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# Concise Workbook in **Practical Microbiology**

*As per the Competency-based Medical Education Curriculum (NMC)*

**Anamika Vyas  
Sheethal S**



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# Culture Media

Date:

**MI1.1:** Describe the different causative agents of infectious diseases, the methods used in their detection.

## SPECIFIC LEARNING OBJECTIVES

- Describe different classifications of culture media used in diagnostic microbiology.
- Describe uses, constituents and sterilization methods of commonly used culture media.
- To choose appropriate culture media and interpret them for identifying causative agents of various infectious diseases.

## INTRODUCTION

Culture media are artificially prepared growth media which contain essential nutrients and other substances to enhance the growth of microorganisms.

## COMMON INGREDIENTS OF CULTURE MEDIA

1. **Water:** It is essential for bacterial growth. Distilled water or demineralized water is used in preparing culture media.
2. **Peptone:** It is obtained from hydrolyzed animal or plant protein which provides nitrogen and amino acids for bacterial growth.
3. **Meat extract:** It provides amino acids, vitamins and mineral salts for bacterial growth.
4. **Yeast extract:** It is used to stimulate the growth of bacteria.
5. **Mineral salts:** Traces of magnesium, potassium, iron and calcium are essential for bacterial enzymatic activity.
6. **Agar:** It is an inert polysaccharide obtained from varieties of sea weed which acts as a solidifying agent at 1-2%. It does not add any nutritive value to culture medium. It melts at temperatures of 95°C or above and solidifies at temperatures of 42°C or below.

## CLASSIFICATION OF CULTURE MEDIA

1. **Based on physical state:**
  - a. Solid media—concentration of agar 1-2% (New-Zealand agar at 1% and Japanese agar at 2%)
  - b. Semi-solid media—concentration of agar 0.2-0.5%
  - c. Liquid media—no solidifying agent (agar or other solidifying agents) is used

**Note:** Certain media are solidified using agents other than agar. For example, Lowenstein-Jensen media and Loeffler's serum slope.
2. **Based on oxygen requirement:**
  - a. Aerobic media, e.g., Nutrient broth, Nutrient agar, Blood agar, etc.
  - b. Anaerobic media, e.g., Robertson's cooked meat media, Thioglycolate broth.
3. **Based on nutritional requirement:**
  - a. Basal media/Simple media
  - b. Complex media
  - c. Synthetic media/Defined media
  - d. Special media
    - ♦ Enriched media
    - ♦ Enrichment media

- ♦ Selective media
- ♦ Differential media
- ♦ Transport media
- ♦ Indicator media
- ♦ Anaerobic media

### Simple/Basal Media

It contains minimum basic ingredients to support the growth of non-fastidious bacteria. For example, Peptone water, Nutrient broth, Nutrient agar.

### Complex Media

All media other than simple media are complex. They contain additional ingredients, such as yeast extract, casein, etc., to provide special environment for the growth of bacteria.

### Synthetic/Defined Media

These media are prepared from pure chemicals whose exact composition are known. They are not very commonly used in medical microbiology.

### Enriched Media

In this media, substances such as blood, serum or egg are added to basal medium to fulfill additional nutritional requirement. Thus, it supports the growth of both non-fastidious and fastidious bacteria. For example, Blood agar, Chocolate agar, Loeffler's serum slope, Lowenstein-Jensen media.

### Enrichment Media

Substances which have a stimulating effect on the bacteria to be grown or an inhibitory effect on those bacteria to be suppressed are added to a liquid medium, the result is an absolute increase in the numbers of the wanted bacteria relative to the other bacteria. They are useful for isolation of the pathogens from specimens which contain normal flora such as sputum and stool. Such media are called enrichment media. For example, Selenite-F broth and Tetrathionate broth for *Shigella* and *Salmonella*.

