

# 5. Culture media and culture methods

## Competency

MI1.1: Describe the different causative agents of infectious diseases and the methods used in their detection, and discuss the role of microbes in health and disease

**Specific Learning Objectives:** At the end the session, the students shall be able to,

- Enlist the basic nutrients required in a culture medium for the growth of bacteria
- Enumerate various culture media used for growing bacteria
- Define different types of culture media (Enriched, Enrichment, Selective, Transport, differential) and explain their uses
- Suggest the use of suitable culture media based on the type of clinical specimen and condition
- Enumerate the media, methods and uses of anaerobic culture
- Describe the media, methods and uses of blood culture
- Discuss the principle of automated blood culture systems with examples
- Compare and contrast the conventional from automated blood culture method

## Exercise 6:

### 1) Explain the purpose of growing the bacteria in the laboratory.

Growing cultures of bacteria on solid media (agar plate or slant) permits us to view and identify colonial characteristics, and also provides a way to separate bacteria in a mixed culture.

### 2) Write the basic chemical constituents in a culture medium.

Sodium chloride, water

- 3) Enumerate different types of transport media and indicate the clinical conditions and type of bacteria that can be supported by such media.

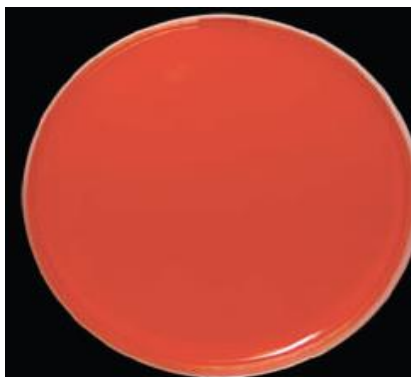
Bacteria	Clinical condition	Transport medium
<b>Aerobic:</b>		
1) <i>Neisseria</i>	Recurrent fever, rash, migratory arthralgias and headaches	Amies medium and Stuart's medium
2) <i>Vibrio cholerae</i>	Watery diarrhea, abdominal cramping, nausea, vomiting and fever	<ul style="list-style-type: none"> <li>• VR (Venkatraman-Ramakrishnan) medium</li> <li>• Autoclaved sea water</li> <li>• Cary Blair medium</li> </ul>
3) <i>Shigella</i> , <i>Salmonella</i>	Abdominal pain, tenesmus, watery diarrhea or dysentery	<ul style="list-style-type: none"> <li>• Buffered glycerol saline</li> <li>• Cary-Blair medium</li> </ul>
<b>Anaerobic:</b>		
1) <i>Clostridium</i>	Fever, dehydration, lower abdominal tenderness or rebound tenderness	Cary-Blair medium

- 4) Define different types of culture media (Enriched, Enrichment, Selective, differential) and explain their uses

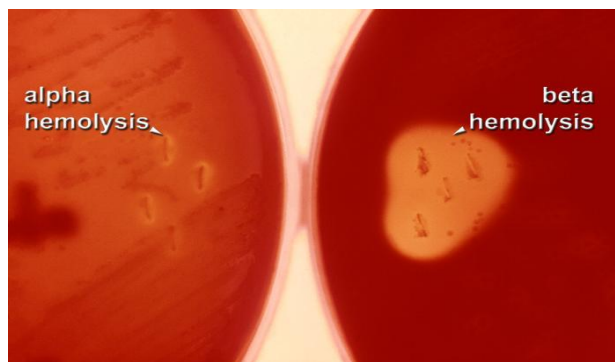
Type of media	Examples	Uses
Enriched media:	<b>Blood agar</b>	Tests the hemolytic property of the bacteria such as: 1) partial or $\alpha$ (green) hemolysis 2) complete or $\beta$ -hemolysis
	<b>Chocolate agar</b>	It supports highly fastidious bacteria, such as <i>Haemophilus influenzae</i> that does not grow on blood agar
	<b>Loeffler's serum slope</b>	It used to isolation of <i>Corynebacterium diphtheriae</i>
	<b>Blood culture media</b>	It used for isolating microorganisms from blood
Enrichment media:	<b>Tetrathionate broth</b>	Used for <i>Salmonella Typhi</i>
	<b>Gram-negative broth</b>	Used for isolation of <i>Shigella</i>
	<b>Selenite F broth</b>	Used for isolation of <i>Shigella</i>
	<b>Alkaline peptone water (APW)</b>	Used for <i>Vibrio cholerae</i>

