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Competency Based Practical Manual for Microbiology

2nd Edition

As per the Revised Competency Based Medical Education Curriculum (NMC)



Upasana Bhumbla

Foreword
Sumit Rai

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CHAPTER 9

Culture Media and Culture Methods

COMPETENCY

MI1.4: Describe the laboratory methods used to detect causative agents of infectious diseases.

Definition

Culture media are required to grow the organism from the infected material to identify the causative pathogen for their antibiotic susceptibility test. Basic constituents of solid culture media are:

- Water : Source of hydrogen and oxygen
- Electrolyte : Sodium chloride or other electrolytes
- Peptone : Complex mixture of partially digested proteins
- Meat extract : Contains protein degradation products, inorganic salts, carbohydrates and growth factors
- **Agar-agar : Prepared from sea weed (algae-gelidium spp.). Used in conc. of 2–3%. It melts at 98°C and solidifies at 42°C. It is an inert substance, neither stimulate nor it inhibit the growth.**
- Blood or serum : Used for enriching culture media. 5–10% sheep blood is used for enriched media to provide extra nutrition to fastidious organisms.

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Classification of Media

1. Based on Physical State

- Liquid media
- Semi-solid media
- Solid media

2. Based on Presence of Molecular Oxygen and Reducing Substances

- Aerobic media
- Anaerobic media

3. Based on Nutritional Factors

- Simple media
- Complex media
- Synthetic media
- Special media
  - Enriched media
  - Enrichment media
  - Selective media
  - Differential media
  - Indicator media
  - Transport media

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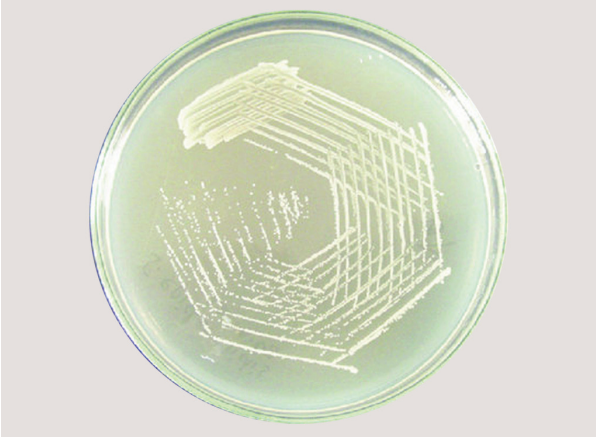
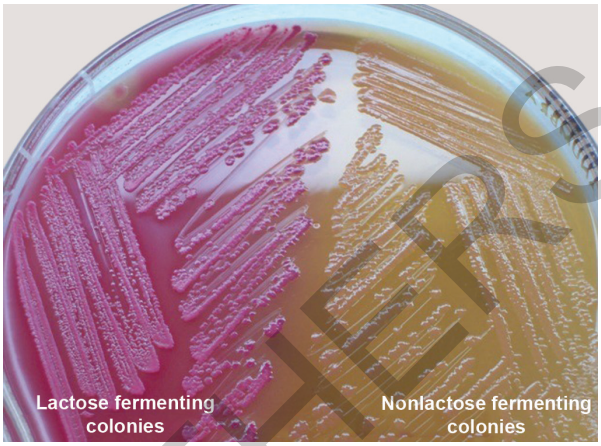


Nutrient agar



MacConkey's agar



Blood agar



Chocolate agar



Loffler's serum slope

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I. CULTIVATION OF AEROBIC BACTERIA

Culture Media (Aerobic)

Solid media: Dispensed in petri dishes/McCartney bottles.