### Selective Media

If specific substances are added to a solid medium, they inhibit the growth of unwanted bacteria and allow the growth of only the wanted bacteria. They are useful for isolation of pathogens from specimens which contain normal flora, such as sputum and stool. Such media are known as selective media. For example:

- ❖ Deoxycholate citrate agar—for isolation of *Salmonella* and *Shigella* in stool sample
- ❖ Wilson and Blair media—for isolation of *Salmonella*
- ❖ Thiosulphate citrate bile salt sucrose agar (TCBS)—for isolation of *Vibrio cholerae*

**Note:** Although both enrichment and selective media are used to isolate organisms from specimens containing normal flora, the distinguishing feature is the consistency of the media. Enrichment are always liquid media whereas selective media are invariably solid in nature.

### Indicator Media

Certain substances whether indicators (neutral red) or reducing substances (potassium tellurite) are added to a medium to produce a visible change in the medium with bacterial growth. They are known as indicator media. For example:

- ❖ Wilson and Blair media—Sulphite
- ❖ McLeod medium—Potassium tellurite
- ❖ MacConkey agar—Neutral red

### Differential Media

These media have substances incorporated in them enabling them to bring out differing characteristics of bacteria thereby helping to distinguish between them. For example, MacConkey agar, TCBS agar, blood agar.

### Transport Media

Media which are used to transport clinical specimens to support delicate organisms from drying or to support organisms which may not survive the time taken for transporting the specimen to the laboratory or to keep organisms viable which may

be overgrow by commensal bacteria are known as transport media. Bacteria only remain viable in these media, they do not multiply. For example:

- ❖ Stuart's medium—*Neisseria gonorrhoeae*
- ❖ Alkaline peptone water, Cary Blair Medium and Venkataraman Ramakrishnan medium—*Vibrio cholerae*
- ❖ Buffered glycerol saline medium—*Salmonella*
- ❖ Pike's medium—*Streptococcus pyogenes*

### COMMONLY USED LIQUID CULTURE MEDIA IN MICROBIOLOGY LABORATORY

1. Peptone water
2. Nutrient broth
3. Sugar media
4. Bile broth and glucose broth
5. Cooked meat broth (CMB)
6. Brain heart infusion (BHI) broth
7. Selenite F broth
8. Alkaline peptone water (APW)
9. Tetrathionate broth

### COMMONLY USED SOLID CULTURE MEDIA IN MICROBIOLOGY LABORATORY

1. Nutrient agar
2. Blood agar
3. Chocolate agar
4. MacConkey agar
5. Loeffler's serum slope
6. Lowenstein-Jensen media
7. Sabouraud's dextrose agar
8. Mueller Hinton agar
9. Cysteine lactose electrolyte deficient agar (CLED)
10. Xylose lysine deoxycholate agar (XLD)
11. Deoxycholate citrate agar (DCA)
12. Thiosulfate citrate bile salt sucrose agar (TCBS)

USES AND CONSTITUENTS OF VARIOUS CULTURE MEDIA

Name of culture media	Type of medium	Important ingredients	Uses of media
Peptone water	Basal medium	Peptone 1%, sodium chloride 0.5%, water 100 mL	For culturing non-fastidious organisms, preparation of sugar media, for testing indole production, for studying motility of organism.
Nutrient broth	Basal medium	Peptone water, meat extract 1%.	Used for culture of common bacteria, basal media used for preparation of cooked meat broth, preparation of bile broth and glucose broth, used for testing of disinfectants.
Nutrient agar	Basal medium	Nutrient broth, agar 2%	For growth of wide range of non-fastidious organisms, acts as a basal medium for preparation of other media like blood agar, salt agar, etc., can be used for antibiotic sensitivity testing, can be used to observe pigment production in <i>Staphylococcus aureus</i> and <i>Pseudomonas</i> , slope of nutrient agar can be used in maintenance of stock culture.
Blood agar	Enriched medium, Differential medium	Nutrient agar, 5–10% sheep blood	Used for culture of fastidious organisms, to demonstrate the hemolytic properties of bacteria and in the preparation of chocolate agar.
Chocolate agar	Enriched medium	5–10% sterile sheep blood is added in to molten nutrient agar at 75°C.	For growth of fastidious organisms, e.g., <i>Neisseria meningitidis</i> , <i>Haemophilus influenzae</i> , <i>Streptococcus pneumoniae</i> .