Selective media:	<b>Lowenstein–Jensen (LJ) medium</b>	It is used for isolation of <i>Mycobacterium tuberculosis</i>
	<b>Thiosulfate citrate bile salt sucrose (TCBS) agar</b>	It is used for isolation of <i>Vibrio</i> species
	<b>DCA (deoxycholate citrate agar and XLD (xylose lysine deoxycholate) agar</b>	They are used for the isolation of enteric pathogens, such as <i>Salmonella</i> and <i>Shigella</i> from stool
	<b>Potassium tellurite agar (PTA)</b>	It is used for isolation of <i>Corynebacterium diphtheriae</i>
Differential media:	<b>MacConkey agar</b>	It is used for the isolation of enteric gram-negative bacteria It differentiates organisms into: • <b>LF or lactose fermenters</b> (produce pink colored colonies, e.g. <i>Escherichia coli</i> ) • <b>NLF or non-lactose fermenters</b> (produce colorless colonies, e.g. <i>Shigella</i> )
	<b>CLED agar (cysteine lactose electrolyte-deficient agar)</b>	It is used as an alternative to combination of blood agar and MacConkey agar, for the processing of urine specimens

5) Draw the colored labeled diagrams of culture media (without and with growth) in the space provided below

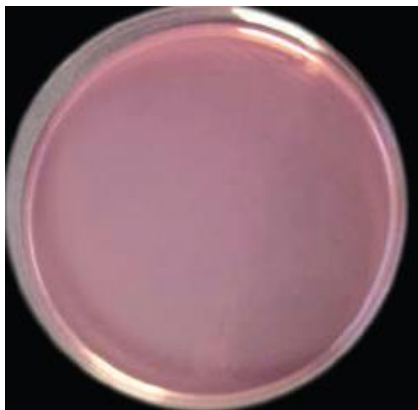


**Blood agar**



**Blood agar with Alpha hemolytic**

**Blood agar with Beta hemolytic colonies**

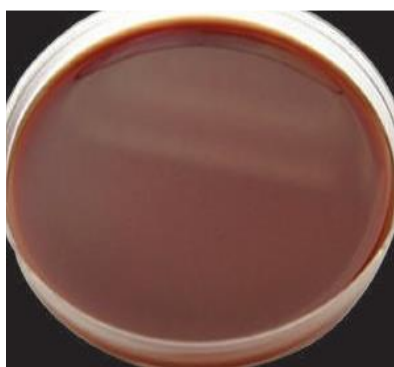


**MacConkey agar**



**MacConkey agar with  
Lactose fermenting  
colonies**

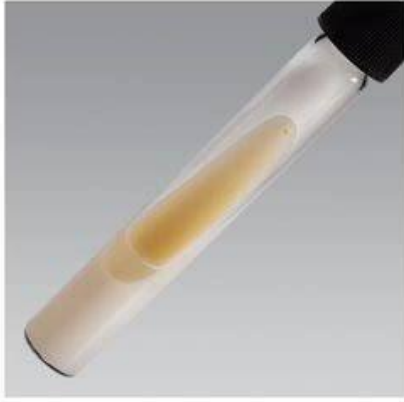
**MacConkey agar  
with non-lactose  
fermenting colonies**



**Chocolate Agar**



**Potassium tellurite agar  
with black colored**



**Loeffler's Serum Slope**



**Lowenstein Jensen medium**



**TCBS agar**



**Deoxycholate citrate agar**

**6) Enlist indications for blood culture. Name the culture media used in conventional blood culture.**

**Indications for blood culture are:** Bacteremia, sepsis, septicemia etc.

**The conventional blood culture media are of two types.**

1. Monophasic medium: It contains brain–heart infusion (BHI) broth

2. Biphasic medium: It has a liquid phase containing BHI broth and a solid agar slope made up of BHI agar. The recovery of organisms in the blood is enhanced by mixing the blood in the broth periodically. If any growth occurs, it can be detected by subcultures

**7) Enumerate the automated blood culture systems.**

There are three automated systems commercially available.

1. BacT/ALERT 3D
2. BacT/ALERT VIRTUO
3. BACTEC

**8) Discuss the advantages and limitations of conventional and automated blood culture systems**

Blood culture system	Advantages	Limitations
Conventional	The recovery of organisms in the blood is enhanced by mixing the blood in the broth periodically. If any growth occurs, it can be detected by subcultures.	From monophasic BHI broth, subcultures are made onto blood agar ,and MacConkey agar periodically for 1 week. There is a higher risk of contamination due to opening of the cap of the bottle every time when subcultures are made
	--	From biphasic BHI broth, subcultures can be made just by tilting the bottles so that the broth runs over the agar slope. There is lower risk of contamination as it obviates the opening of the cap of the bottle
Automated	Continuous automated monitoring: Following inoculation, the culture bottles are loaded inside the automated culture system The incubated bottles are periodically tilted automatically every 10 minutes, which allows mixing of blood with broth which fastens the recovery Bottles are periodically monitored for the microbial growth once in every 10 minutes by the instrument. Once positive for microbial growth, the	high cost of the instrument and culture bottles high cost of the instrument and culture bottles