Type of medium	Name of medium	Composition	Laboratory use
A. Simple	***Nutrient agar***	Peptone + Meat extract + Agar + NaCl	Culture and demonstration of pigmentation of different bacteria
B. Complex/Special a. Differential	***MacConkey's agar***	Lactose + Peptone + NaCl + Bile salts + Neutral red + Agar	Differentiate between lactose and nonlactose fermenters
b. Enriched	***1. Blood agar***	Nutrient agar + 5% Sheep blood	Differentiate between hemolytic and nonhemolytic bacteria
	2. Chocolate agar	Blood agar heated at 75°C for 10–15 minutes leads to release of X and V factors	*H. influenzae, Neisseria*
	3. Loeffler's serum slope	Nutrient broth + glucose + horse serum	*Corynebacterium diphtheriae*

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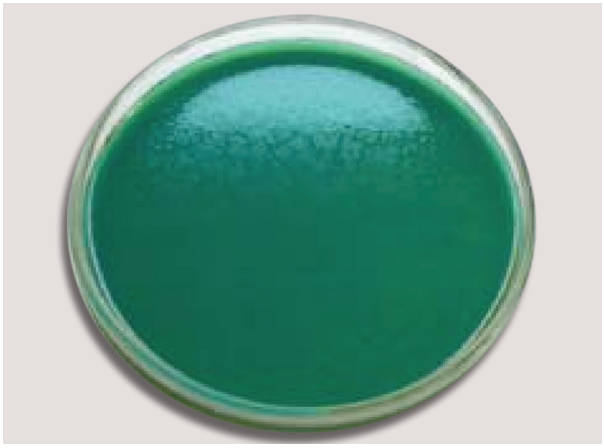
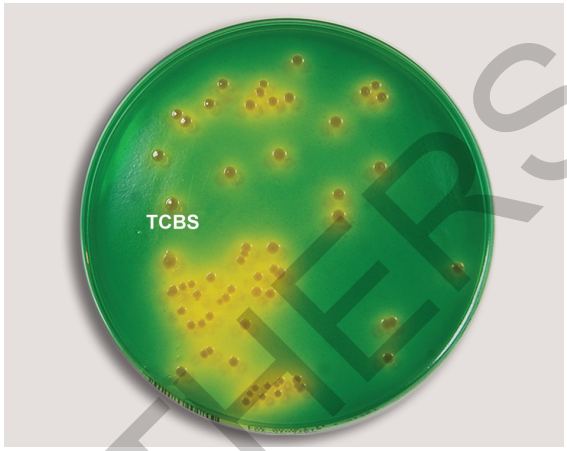
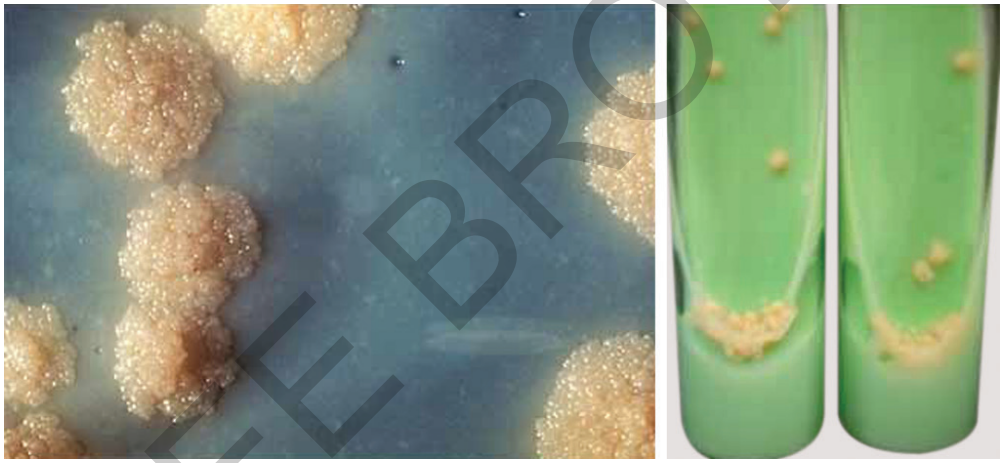


