

Bacterial taxonomy

Lab:8

Differential Media

Differentiation between aerobic and anaerobic bacteria

oxygen requirement

According to oxygen requirement, bacteria can be divided into five types:

1-Obligate aerobes:

Definition: Bacteria that require oxygen to grow; they cannot survive without it.

Examples: *Mycobacterium tuberculosis*,
Pseudomonas aeruginosa

2-Obligate anaerobes:

Definition: Bacteria that cannot tolerate oxygen; they die in its presence.

Examples: *Clostridium botulinum*, *Bacteroides fragilis*

3-Facultative anaerobes:

Definition: Bacteria that can grow with or without oxygen, but grow better in the presence of oxygen.

Examples: *Escherichia coli*, *Staphylococcus aureus*

4-Microaerophiles:

Definition: Bacteria that require a small amount of oxygen for growth, but high concentrations are toxic.

Examples: *Helicobacter pylori*, *Campylobacter jejuni*

5-Aerotolerant anaerobes:

Definition: Bacteria that do not use oxygen but can tolerate its presence.

Examples: *Lactobacillus* spp, *Streptococcus pyogenes*

obligate
aerobes



A

obligate
anaerobes



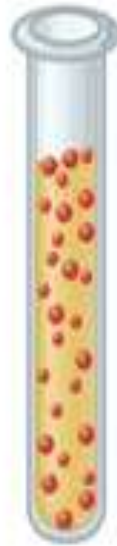
B

facultative
anaerobes



C

aerotolerant
anaerobes

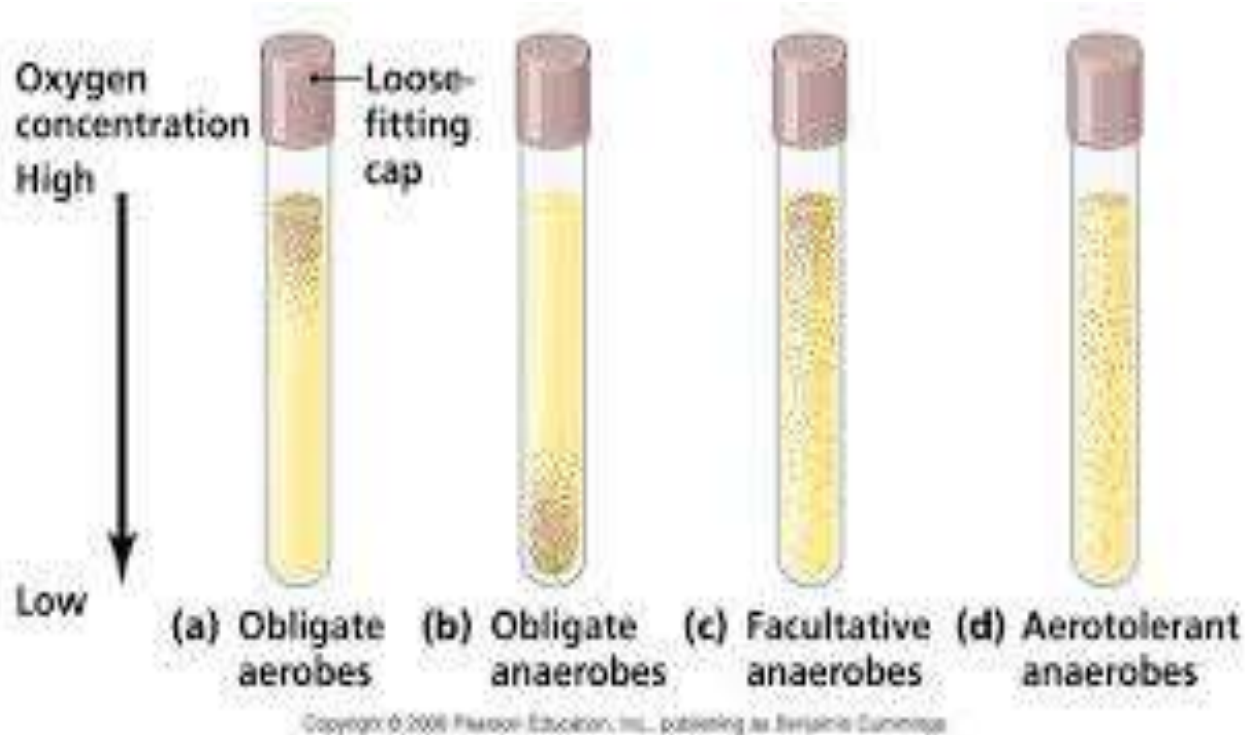


D

microaerophiles



E



Culture media

Culture media can be divided according to oxygen requirement into two main types:

1-Aerobic culture media:

These media are used for the growth of microorganisms that require oxygen for their metabolism and growth. Examples include **nutrient agar**, **blood agar**, and **tryptic soy agar** incubated under aerobic conditions.

2-Anaerobic culture media:

These media are designed for the cultivation of microorganisms that grow in the absence of oxygen or are inhibited by its presence.

Examples include **thioglycolate broth**, **Robertson's cooked meat medium**, and **anaerobic blood agar**.



Media used in anaerobic bacteriology can be freshly prepared or purchased from commercial suppliers. Media for anaerobic culture prepared in the laboratory should be used within two weeks of preparation as long storage degrades the quality of the media due to peroxide accumulation and dehydration. Anaerobic culture media contains reducing agents such as cysteine.

