.

Alternatively blood agar plates are kept in hot air oven at 55°C for 1–2 hours till they become chocolate brown in color.

Uses:

- For growth of meningococci, gonococci, *H. influenzae, Str. pneumoniae.*

c. *Loeffler's serum slope:*

Contents:

- Horse serum 300 mL
- Nutrient broth 100 mL
- Glucose 1 g

First dissolve glucose in NB. Then autoclave it and add serum with sterile precautions and dispense in the screw capped bottles. Then these bottles are kept in serum inspissator by making slope. Temperature is maintained to 80°C for 2 hours. Then the serum coagulates. The medium becomes solid and looks yellowish white.

Use:

- For the growth of *C. diphtheriae.*
- Advantage of this medium is that *C. diphtheriae* grows on this medium within 6 to 8 hours. While other bacteria require more than 16 hours for growth.

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C. Enrichment Media

- APW
- Tetrathionate broth
- Selenite F broth.

a. *APW (Alkaline Peptone Water):*
   - It is a liquid medium
   - Contents are same as that of peptone water only
   - pH is alkaline
   - pH is 8.6–9.0.

   Use:
   - For *V. cholerae.*

b. *Tetrathionate broth:*
   It is a liquid medium. It inhibits growth of coliform bacilli. But it permits growth of typhoid and paratyphoid organisms.
   Contents:
   - Calcium carbonate 2.5 g
   - Nutrient broth 78 mL
   - Thiosulfate solution 15 mL
   - Iodine solution 4 mL
   - Phenol red 3 mL

   Use:
   Enrichment medium for *S. typhi* and *S. paratyphi A* and *S. paratyphi B.*

c. *Selenite F Broth:*
   Contents:
   - Sodium hydrogen selenite—4 g
   - Peptone—5 g
   - Lactose—4 g
   - Disodium hydrogen phosphate—9.5 g
   - Sodium dihydrogen phosphate—0.5 g
   - Distilled water—1000 mL

   Use:
   - Same as tetrathionate broth.

D. Differential Media

- MacConkey's Agar
- DCA

a. *MacConkey's Agar: (Bile Salt– Lactose Neutral Red Agar)*
   It is used for isolation of intestinal pathogens. Bile salt inhibits growth of gram positive cocci. While gram negative bacilli and enteric organism are grown. It also contains lactose and neutral red to distinguish between lactose-fermenting and non-lactose fermenting colonies.

   Enteric organisms, i.e. *S. typhi* and *S. paratyphi A* and B and *Shigella* do not ferment lactose. Hence, they produce pale or colorless (non Lactose Fermenting) colonies. While other gram negative bacilli like *E. coli*, *Klebsiella* ferment lactose and produce pink color colonies (Lactose fermenting).

   In this way these organism, i.e. lactose fermenting can be differentiated from non-lactose fermenting by colony morphology on this medium.

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Contents:

- Agar – 2%
- Sodium taurocholate – 0.5%
- Peptone powder – 2%
- Distilled water – 100 mL

Preparation:

Mix all contents in the sterile flask. Autoclave it then cool it and adjust the pH to 7.4–7.5. Then add 1% neutral red indicator. Then add 1% lactose sugar. Sterilize it by keeping in Arnolds steam sterilizer (100°C) for one hour, then at 115°C for 15 minutes and pour the plates.

Use:

- For the growth of gram negative organism like *E. coli*, *Klebsiella* which form lactose fermenting (pink color) colonies and enteric organisms which form non-lactose fermenting (colorless colonies).
- Now-a-days ready to use dehydrated media are available, which can be prepared by adding distilled water and autoclaving at 121°C for 15 minutes.

b. *Deoxycholate Citrate Agar (DCA):*

It is particularly suitable for isolation of *Shigella, S. typhi and S. paratyphoid* A and B organisms.

Contents:

- Neutral Red Lactose Agar
- Solution A
- Solution B.

Preparation:

- 0.5% beef extract
- 1% Peptone powder
- 100 mL distilled water
- 2% Agar powder
- Mix well then keep in autoclave remove it and adjust the pH 7.4 then add 1% Lactose sugar. This becomes lactose sugar agar. Then add :
- 5 mL solution A
- 5 mL solution B
- Shake well add neutral red indicator (1%) shake well and pour in petri dishes.

Solution A:

- Sodium citrate
- Sodium thiosulfate
- Ferric ammonium citrate
- Distilled water.

Solution B:

- Sodium deoxycholate
- Distilled water.

Use:

- For isolation of *Shigella* and enteric group of organisms.

Note:

DCA is a selective medium for *Shigella* and *Salmonella* organisms. But, it may be called as differential medium as lactose fermenting organisms, such as *E. coli*, *Klebsiella* produce pink (LF) color colonies and *Salmonella, Shigella* produce colorless (NLF) colonies on it.