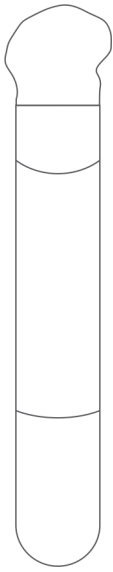
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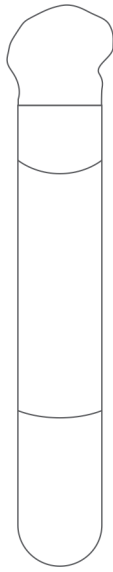
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Name of culture media	Type of medium	Important ingredients	Uses of media
MacConkey agar	Differential medium, Selective medium, Indicator medium	Peptone 2%, lactose, agar 2%, neutral red (indicator), sodium taurocholate 5%	For culture of members of family <i>Enterobacteriaceae</i> and differentiating lactose fermenting from non-lactose fermenting organisms.
Xylose lysine deoxycholate agar (XLD)	Selective medium, Differential medium	Yeast extract, xylose, lysine (0.5%), lactose, sucrose, sodium chloride, sodium deoxycholate (0.25%), sodium thiosulfate, ferric ammonium citrate, phenol red (0.008%), agar, water	Used for growth and isolation of <i>Salmonella</i> and <i>Shigella</i> spp. Red colonies with black centers: <i>Salmonella</i> spp. Red colonies without black centers: <i>Shigella</i> spp. Yellow colonies: <i>E. coli</i> , <i>Klebsiella</i> spp., <i>Proteus</i> spp.
Deoxycholate citrate agar (DCA)	Selective medium, Differential medium	Meat extract, peptone, agar, neutral red, sodium citrate, lactose, sodium thiosulfate, ferric ammonium citrate, sodium deoxycholate (0.5%)	Used for growth and isolation of <i>Salmonella</i> and <i>Shigella</i> spp.; <i>Salmonella</i> and <i>Shigella</i> spp. produce pale, non-lactose fermenting colonies (with black centers only in <i>Salmonella</i> ).
Thiosulfate citrate bile salt sucrose (TCBS) agar	Selective medium, Differential medium	Yeast extract, peptone, sodium citrate, ferric citrate, ox bile (0.8%), sucrose (2%), sodium chloride, bromothymol blue (0.004%), agar, water	Used for growth and isolation of <i>Vibrio</i> spp. Sucrose fermenting <i>Vibrios</i> ( <i>V. cholerae</i> ) form yellow color colonies whereas non-sucrose fermenting <i>Vibrios</i> ( <i>V. parahaemolyticus</i> ) form green color colonies.
Lowenstein—Jensen (LJ) medium	Enriched medium	Mineral salts, asparagine, glycerol, malachite green and hen egg	Used for the cultivation and isolation of <i>Mycobacterium</i> species.
Robertson's cooked meat medium (RCM)/ Cooked meat broth (CMB)	Anaerobic medium, Differential medium	Cooked meat particles, nutrient broth	Used for the growth and isolation of anaerobic microorganisms, used for maintenance of stock culture for aerobic bacteria, used to differentiate between proteolytic clostridia and saccolytic clostridia.
Mueller Hinton agar (MHA)	Enriched medium	Beef infusion (0.3%), casein hydrolysate (1.75%), starch, agar, distilled water. Starch absorbs bacterial toxins so that it does not interfere with antibiotic diffusion through the agar	Used to perform antimicrobial susceptibility testing of most of the bacterial pathogens.
Alkaline peptone water	Enrichment medium	Peptone water (ph 9.0)	Culture of <i>Vibrio</i>
Selenite-F broth	Enrichment medium	Peptone water, sodium selenite	For culture of <i>Salmonella</i> and <i>Shigella</i>
Tetrathionate broth	Enrichment medium	Nutrient broth, sodium thiosulfate, calcium carbonate, bile salts	For culture of <i>Salmonella</i> and <i>Shigella</i>
Loeffler's serum slope	Enriched medium	Glucose broth, horse serum	Cultivation of <i>Corynebacterium diphtheria</i> , water of condensation is used for testing the toxigenicity in guinea pig.
Sabouraud dextrose agar	Selective medium	Dextrose, peptone, agar, water	Used for cultivation of fungi and yeast.
Bile broth and Glucose broth	Enrichment medium	Nutrient broth, bile/glucose	Primary medium for blood culture (bile broth for isolation of <i>Salmonella</i> from blood and glucose broth for isolation of pyogenic bacteria from blood).
Sugar media	Indicator medium	1% sugar (glucose, lactose etc.) in sterilized peptone water, Andrade's indicator. This media is dispensed in test tubes containing inverted Durham's tube.	Used for testing the various sugar fermentation reaction for identification of organism. Gas producing bacteria are identified by observing air column in Durham's tube.
Cysteine lactose electrolyte-deficient agar (CLED)	Differential medium	Lactose, pancreatic digest of gelatin, beef extract, cysteine, bromothymol blue and agar	Used for processing urine samples, can be used as alternative to MacConkey agar and blood agar for urine samples.

