2nd Edition

Exam Preparatory Manual for Undergraduates

MICROBIOLOGY (VIRAT)

Thanveer P • Ramki M • Vivek VK

Contents

3 Culture Media and Methods

TYPES OF CULTURE MEDIA

- Solid media, liquid media, semisolid media
- Aerobic media, anaerobic media
- Simple media, complex media, synthetic media, semisynthetic media, special media.

Special Media

- Enriched media
- Enrichment media
- Selective media
- Indicator media
- Transport media
- Sugar media.

Liquid Media

Uses

- For obtaining bacterial growth from blood.
- For preparing bulk cultures of antigens or vaccines.

Disadvantages

- Bacteria may not exibit special characteristics
- Difficult to isolate different types of bacteria, e.g. nutrient broth, alkaline peptone water.

Solid media

- Bacteria have distinct colony morphology when cultured, e.g. cooked cut potato (earliest media), Agar, Peptone.

Simple media

- Nutrient broth – Peptone + meat extract + NaCl + Water.
- Nutrient Agar – Nutrient broth + 2% Agar.

Increasing concentration of agar to 6% prevents swarming.

SPECIAL MEDIA

1. Enriched Media

- Substance like blood, serum/egg added to basal media.
- For growth of bacteria which are more extracting in nutrient needs, e.g. a. Brain – heart infusion agar Broth: for typhoid. b. Blood-agar.
- Consists of blood, agar and peptone.
- Example for enriched, indicator, nonselective and differential medium.
- To differentiate *grampositive cocci* based on hemolysis.
- Alpha hemolysis – greenish yellow discoloration around colony.
- Beta hemolysis – clearing around colonies.
- Gamma hemolysis – no change in medium. c. Chocolate agar
  - By heating a mixture of sheep red cells and nutrient agar.
  - RBC disrupted released contents Hb, hemin (X), NADC (factor V), e.g. H. influenza, Niesseria, Moraxella.

2. Enrichment Media

- Liquid media
- In mixed culture, bacterium to be isolated overgrown by unwanted bacteria
- Normally nonpathogenic species overgrows
- Substance that have stimulating effect on bacteria to be isolated and inhibitory effect on unwanted is incorporated into medium, e.g. Selenite F- Broth – dysentery bacilli and Tetrathionate broth – for typhi and paratyphi.

3. Selective Media

- When above substance is incorporated into solid medium.
- Greater number of required bacterial colonies are produced, e.g. Desoxycholate Citrate for dysentery bacteria
  - TCBS for vibrio
  - Thayer martin for Neisseria
  - Lowenstein- Jensen medium for Mycobacterium tuberculosis.

4. Indicator Media

- Medium which has indicator which change color when bacteria grows, e.g. Wilson blair medium – Salmonella typhi and Mcleod's medium – Pottasium tellurite to tellurium, Diphtheria bacilli.

5. Differential Media

- Has substance incorporated in it which about differing characteristics of bacteria, e.g. McConkey agar

Differentiate → Lactose fermenters – pink colonies
Differentiate → Nonlactose fermenters – colorless colonies.

6. Transport Media

- Delicate organism that cannot withstand time taken for transporting the specimen to laboratory, e.g. Stuart's media: nonnutrient soft agar gel that contain reducing agent and charcoal for enterococci.
  - 'Buffered Glycerol saline – for enteric bacilli'.

Anaerobic Media

- To grow anaerobic organisms, e.g.
  - Robertson's cooked meat medium
  - Thioglycollate medium
  - Sabouraud dextrose agar.

Aerobic Culture Methods

- Streak culture (Surface plating)
- Lawn or carpet culture
- Stroke culture
- Stab culture
- Pour plate culture
- Liquid culture.

ANAEROBIC CULTURE METHODS

Culture Methods

- Strict anaerobes like clostridium tetani form growth if only Oxygen tension < 2 mm Hg.