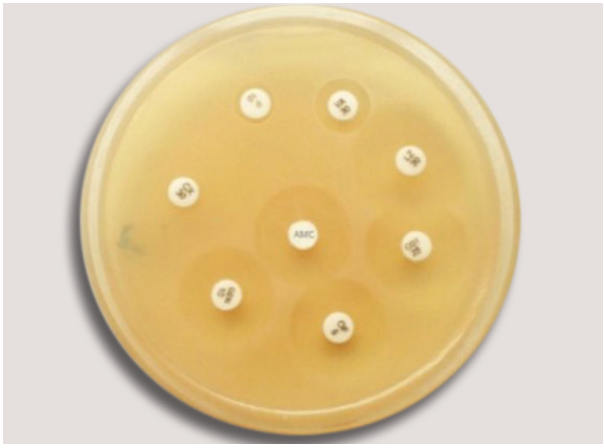
Wilson and Blair medium



TCBS medium



Lowenstein-Jensen medium



Mueller-Hinton agar

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Type of medium	Name of medium	Composition	Laboratory use
C. Selective	***1. Thayer-Martin medium***	Agar base + Hb + growth factor + antibiotics like Vancomycin, Colistin + Nystatin	Isolation of *Neisseria gonorrhoeae*
	2. Potassium tellurite agar	Blood agar + potassium tellurite	For *Corynebacterium diphtheriae*
	3. Wilson and Blair medium	Nutrient agar + glucose phosphate + brilliant green + bismuth sulphate	Isolation of *Salmonella* spp.
	4. Deoxycholate citrate agar (DCA)	Peptone + Lactose + Sodium deoxycholate + sodium citrate+ neutral red	Isolation of *Salmonella* and Shigella
	5. Thiosulphate citrate bile salt sucrose agar (TCBS) medium	Peptone+ sodium thiosulphate+ sodium citrate + NaCl + sucrose + ox bile + bromothymol blue	Isolation of *Vibrio cholerae*
	6. Lowenstein-Jensen medium	Egg + glycerol + NaCl + malachite green	Isolation of *Mycobacterium tuberculosis*
	7. Mueller-Hinton agar	Beef extract + casein hydro lysate + starch	For antibiotic susceptibility testing

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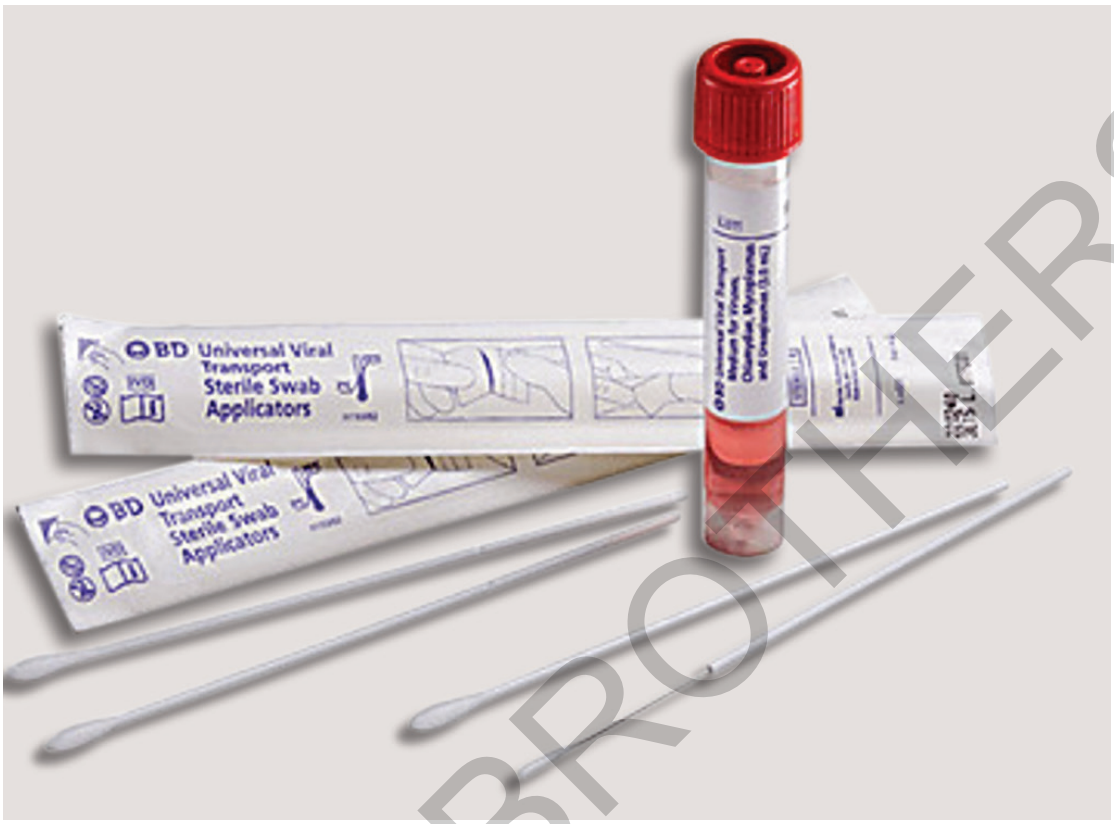