**Exercise:** Complete the stencils using appropriate colored pencils to match the labeled media.



Peptone water



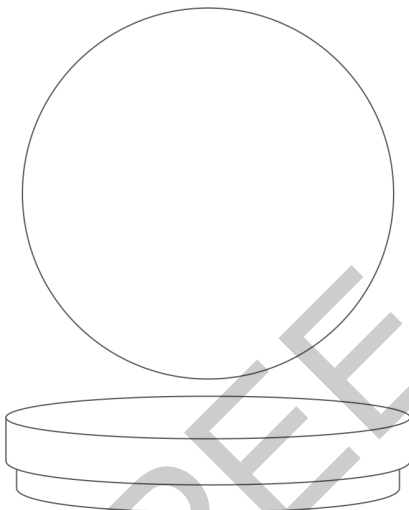
Nutrient broth



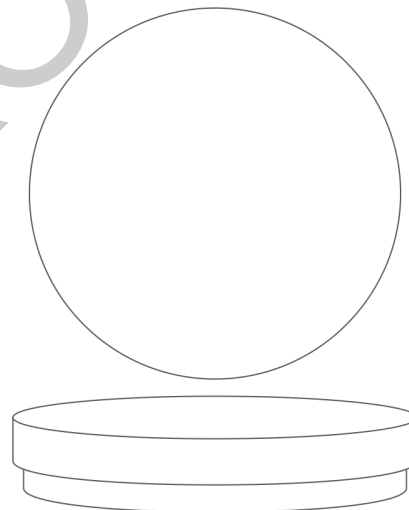
Glucose broth



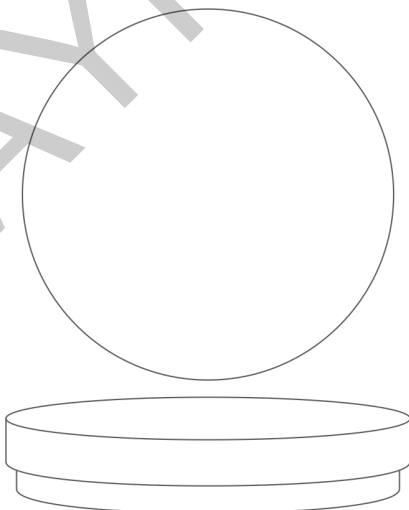
Selenite-F broth



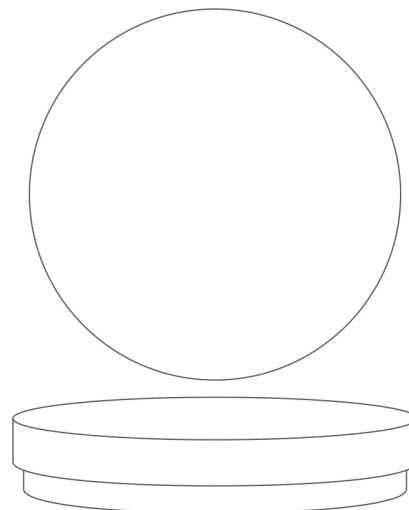
Blood agar



Chocolate agar

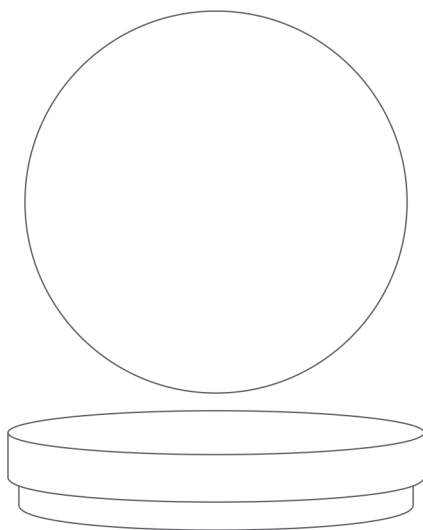


