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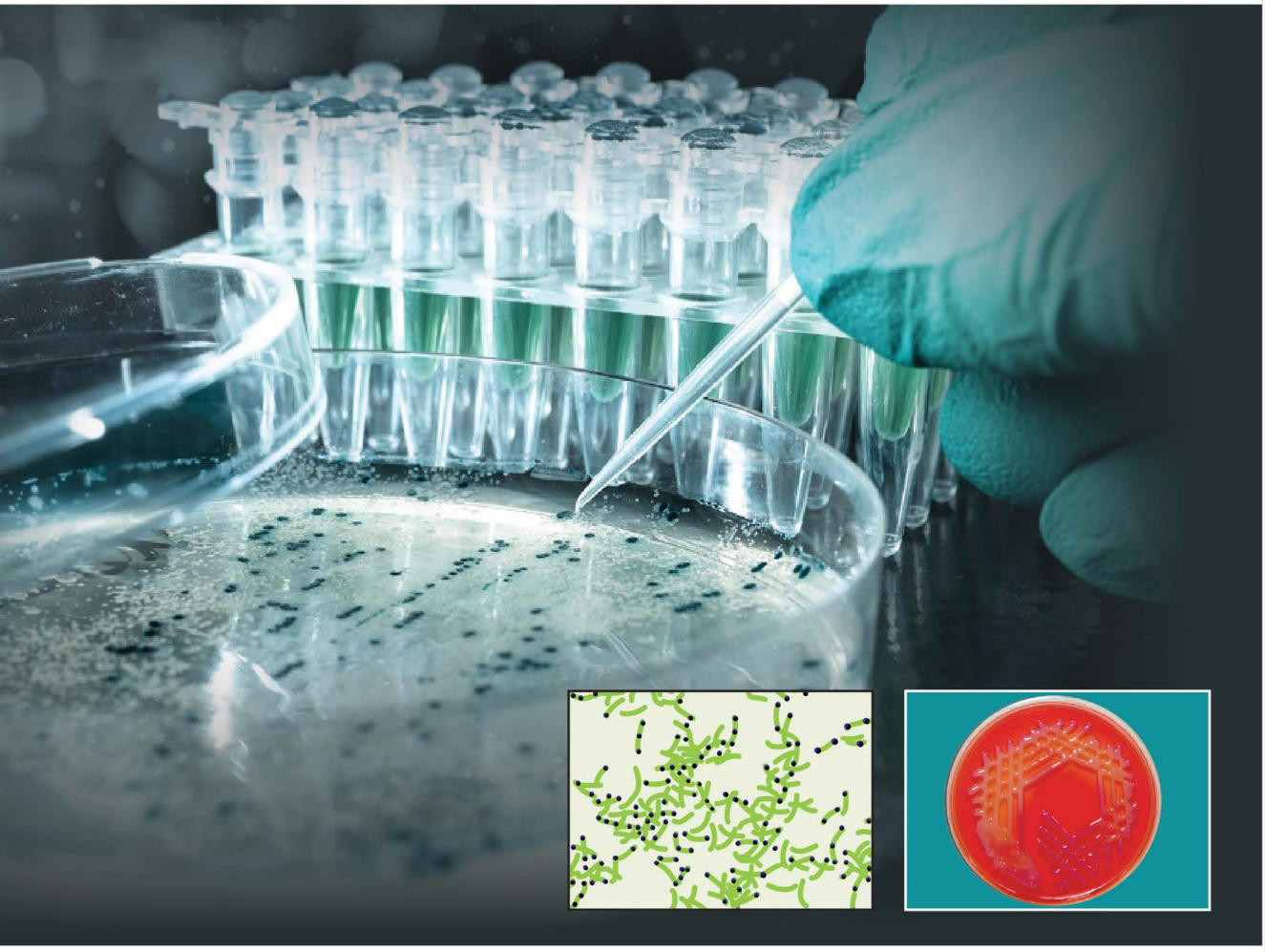
Practical MICROBIOLOGY