Pre-reduced, anaerobically sterilized media are produced by different commercial suppliers, which have extended shelf life up to six months. The primary plating media for inoculating anaerobic specimens includes a non-selective **blood agar and one or all of the following mentioned selective media.**



Clostridium perfringens in anaerobic blood agar



Clostridium perfringens in egg yolk agar



Bacteroides bile esculin agar

Anaerobic Culture Media



Robertson's cooked meat broth



Anaerobic phenylethyl alcohol blood agar



C. difficile in cycloserine cefoxitin fructose agar

1-Nonselective media used in anaerobic bacteriology:

- 1. Cooked meat broth (e.g. Robertson's Cooked Meat Medium):** Non-selective for the cultivation of anaerobic organisms; with the addition of glucose, can be used for gas-liquid chromatography.
- 2. Anaerobic blood agar:** It is a non-selective medium for the isolation of anaerobes and facultative anaerobes.
- 3. Egg-yolk agar (EYA):** Non-selective for determination of lecithinase and lipase production by Clostridia and Fusobacteria.
- 4. Peptone-yeast extract glucose broth (PYG):** Non-selective for the cultivation of anaerobic bacteria for gas-liquid chromatography.

2-Selective and differential media used in anaerobic bacteriology:

1. **Bacteroides bile esculin agar (BBE):** It is selective and differential for *Bacteroides fragilis* group and good for presumptive identification.
2. **Laked Kanamycin-vancomycin blood agar (LKV):** It is selective for isolation of *Prevotella* and *Bacteroides* spp.
3. **Anaerobic phenylethyl alcohol agar (PEA):** Selective for inhibition of gram-negative rods and swarming by some *Clostridia*.
4. **Cycloserine cefoxitin fructose agar (CCFA):** Selective for *Clostridium difficile*.
5. **Thioglycollate broth:** Nonselective for the cultivation of anaerobes; as well as facultative anaerobes and aerobes.



bottles of aerobic and anaerobic media that can be used to detect microbes using dilution and timing Captions are provided by our contributors.

Anaerobic Blood Agar

- Composition
- Principle
- Preparation
- Method of Use
- Result Interpretation
- Uses
- Limitations



Anaerobic Blood Agar- Composition, Principle, Preparation, Results, Uses

Anaerobic Blood Agar is a solid media recommended for use in qualitative procedures for primary isolation and cultivation of anaerobic organisms, including fastidious strains. Anaerobic Blood Agar supports good growth and typical pigmentation of fastidious and slow-growing anaerobes, as well as other anaerobes of significant clinical importance.

Composition of Anaerobic Blood Agar

Ingredients	Gm/liter
Casein enzymic hydrolysate	15.000
Papaic digest of soybean meal	5.000
Yeast extract	5.000
Sodium chloride	5.000
L-Cysteine	0.500
Hemin	0.005
Agar	13.50

Principle of Anaerobic Blood Agar

- Anaerobic Blood Agar base serves as a nutritious, nonselective medium allowing the cultivation of not only fastidious anaerobes but also of aerobic and microaerophilic microorganisms.**
- It contains peptones which supply nitrogenous substances and amino acids necessary for the growth of anaerobic bacteria.**
- Yeast extract provides B-complex vitamins and serves as a growth enhancer.**
- Hemin, vitamin K, and sheep blood stimulate the growth of anaerobes like Bacteroides species and gram-positive spore bearers like Clostridium species.**
- Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium.**

- . Addition of blood provides nutrients and helps to differentiate hemolytic organisms.**

Preparation and Method of Use of Anaerobic Blood Agar

- 1.Suspend 44.0 grams in 1000 ml distilled water.**
- 2.Heat to boiling to dissolve the medium completely.**
- 3.Add the rehydrated contents of 1 vial of Vitamin K1 solution.**
- 4.Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.**
- 5.Cool to 45-50°C.**
- 6.Aseptically add 5% v/v sterile defibrinated sheep blood.**
- 7.Mix well and pour into sterile Petri plates.**

8. Prior to use, reduce the plates for a minimum of 24 hours by placing them in an anaerobic environment at room temperature.

9. Inoculate specimens for anaerobic culture on both selective and non-selective media as soon as possible after receipt in the laboratory; streak plates for isolation.

10. Incubate anaerobically at 33-37°C for 48-72 hours.

11. Confirm anaerobic growth by subculture to anaerobic blood agar plate.

Luxuriant Growth: Growth of microorganisms that is very abundant, dense, or vigorous on a culture medium.

Result Interpretation of Anaerobic Blood Agar

Organisms	Growth
<i>Bacteroides fragilis</i>	Luxuriant Growth
<i>Bacteroides melaninogenicus</i>	Luxuriant Growth
<i>Peptostreptococcus anaerobius</i>	Luxuriant Growth
<i>Clostridium perfringens</i>	Growth, double zone β-hemolysis
<i>Fusobacterium nucleatum</i>	Growth
<i>Prevotella melaninogenica</i>	Growth; pigment (tan to brown)
<i>Escherichia coli</i>	Growth
<i>Staphylococcus aureus</i>	Growth

Uses of Anaerobic Blood Agar

1-Anaerobic Blood Agar Base is recommended for the cultivation of anaerobic microorganisms, including very fastidious organisms from clinical specimens.

2-It promotes both typical pigment formation in *Bacteroides melaninogenicus* and displays double hemolytic reaction in *Clostridium perfringens* with added blood to the medium base.

3-The media supports typical pigment production by pigmented *Prevotella* and *Porphyromonas*

Limitations of Anaerobic Blood Agar

1-The media must be prepared, dispensed, and packaged under oxygen-free conditions to prevent the formation of oxidized products prior to use.

2-This Agar will not provide complete information for identification of bacterial isolates. Additional test procedures and media are required for complete identification.

3-It is recommended that a selective media such as Anaerobic Brucella Laked Blood Agar with Kanamycin and Vancomycin and/or Anaerobic Brucella Blood Agar with Phenylethyl Alcohol also be inoculated with clinical specimens to assure growth of all species present.