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TRANSPORT MEDIA

- Carry-Blair medium
- Thioglycollate medium
- VR medium

a. *Carry-Blair Medium:*
It is a buffered solution of:
- Sodium chloride—2.5 g
- Sodium thioglycollate—0.75 g
- Disodium hydrogen phosphate—0.55 g
- Calcium chloride—10 g/L (1% w/v)—4.5 mL
- Distilled water—495 mL
- pH—8.4 (Alkaline)

Use:
It is used as transport medium for *Salmonella, Shigella, Vibrio* and *Campylobacter*.

b. *Thioglycollate medium:*
It contains reducing agents, i.e. sodium thioglycolate with semisolid agar and indicator, i.e. methylene blue or resazurin. The indicator shows that the media is anaerobic except on the surface layer. To transport the specimen anaerobically.

c. VR Medium:
- Crude sea salt 20 g
- Peptone 5 g
- Distilled water 1000 mL
- pH 8.6

Use:
- Transport medium for *V. cholerae.*

SELECTIVE MEDIA

- LJ
- BPT
- RCM
- Sabouraud's
- TCBS
- Bile salt agar
- Monsur's GTTA
- DCA
- Wilson Blair medium
- XLD Agar
- *Salmonella-Shigella* agar

a. *LJ Media:*
Contents:
- Mineral salt solution 600 mL (containing potassium dihydrogen phosphate, magnesium sulfate, magnesium citrate, aspargine, glycerol and water)
- Malachite green 20 mL
- Beaten eggs (20–22) 1 L.
- Glycerol
- Aspargine

Due to malachite green, the medium becomes selective as it inhibits the growth of other organisms except *M. tuberculosis.* Due to glycerol growth of human type of mycobacteria is enhanced. First sterilize mineral salt solution in autoclave. Then add sterile malachite green solution. Then with sterile precaution add egg materials into it. Mix completely and distribute in screw capped bottle in 5 mL amount. Keep in the serum inspissator by making slope at 80°C for one hour. Afterwards it becomes solid.

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Uses:
- For culture of *M. tuberculosis*, *M. bovis*, atypical mycobacteria
- For culture of *Nocardia*
- For drug sensitivity testing for *M. tuberculosis.*

b. *BPT (Blood Potassium Tellurite Medium):*
Contents:
- Nutrient agar 100 mL
- Sterile defibrinated blood 10 mL
- Sterile potassium tellurite solution 1 mL (1%)

Procedure:
Take 100 mL of melted nutrient agar, cool it in water bath at 55°C, adjust the pH 7.6. Then add fresh defibrinated blood in 10 mL of potassium tellurite solution, shake well and distribute in the petri dishes.

Use:
- For culture of *C. diphtheriae.*

Advantage:
- It is selective medium for *C. diphtheriae*
- We can differentiate the three types of *C. diphtheriae*, i.e. *gravis, mitis* and *intermedius* on colony morphology.

Disadvantage:
- For growth of *C. diphtheriae* we have to wait for about 72 hours.

Note: C. diphtheriae produce black color colonies on BPT as tellurite is reduced to mellallic tellurium by the *C. diphtheriae* and colonies appear black. BPT and blood agar appear red but BPT is transparent and blood agar is opaque.

c. *RCM (Robertson's Cooked Meat Medium):*

Contents :
- Minced meat
- Nutrient broth.

Procedure:
Minced meat is boiled in alkaline water. Then soften meat is dried with filter papers and added to nutrient broth. The prepared medium is autoclaved.

Use:
- For culture of anaerobic organisms.

d. *Sabouraud's dextrose agar:*

Contents:
- Dextrose 40 g
- Peptone 10 g
- Agar 20 g
- Distilled water 1 L
- Sterilization By autoclave
- pH 5.4 (Acidic)

Use:
- Used for culture of fungi.

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Disadvantage:

- Saprophytic fungi may grow obscuring the true pathogen. The acidic pH may inhibit some significant fungal species
- Alternatively, Neutral Sabouraud's dextrose agar (NSDA)-(Emmons modification) with neutral pH, i.e. 6.8 to 7.0, Neopeptone instead of peptone and 2%, i.e. 20 g of dextrose which induces optimum growth of most pathogenic fungi. Bacterial contamination is controlled by adding antibiotics.

e. *TCBS Media:* The important ingredients are as given below. Since it is complicated and laborious to prepare and commercially prepared media are available the details are not mentioned here. However, the important constituents are given below:

Contents:

- Sodium thiosulfate
- Sodium citrate
- Bile salts
- Sucrose

Sterilization—By autoclave.