for MBBS Students



Surinder Kumar

JAYPEE

Contents

Unit 1. Basic Principles of Practical Microbiology

1. Introduction **3**

Universal Precautions *4*
Laboratory Instructions to the Students *4*

2. Microscopy **6**

Microscopy: Instruments *6*

3. Commonly Used Procedures in Practical Microbiology **11**

Hanging Drop Preparation *11*

4. Culture Media **15**

Phases of Growth Media *15*

Unit 2. Practical Exercises in Microbiology

5. Staining Exercises **22**

Staining Methods *22*
Staining for Practical Examination *22*

6. Identification of Bacterial Culture—An Overview **44**

Identification Scheme *44*

7. Bacterial Culture Identification—A Specific Approach **46**

Nutrient Agar *46*
Blood Agar *48*
MacConkey Agar *51*

8. Stool/Feces Examination **80**

Stool/feces Examination *80*
Identification of Eggs and Cysts *81*

9. Spots **90**

Identification of the Spots *90*

Unit 3. Appendices

Appendices **133**

Appendix I: Gram-positive Cocci (GPC), Nonmotile *133*
Appendix II: Gram-negative Cocci (GNC), Nonmotile *133*
Appendix III: Gram-positive Bacilli (GPB) *133*
Appendix IV: Gram-negative Bacilli (GNB) *134*
Appendix V: Lactose-Fermenter (LF) Bacteria *134*
Appendix VI: Nonlactose Fermenter (NLF) Bacilli *135*
Appendix VII: Characteristics of Eggs *135*
Appendix VIII: Parasites Infecting Different Tissues *136*
Appendix IX: Microfilariae *136*

Index *137*

4

CHAPTER

Culture Media

Learning Objectives

After reading and studying this chapter, student you be able to:

1. Describe liquid (broth) media such as peptone water, nutrient broth and glucose broth.
2. Identify solid media such as nutrient agar, blood agar, and MacConkey agar.

INTRODUCTION

A nutrient material prepared for the growth of microorganisms in a laboratory is called a culture medium.

PHASES OF GROWTH MEDIA

Growth media are used in either of two phases: liquid (broth) or solid (agar).

A. Liquid (broth) media (Table 4.1)

1. Peptone water
2. Nutrient broth
3. Glucose broth.

B. Solid agar media (Table 4.2)

1. Nutrient agar
2. Blood agar
3. Chocolate agar
4. Loeffler's serum slope (LSS)
5. MacConkey agar
6. Deoxycholate citrate agar (DCA)
7. Thayer Martin medium.

Composition of media

A. Liquid (broth) media (Table 4.1)
B. Solid (agar) Media (Table 4.2)

Identification of media

A. Liquid media

Glucose broth is yellowish in color while peptone is colorless or less yellowish than

Table 4.1: Representative types of liquid media

Medium	*Composition*	*Characteristics*
1. **Peptone water**	Peptone ——— 10 gm Sodium chloride (NaCl) ——— 5 gm Water ——— 1 Liter (pH 7.4-7.5)	i. Basis for carbohydrate fermentation media ii. For testing the formation of indole
2. **Nutrient broth**	Peptone water Meat extract	i. For routine culture ii. As a base to prepare many other culture media iii. To study growth curve
3. **Glucose broth**	Nutrient broth + Glucose (1% commonest)	Blood culture Promotes luxuriant growth of many organisms Glucose acts as reducing agent

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Table 4.2: Representative types of solid (agar) media

Medium	*Composition*	*Characteristics*
A. Simple medium		
Nutrient Agar	Nutrient broth, Agar (2-3%)	Complex medium used for **routine laboratory work**. Supports the growth of a variety of **non-fastidious bacteria**
B. Enriched media		
1. Blood Agar	Nutrient agar, Sheep blood (5-10%)	In addition to being **enriched medium**, it is an **indicator medium** showing the hemolytic properties of bacteria such as *Streptococcus pyogenes*
ii. Chocolate agar	Heated blood agar	This medium is used to culture **fastidious bacteria**, such as *Haemophilus influenzae*, *Neisseria* and *Pnemococcus*
iii. Loeffler's serum slope (LSS)	Nutrient broth Serum (of ox, sheep or horse) Glucose	Culture of *Corynebacterium diphtheriae*
C. Indicator media		
MacConkey agar	Peptone Sodium taurocholate, Agar Neutral red Lactose	Isolation and differentiation of **lactose fermenting (LF)** and **nonlactose fermenting (NLF)** enteric bacilli **Selective** because bile salts inhibits non-intestinal bacteria and lactose with neutral red to distinguish the lactose fermenting (LF) coliforms from the nonlactose fermenting (NLF) *Salmonella* and *Shigella* group **Differential** because the pH indicator turns red when the sugar in the medium, lactose, is fermented
D. Selective media		
Deoxycholate citrate agar (DCA)	Nutrient Agar Sodium deoxycholate, Sodium citrate, Lactose, Neutral red	Suitable for the isolation of *Salmonella* and *Shigella*
Thayer-Martin medium	Chocolate agar + vancomycin, colistin, nystatin, trimethoprim	Selective for *Neisseria* spp., which are fastidious. Selective-contains antibiotics (vancomycin, colistin and nystatin) that inhibit most organisms except *Neisseria* spp. Trimethoprim inhibits swarming *Proteus* spp.

glucose broth. Glucose broth is darker and more yellowish in color as compared to peptone water. Both the media are dispensed in test tubes with cotton plug.