MacConkey agar

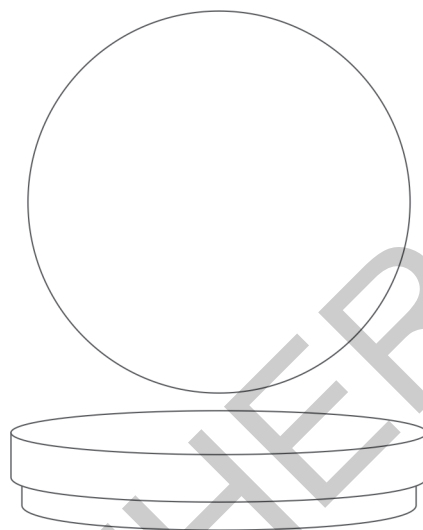


Xylose Lysine deoxycholate agar (XLD)

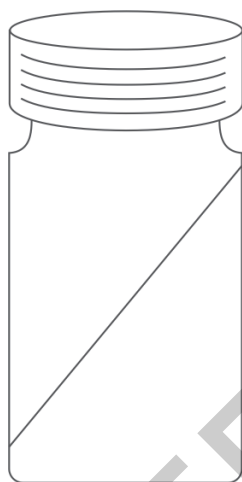




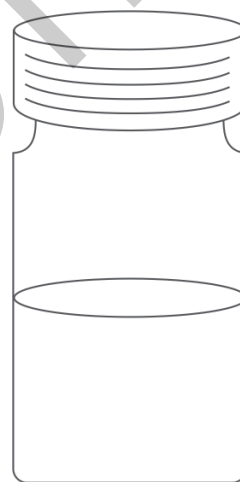
Nutrient agar



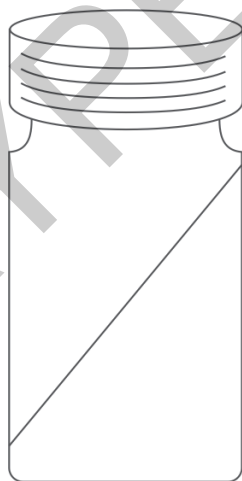
Thiosulfate citrate bile salt sucrose agar (TCBS)



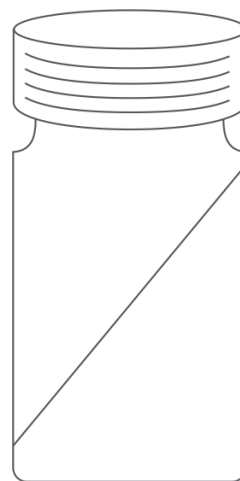
Sabouraud Dextrose agar



Robertson's cooked meat broth (RCMB)