	instrument gives a signal	
	<p>Composition: Automated blood culture bottles contain:</p> <p>Tryptic soy broth and/or brain heart infusion broth (as enriched media) added with Polymeric resin beads which adsorb and neutralize the antimicrobials present in blood specimen.</p> <p>Specimens: In addition to blood, these bottles can also be used for culture of bone marrow, sterile body fluids such as CSF, peritoneal, pleural and synovial fluid</p> <p>More sensitive: It gives a higher yield of positive cultures from clinical specimens</p> <p>Rapid: It takes less time than conventional methods</p> <p>Less labor intensive, as fully-automated.</p>	inability to observe the colony morphology as liquid medium is used

## 9) List the anaerobic culture media

Robertson's cooked meat (RCM) broth

- Thioglycollate broth
- Anaerobic blood agar
- BHIS agar (Brain-heart infusion agar) with supplements (vitamin K and hemin)
- Neomycin blood agar
- Egg yolk agar
- Phenyl ethyl agar
- *Bacteroides* bile esculin agar (BBE agar).

## 10) Describe the anaerobic culture methods and write their uses.

Obligate anaerobic bacteria can grow only in the absence of oxygen, hence for the growth of such bacteria, anaerobic environment is needed. The following are the methods used to create anaerobiosis.

Evacuation and Replacement: This involves evacuation of the air from jar and replacement with inert gas like hydrogen followed by removal of the residual oxygen by use of a catalyst. It is carried out either by:

Manual method by using McIntosh and Filde's anaerobic jar. It was the most popular method for creating anaerobiosis in the past, now not in use.

**Automated system (Anoxomat):** It automatically evacuates air and replaces by hydrogen gas from a cylinder.

- The catalyst used to combust residual oxygen is a sachet containing aluminum pellets coated with palladium.
- It is easier to operate than McIntosh jar method and claims to be highly effective for creating anaerobiosis.

### **Absorption of Oxygen by Chemical Methods**

GasPak system (BD diagnostics) works on this principle. It is the the most commonly used method for anaerobiosis, especially for laboratories with less sample load.

- Here, the oxygen is removed by chemical reactions, instead of evacuation and replacement technique used in Anoxomat
- It uses a sachet containing sodium bicarbonate and sodium borohydride which react chemically in presence of water, to produce hydrogen and CO<sub>2</sub> gas
- The traces of oxygen is removed by using the same catalyst used for Anoxomat (aluminium pellets coated with palladium) placed below the jar lid

**Indicator of anaerobiosis:** The effectiveness of anaerobiosis can be checked by:

- Chemical indicator: Reduced methylene blue remains colorless in anaerobic conditions, but turns blue on exposure to oxygen
- Biological indicator using obligate aerobe such as *Pseudomonas*: Absence of its growth indicates that complete anaerobiosis has been achieved.

**GENbag (bioMérieux):** It consists of an airtight transparent bag with a generator sachet, which rapidly produces carbon dioxide and creates an anaerobic environment. Its application is similar to that of GasPak system.

### **Anaerobic Glove Box and Anaerobic Work Station**

These systems provide facility for easy processing, incubation and examination of the specimens without exposure to oxygen.



### **Reducing Agents**

Oxygen in culture media can be reduced by various reducing agents, such as glucose, thioglycollate, cooked meat pieces, cysteine and ascorbic acid. Robertson cooked meat broth is the most widely employed anaerobic culture medium which uses chopped meat particles (beef heart) as reducing agent.

### **Pre-reduced Anaerobically Sterilized (PRAS)**

PRAS media are prepared entirely under oxygen-free conditions from initial sterilization to packaging in sealed foil packets.

**11) Give examples for presumptive identification of bacteria based on their colony characteristics.**

<b>Colony characters</b>	<b>Probable bacteria</b>
Lactose fermenting, moist colonies on MacConkey's agar	<i>Escherichia coli</i>
Lactose fermenting, mucoid colonies on MacConkey's agar	<i>Klebsiella spp.</i>
Non-lactose fermenting, spreading colonies on MacConkey's agar	<i>Pseudomonas aeruginosa</i>
Beta haemolytic colonies on blood agar	<i>Staphylococcus</i>

**Date:**

**Faculty Name & Signature:**