Description of appearance of growth in liquid media (Table 4.3)

1. Degree; 2. Turbidity; 3. Deposit; 4. Surface growth.

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Table 4.3: Description of appearance of growth in liquid media

1. **Degree**—Non-scanty, moderate. abundant or profuse.
2. **Turbidity**—If present, slight, moderate or dense; uniform, granular or flocculent. Most of the Gram-negative bacteria grow in uniform turbidity form.
3. **Deposit**—If present, slight, moderate or abundant; powdery, granular, flocculent, membranous or viscid; disintegrating completely or incompletely on shaking, e.g. Streptococci growth occurs as a granular turbidity with a powdry deposit.
4. **Surface growth**—All aerobes have tendency to grow on surface of media due to more content of oxygen present on the surface, e.g. *Pseudomonas* spp.

B. Solid (agar) Media (Table 4.2)

1. Nutrient Agar

Nutrient agar is prepared by adding agar at a concentration of 2% to the nutrient broth. It is the simplest and commonest medium used routinely in microbiology laboratories, to grow non-fastidious bacteria. Nutrient agar is commonly referred to as "agar medium". Nutrient agar is used in the form of slopes, butts in test tubes or plates in petri dishes.

It is semi-transparent and pale, yellowish in color **(Fig. 4.1).**

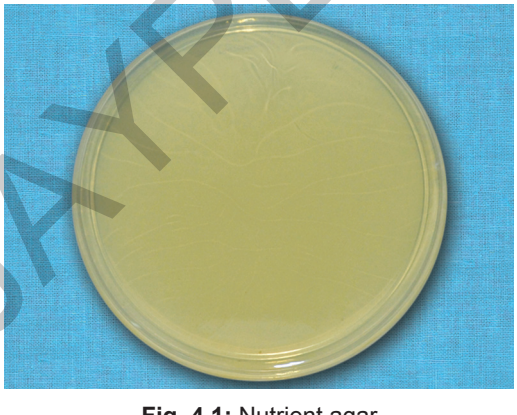

Fig. 4.1: Nutrient agar

Uses of Nutrient Agar

i. For routine laboratory work.
ii. To study colony characteristics.
iii. To detect pigment formation.
iv. For antibiotic sensitivity testing.
v. To maintain stock cultures.
vi. For slide agglutination and other tests—Pure growth of the bacterium on nutrient agar medium is used.

2. Blood Agar

Blood agar is bright red in color and is opaque when viewed by transmitted light if this consists entirely of 10% blood agar and hemolysis is difficult to see **(Fig. 4.2).** Double-layer blood agar overcomes this difficulty. A thin layer of melted blood agar is poured in a Petri dish and allowed to set . Then a similar thin layer of blood agar is poured on the top of the first layer. Thus two layers are present in blood agar medium, one above the other. The lower layer is of nutrient agar and upper layer is of sheep blood.

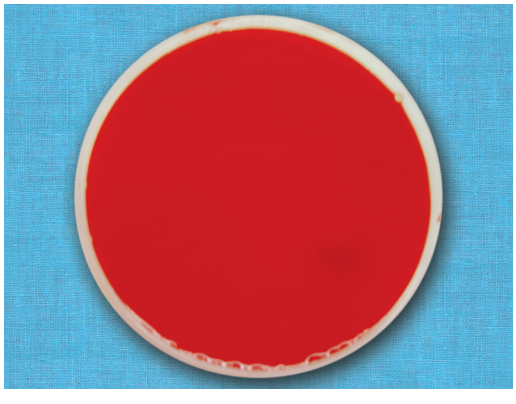

Fig. 4.2: Blood agar

3. MacConkey Agar

MacConkey agar is distinct reddish-brown color and is transparent **(Fig. 4.3).** If you keep your finger outside below the culture plate and

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try to see your finger through the medium, it is clearly visible; whereas the finger cannot be seen clearly through the blood agar. Absence of two layers in MacConkey agar is another differentiating feature in double layer blood agar. All the three media are dispensed in a petri dish.



Fig. 4.3: MacConkey agar

Table 4.4 shows differentiating features of nutrient agar, blood agar and MacConkey agar.