1. Mcintosh – Fildes anaerobic jar

- Most reliable and widely used.
- Parts:
  - Stout glass/metal jar with metal lid of air tight screws.
  - Two tubes with taps – one inlet and one outlet.
  - Beneath lid – two terminals that can be connected to electric supply which is connected to palladinised asbestos.
- Working:
  - Inoculated culture plates are placed inside the jar in medium in bottom half of plates.
  - Outlet tube connected to vaccum pump and air inside is evacuated.
  - Inlet tube to hydrogen supply – terminals connected to electric supply.
  - Palladinised asbestos acts as catalyst and residual oxygen reacts with hydrogen.
  - Aluminium pellets coated with palladium – in gauze sachet suspended from lid of jar act as catalyst at room temperature.

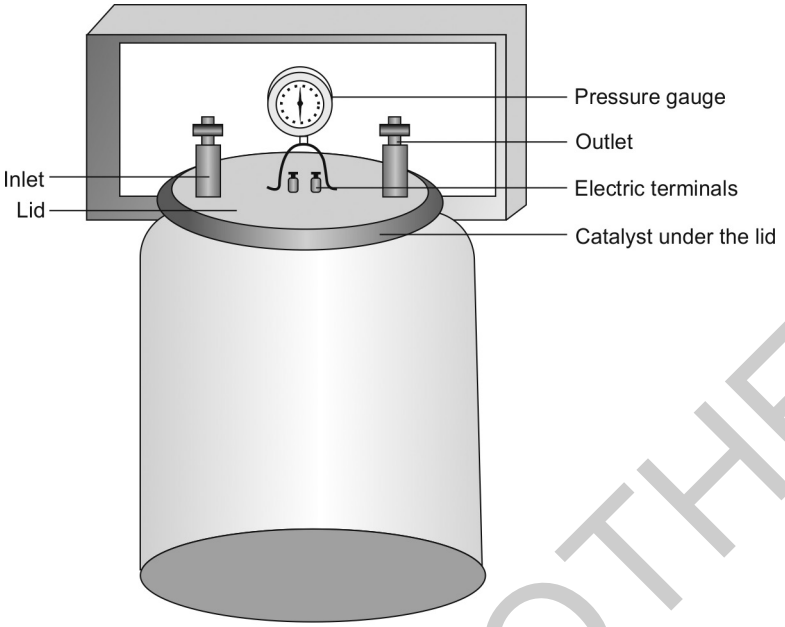

Fig. 3.1: Mcintosh and fildes anaerobic jar

2. Gaspak

- Method of choice for anaerobiosis
- Disposable envelope containing chemicals which generate hydrogen and carbon dioxide on addition of water
- It is simple and effective, eliminating the need for drawing a vacuum and adding hydrogen.

3. Prereduced anaerobic system (PRAS)

- For fastidious anaerobes.

4. Anaerobic chamber (glove box)

5. Robertson's cooked meat medium

- Most widely used fluid medium for culture of anaerobes
- Fat free minced cooked meat in broth
- Unsaturated fatty acids take up oxygen, reaction catalysed by hematin in meat and sulphydryl compounds bring about reduced oxidation reduction potential
- Saccharolytic species – Pink
- Proteolytic species – Black.

6. Other methods

- Reducing agents – 1% glucose, 0.1% ascorbic acid
- Broth – Smith Noguchi medium.
- Thioglycolate broth.

Exam Preparatory Manual for Undergraduates

MICROBIOLOGY (VIRAT)

Salient Features

- Chapterwise concise text on each topic, which helps in easy revision
- Emphasized on selected topics, which are frequently asked in the university examinations
- Large number of diagrams and flowcharts added
- Life cycles of parasites made easy
- Appendices at the end of book include additional topics
- Separate sections for vaccines and staining methods helpful for practical examinations
- Previous years' KUHS-pattern question papers
- Also useful for the final year MBBS students.

The authors are MBBS graduates from Government Medical College, Thrissur, Kerala, India.

They are well-known personalities among the faculties and students of Thrissur Medical College, Thrissur. The authors are the emerging young doctors, who are engaged in teaching others. The synopsis of microbiology is an attempt for the same.

Thanveer P

Ramki M

Vivek VK

Available at all medical bookstores or buy online at www.jaypeebrothers.com

JAYPEE BROTHERS
Medical Publishers (P) Ltd.
www.jaypeebrothers.com

Join us on facebook.com/JaypeeMedicalPublishers

Shelving Recommendation
MICROBIOLOGY

ISBN 978-93-85891-77-9