Loeffler's serum slope



Lowenstein-Jensen medium (LJ)

## QUESTIONS

1. Write uses and method of sterilization of following media:

a. Nutrient agar

b. Blood agar

c. Thiosulfate citrate bile salt sucrose agar (TCBS)

d. Lowenstein-Jensen (LJ) medium

e. Sabouraud Dextrose agar (SDA)

f. MacConkey agar

g. Robertson's cooked meat broth (RCMB)

h. Glucose broth

i. Loeffler's serum slope

2. Which blood is preferred for preparing blood agar? Why?

3. Enumerate media which are solid without use of agar.

4. How is chocolate agar prepared?

5. Enumerate the varieties of agar.

6. Enlist any four transport media.

7. What is the function of malachite green in LJ media?

8. Enlist various types of hemolysis giving suitable examples for each. Which media is used to see this property of bacteria?
9. Enlist pigment producing bacteria. Which media is used to demonstrate this property of bacteria?
10. What are the uses of semi-solid media?

# Concise Workbook in Practical Microbiology

## Salient Features

- *Concise Workbook in Practical Microbiology* has designed to help students, learn and work on the practical aspects of Microbiology to perform on their own, hence introducing them to possible questions they may face in exams.
- First of a kind workbook styled practical manual in Microbiology for second year MBBS students
- AETCOM exercises as per CBME syllabus
- Skill certification templates are available for staining, stool examination, hand hygiene and personal protective equipment
- Chapters are designed in simple language for easy understanding of practical approach to Microbiology
- Question- and answer-based format of exercises for making second year MBBS students exam ready
- Dedicated section to system-wise clinical case discussion scenarios for integrated approach in identifying common clinical syndromes.

**Anamika Vyas** completed both her MBBS and MD (Microbiology) from Rabindranath Tagore Medical College, Udaipur, Rajasthan, India. Her fields of interest are Clinical Microbiology and Infection Prevention and Control. Currently, she is working as Professor and Head in the Department of Microbiology, Geetanjali Medical College and Hospital, Udaipur where she is also the Member Secretary of the institute's Hospital Infection Control Committee. She started her journey in Geetanjali Medical College and Hospital, Udaipur in 2008 and has been part of the Department of Microbiology since the institute was founded. She is an enthusiastic microbiologist and a keen academican. She has been teaching MBBS students from 2008 and since 2012 has been a thesis guide to nearly 20 MD (Microbiology) and MSc (Medical Microbiology) students so far. She has authored 21 publications in many noted journals. This book has been in the pipeline for some time, now conceptualized to assist students in grasping the practical importance of microbiology in medicine.

**Sheethal S** is a Clinical Microbiologist and an Infection Preventionist. She completed her MBBS from Vydehi Institute of Medical Sciences and Research Centre, Bengaluru and MD (Microbiology) from Karnataka Institute of Medical Sciences, Hubballi, Karnataka, India. She is also a recipient of the postgraduate diploma in Infection Prevention and Control awarded jointly by the University of Hyderabad (UoH) and Infection Control Academy of India (IFCAI). She is a big dreamer and lets nothing stop her in achieving what she sets her mind to. Currently, she is pursuing a Fellowship in Clinical Microbiology and Infectious Diseases from Deenanath Mangeshkar Hospital, Pune, Maharashtra. She has worked as a Clinical Microbiologist and Infection Control Officer in HCG-NMR Curie Center of Oncology, Hubballi and as an Assistant Professor and Infection Control Officer in the Department of Microbiology in Geetanjali Medical College and Hospital, Udaipur. In her short career in the field as an Astute Teacher, she has mentored 3 MD (Microbiology) postgraduates, 11 MSc (Medical Microbiology) students and about 4 batches of MBBS undergraduates. She has also been a Co-guide to 4 MD/MSc (Medical Microbiology) students with 10 publication articles to her name. This book is just a small effort from her to help second year MBBS students who might find clinical microbiology hard to understand.

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