Table 4.4: Differentiating features of nutrient agar, blood agar and MacConkey agar

Characters	*Nutrient agar*	*Blood agar*	*MacConkey agar*
1. Color	Pale, yellowish	Bright red	Reddish-brown
2. Opacity	Semi-transparent	Opaque	Transparent
3. Double-layer in the medium	Absent	May be present	Absent

Description of appearance of growth on solid media (Table 4.5)

1. Shape; 2. Size; 3. Elevation; 4. Margins; 5. Surface; 6. Edges; 7. Color; 8. Structure; 9. Consistency; 10. Emulsifiability; 11. Differentiation.

Table 4.5: Description of appearance of growth on solid media

1. **Shape**—circular, irregular, or rhizoid
2. **Size-Pin-head size** is characteristic of staphylococci and **pin-point size** is a feature of streptococcal colonies
3. **Elevation**—effuse, elevated, convex; concave, umbonate or umbilicate
4. **Margins**—bevelled or otherwise
5. **Surface**—smooth, rough, granular, papillate or glistening
6. **Edges**—entire, undulate, crenated, fimbriate or curled
7. **Color**—pigment production
8. **Structure**—opaque, translucent or transparent
9. **Consistency**—membranous, friable, butyrous or viscid
10. **Emulsifiability**—easy or difficult
11. **Differentiation**—differentiated into a central and a peripheral portion

KEY POINTS

1. Identify the solid culture media (nutrient agar, blood cuture and MacConkey agar) with their characters.
2. Remember the composition of various media with the name of indicator, if any, present in the medium.

QUESTIONS RELATED TO CULTURE MEDIA

Q.1. What are phases of growth media?

Ans. Growth media are used in either of two phases: liquid (broth) or solid (agar).

Q.2. What are the representative types of liquid media?

Ans. Peptone water, nutrient broth and glucose broth.

Q.3. What are the advantages of solid media agar?

1. It is used to isolate the bacteria in pure form.
2. To study colony characteristics.

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Q.4. What are various types of agar media?

Ans. Nutrient agar, blood agar, chocolate agar, Loeffler's serum slope (LSS) and MacConkey agar.

Q.5. Name simple media, enriched media and indicator media?

Ans. Simple medium—Nutrient agar;

Enriched media—Blood agar, chocolate agar, Loeffler's serum slope (LSS)

Indicator media-MacConkey agar.

Q.6. How you will differentiate nutrient agar, blood agar and MacConkey agar?

Ans. See Table 4.3.

Q.7. How will you describe appearance of growth on solid media?

Ans. See Table 4.4.

1. Shape; 2. Size; 3. Elevation; 4. Margins; 5. Surface; 6. Edges; 7. Color; 8. Structure; 9. Consistency; 10. Emulsifiability; 11. Differentiation.

Q.8. Which indicator is used in MacConkey agar?

Ans. Neutral red.

Q.9. What are the differences between enriched media and enrichment media with examples?

Ans. **Enriched media** are solid while enrichment media are liquid media.

Enriched media are prepared to meet the nutritional requirements of more exacting bacteria by the addition of substances such as blood, serum or egg to a basal medium. Examples of enriched media: 1. Blood agar; ii. Chocolate agar; iii. Loeffler's serum slope.

Enrichment media-When a substance is added to a liquid medium which inhibits the growth of unwanted bacteria and favors the growth of wanted bacteria is known as enrichment medium. This medium for an enrichment culture is usually liquid and provides nutrients and environmental conditions that favor the growth of a particular microbe but not others.

Examples: a. Tetrathionate broth; b. Selenite F (F for Faeces) broth; c. Alkaline peptone water.

Q.10. What is indicator media?

Ans. Indicator media contain an indicator which changes color when a bacterium grows in them, e.g. MacConkey agar indicates lactose fermenting (LF) property. LF produce pink colonies and nonlactose fermenter (NLF) produce colorless colonies due to neutral red indicator.

Q.11. What is differential media?

Ans. A medium which has substances incorporated in it, enabling it to bring out differing characteristics of bacteria and thus helping to distinguish between them, is called a **differential medium. MacConkey agar** is both differential and selective. Some bacteria that ferment the sugar produce acid, which decreases medium pH and causes the neutral red indicator to give a pink or red color to colonies which are known as lactose fermenters (LF). Non-lactose fermenters (NLF) form colorless, pale colonies.

Q.12. What is selective media?

Ans. When a substance is added to a **solid medium** which inhibits the growth of unwanted bacteria but favors the growth of wanted bacteria it is known as **selective media**. Inhibitory agents used for this purpose include dyes, bile salts, alcohols, acids, and antibiotics. These media are used to isolate particular bacteria from specimens where mixed bacterial flora is expected, e.g. Lowenstein and Jenson medium is used for *Mycobacterium tuberculosis*.

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ISBN 978-93-5270-190-2