Viral transport medium

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Transport media: It is devised to maintain the viability of a pathogen and to avoid overgrowth of other contaminants during transit of sample from patient to laboratory. It is available as sterile disposable swab kits.

Name of the medium	Composition	Laboratory use
Stuart transport medium	Sodium thioglycolate + sodium glycerophosphate + calcium chloride + agar + methylene blue + distilled water	For maintaining the viability of *Neisseria gonorrhea* on swab
Amie's transport medium	Sodium thioglycolate + NaCl+ KCl + $CaCl_2$ + disodium hydrogen phosphate + potassium dihydrogen phosphate + charcoal + agar + distilled water	For maintaining the viability of *Neisseria gonorrhea* on swab
Pike's medium	Blood agar containing crystal violet + sodium azide	For *Strep. pyogenes, H. influenzae* swabs
Glycerol saline transport medium	Glycerol + NaCl + disodium hydrogen phosphate + phenol red 0.02% + water	For *Salmonella typhi* feaces culture
Venkatraman Ramakrishna medium	Crude sea salt + peptone + distilled water at pH- 8.6–8.9	For *Vibrio cholerae*
Viral transport medium (VTM)	Modified Hank's Balanced Salt Solution supplemented with bovine serum albumin, sucrose, glutamic acid, and gelatin.	For transporting H1N1, Novel coronavirus (nCOVID) samples

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Liquid media: Dispensed in tubes.

Type of medium	Name of medium	Composition	Laboratory use
a. Basal	***Peptone medium***	Peptone + NaCl + water	For sugar fermentation test
B. Special **a. Enriched**	***1. Glucose broth***	Nutrient broth + glucose	For *Streptococcus*
	2. Hiss serum	Serum + peptone water	For *Corynebacterium*
b. Enrichment	***1. Selenite F broth***	Peptone water + sodium selenite	Isolation of *Salmonella* and *Shigella*
For specimens other than blood	***2. Tetrathionate broth***	Nutrient broth + sodium thiosulphate + calcium carbonate + iodine solution	For *Salmonella*
	3. Alkaline peptone water	Peptone water pH = 8.6	For *Vibrio cholerae*
Liquid media used for blood culture	***1. Brain heart infusion broth***	Nutrient broth + dehydrated heart meat	Blood culture
	2. Liver infusion broth	Nutrient broth + crushed lean liver of sheep	Blood culture of pyogenic bacteria and enteric bacilli
	3. Glucose broth	Nutrient broth + glucose	For pyogenic organism
	4. Bile broth	Nutrient broth + bile	For *Salmonella*

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Biphasic media: Dispensed in blood culture bottles.