Use:

- For culture of *V. cholerae*. They produce large, yellow convex colonies, which become green after further incubation.

f. *Wilson and Blair Medium:*

Contents:

- Sterile nutrient agar 100 mL
- Bismuth sulfite
- Glucose phosphate mixture 20 mL
- Iron citrate brilliant green mixture 4.5 mL

Use:

For isolation of *Salmonella*. It is a selective medium for *Salmonella*. *Salmonella* produce green colonies with black center except *S. paratyphi* A as it does not produce H_2S.
Due to brilliant green and bismuth sulfite growth of *E. coli* is inhibited.

OTHER MEDIA

- Sugar media
- Hiss's serum sugar
- Glucose broth and bile broth
- TSI (Triple sugar iron agar)
- Biochemical media.

a. *Sugar media (LGMS):*

- Take prepared peptone water.
- Take 4 sterile conical flasks and distribute 100 mL in each flask respectively.
- Add 1% respective sugars.
- Take freshly prepared Andrade's indicator.

Preparation of Andrade's Indicator:

Take 100 mL of distilled water + 1% Acid fuschin. Then add 4% sodium hydroxide until the water become yellow.

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After preparation of Andrade's indicator, it is added in each 1% sugar. Filter it and pour in test tubes containing Durham's tube. Sterilize all in Arnold's or Koch's steam sterilizer (100°C) for 20 minutes for 3 successive days.

Use:
To study fermentative reaction of different organisms.

b. *Hiss's Serum Sugars:*
Prepare 1% sugar media with Andrade's indicator then add 25% serum (Horse, Sheep, Ox). Then sterilize with Arnold's or Koch's steam sterilizer at 100°C for 20 minutes for 3 successive days.

Use:
- To see fermentative reaction of *C. diphtheriae* and *Str. pneumoniae.*

c. *Glucose broth and Bile broth:*

Glucose broth:

Contents:
Nutrient broth + 1% glucose. Mix well and sterilize in Arnold's or Koch's steam sterilizer at 100°C for 20 minutes for 3 successive days.

Use:
Enrichment media for gram positive cocci, i.e. staphylococci, streptococci, pneumococci as well as for meningococci from blood, i.e. septicemia caused by gram positive cocci and meningococci also.

Bile Broth:
Contents:
- Sodium taurocholate 0.5 g
- Sodium chloride 0.5 g
- Peptone powder 2 g
- Distilled water 100 mL

Use:
Enrichment media for *Salmonella* organism.

d. *Triple Sugar Iron media (TSI) (Table 1)*

Contents:
- Lactose—10 parts
- Glucose—1 part
- Sucrose—10 parts
- Ferrous sulfate—Indicates H_2S production
- Sodium chloride
- Sodium thiosulfate
- Peptone
- Yeast extract
- Agar
- Phenol red (indicator)—Indicates acidification
- Distilled water.

Method of Sterilization:
- It is sterilized in Arnold's or Koch's steam sterilizer.

Use:
- To study fermentative reaction of different organisms.

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Table 1: Interpretation of triple sugar iron (TSI)

1.	Butt	Acid	*Escherichia*
	Slant	Acid	*Klebsiella*
	Gas	++	*Enterobacter*
2.	Butt	Acid	*Citrobacter freundi*
	Slant	Acid	
	Gas	++	
	H_2S	+	
3	Butt	Acid	*Salmonella*
	Slant	Alkaline	*Proteus*
	Gas	+	*Citrobacter freundii*
	H_2S	+	*Edwardsiella*
4	Butt	Acid	*Shigella*
	Slant	Alkaline	*Providencia*
	Gas	No Gas	*Yersinia*
	H_2S	No H_2S	
5	Butt	Alkaline slant	*Pseudomonas*
	Slant	Alkaline butt	*Flavobacterium*
	Gas	No Gas	*Alcaligenes*

Ref: Chapter 6. The Enterobacteriaceae. Koneman's Atlas and Textbook of Diagnostic Microbiology. 6th Edition.

Note:

1. Acid—Yellow color
   Alkaline—Pink color
2. If "Butt" is acidic and Slant is alkaline it indicates that glucose is fermented.
3. If "Butt" is acidic and Slant is acidic then lactose, glucose, sucrose are fermented.
4. No change in Butt and Slant (or alkaline slant and butt) indicates no fermentation of any sugar.
5. H_2S production is noted by black color of Butt or Slant or both.
6. Gas production is noted by gas bubbles in Butt or Slant or in both and elevation of Butt.

e. *Media for biochemical (IMVIC set)*

i. *Indole Medium*

Contents:

Peptone water + 1% tryptone

Principle of Indole test:

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane to indole which accumulates in the medium. For detecting the presence of indole, Kovac's reagent (dimethylaminobenzaldehyde) is added to the medium. It reacts with Indole to give a red colored compound.

Result:

- Red color ring—Positive Indole test
- No red colored ring—Negative Indole test.

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