Name of medium	Composition	Laboratory use
Castaneda medium	***Brain heart infusion broth and agar***	For blood culture for Brucellosis

II. CULTIVATION OF ANAEROBIC BACTERIA

1. Culture Media (Anaerobic)

S. No.	Type of medium	Name of medium	Composition	Laboratory use
1.	Liquid medium	*Robertson's cooked meat medium*	Nutrient broth + Minced meat	Growth of anaerobic bacteria
2.	Thioglycolate broth	*Thioglycolate medium*	Nutrient broth + 1% thioglycolate + Resazurin	Growth of anaerobic bacteria



Cooked M Medium (RC medium) (M149)

1. Control
2. *Clostridium perfringens* ATCC 12924
3. *Clostridium sporogenes* ATCC 11437

Robertson cooked medium

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2. Inoculation Methods

Inoculation is done with a loop made of Nichrome wire of 24 S. W. G size; loop 2–4 mm in diameter with a wire 2 to 3" long and is sterilized in the Bunsen flame. Various methods of inoculation are employed:

S. No.	Name of method	Diagram	Procedure	Indication
1.	***Streak culture***		Loop is sterilized on flame and inoculum is taken and smeared on a well From there create a parallel streaks A, B, C and the tail	To get isolated colonies
2.	***Lawn culture***		Hooding the plate with liquid culture suspension	For antibiotic sensitivity pattern
3.	***Stroke culture***		Streaking on a nutrient agar slope	Isolation of pure growth of bacterium for slide agglutination test
4.	***Pour plate method***		Serial dilutions of bacteria into molten agar	Bacterial count and quantitative analysis of urine
5.	***Stab culture***		Performed by straight wire, by puncturing deep inside the agar	For demonstration of gel liquefaction, stock culture and for of test

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Q1. HOW ARE BACTERIA CATEGORISED ON THE BASIS OF THEIR OXYGEN AND TEMPERATURE REQUIREMENT?

On basis of their oxygen requirement

- **Obligate aerobes:** Those bacteria which require only oxygen for their growth, e.g., *Pseudomonas aeruginosa*
- **Facultative anaerobes:** Aerobes but can also grow without oxygen, e.g., *Staphylococcus* spp., *Escherichia coli*
- **Microaerophilic:** It can grow in presence of traces of oxygen, e.g., *Campylobacter jejuni, H. pylori*
- **Obligate anaerobes:** Obligate or strict anaerobes can grow only in the absence of oxygen, e.g., *Clostridium tetani*
- **Capnophilic:** Some bacteria require 5–10% amount of carbon dioxide for their growth, e.g., *Brucella abortus.*

On basis of their temperature

Mainly bacteria grow at 37°C. Bacteria are further grouped as:

- **Mesophiles:** Grows at temperature between 25°C and 40°C, e.g., all pathogenic bacteria
- **Psychrophiles:** Grows at temperature below 20°C, e g., Soil and water saprophytes
- **Thermophiles:** Grows at temperature between 55°C and 80°C, e.g., *Bacillus stearothermophilus*.

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Incubator



In microbiology, an incubator is a device used to grow and maintain microbiological cultures or cell cultures.

The incubator maintains optimal temperature, humidity and other conditions.

For routine bacterial cultures , temperature is maintained at 37°C.

McIntosh and Fildes' Anaerobic Jar

McIntosh and Fildes' anaerobic jar



McIntosh and Fildes' anaerobic jar is an instrument used in the production of an anaerobic environment (anaerobiosis).

Hydrogen gas is passed and the catalyst helps to combine hydrogen and oxygen.

The presence of air is deleterious for many anaerobic bacteria and must be incubated in its absence. The inoculated culture plates are placed inside a metal jar and the lid clamped tight. The air inside is removed using a vacuum pump. The pressure inside the chamber is reduced to 100 mm below mercury.

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Competency Based Practical Manual for Microbiology

This book is designed according to the newer guidelines of Competency Based Medical Curriculum for Indian Medical Graduates implemented by the National Medical Council of India.

Salient Features

- It is based on a newer competency based curriculum for undergraduates.
- The book provides essential information about the practical aspects of Microbiology.
- Moreover, it covers all the topics, including Clinical and Applied Microbiology.
- It also gives opportunity to students to practice and learn Microbiology.

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Shelving Recommendation
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