Blood Agar

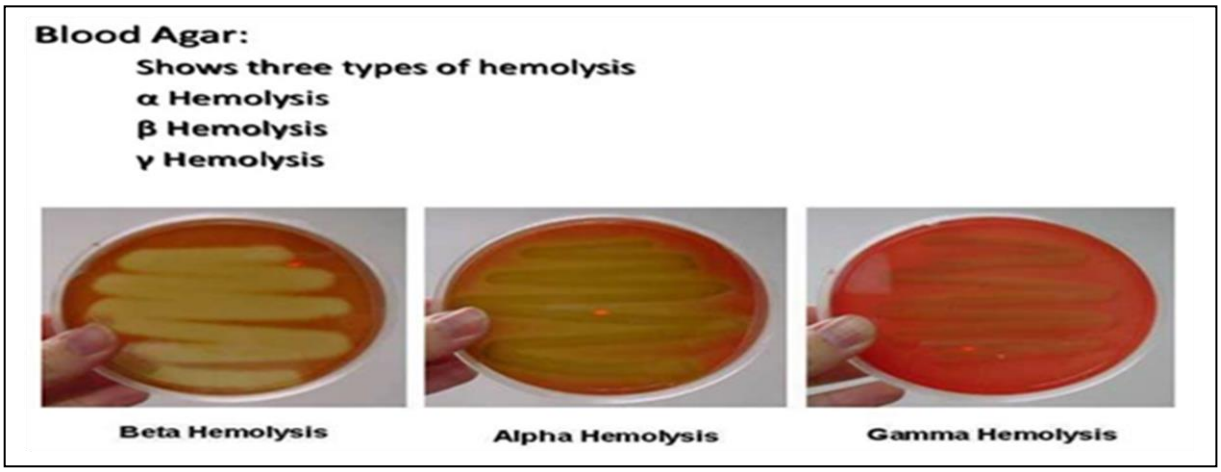
Blood Agar (BA) are enriched medium used to culture those bacteria or microbes that do not grow easily. Such bacteria are called “fastidious” as they demand a special, enriched nutritional environment as compared to the routine bacteria.

Blood Agar is used to grow a wide range of pathogens particularly those that are more difficult to grow such as Hemophilus influenzae, Streptococcus pneumoniae and Neisseria species. It is also required to detect and differentiate hemolytic bacteria, especially Streptococcus species. It is also a differential media in allowing the detection of hemolysis (destroying the RBC) by cytolytic toxins secreted by some bacteria, such as certain strains of Bacillus, Streptococcus, Enterococcus, Staphylococcus, and Aerococcus.

Blood agar can be made selective for certain pathogens by the addition of antibiotics, chemicals or dyes.

Preparation of Blood Agar:

1. Suspend 28 g of nutrient agar powder in 1 liter of distilled water.
2. Heat this mixture while stirring to fully dissolve all components.
3. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.
5. When the agar has cooled to 45-50 °C, add 5% (vol/vol) sterile defibrinated blood that has been warmed to room temperature and mix gently but well.
6. Avoid Air bubbles.
7. Dispense into sterile plates while liquid.



Hemolysis is the breakdown of red blood cells (RBC). A substance that causes hemolysis is a hemolysin. Brown (1919) introduced three terms alpha, beta and gamma to indicate three types of streptococci based on haemolytic reactions observed on blood agar plates.

Beta-hemolytic:

Beta-hemolysis (β-hemolysis) is associated with complete lysis of red cells surrounding the colony. Beta hemolysis is caused by two hemolysins O and S; the former is inactive in the presence of oxygen. Thus, stabbing of the plate increases the intensity of the hemolysis reaction. S is an oxygen-stable cytotoxin.

Examples: Streptococcus pyogenes, or Group A beta-hemolytic Strep (GAS).

Alpha-hemolysis:

Alpha-hemolysis (α-hemolysis) is a partial or "green" hemolysis associated with reduction of red cell hemoglobin. Alpha hemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green methemoglobin.

Examples: Streptococcus pneumoniae and a group of oral streptococci (Streptococcus viridans or viridans streptococci)

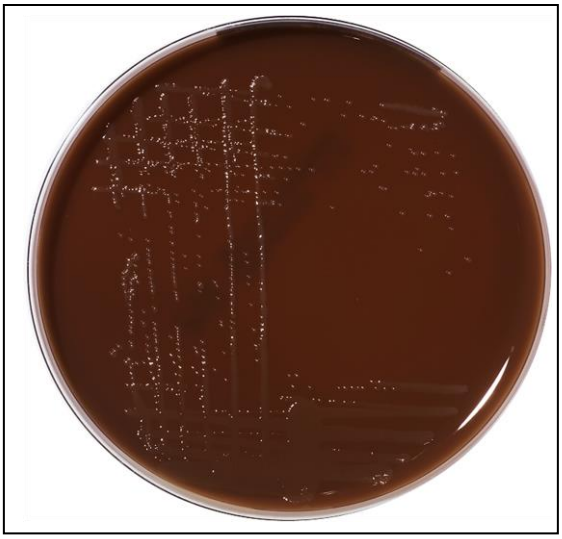
Gamma-hemolytic (Non-Haemolytic):

Colonies show neither typical alpha nor beta haemolysis. There may be, however, slight discoloration in the medium. The streptococci included in this group are usually not pathogenic.

Examples: Enterococcus faecalis (formerly called "Group D Strep")

Chocolate agar

Chocolate agar (CHOC) or chocolate blood agar (CBA), is a nonselective, enriched growth medium used for isolation of pathogenic bacteria.[1][2][3] It is a variant of the blood agar plate, containing red blood cells that have been lysed by slowly heating to 80°C. Chocolate agar is used for growing fastidious respiratory bacteria, such as Haemophilus influenzae and Neisseria meningitidis.[4] In addition, some of these bacteria, most notably H. influenzae, need growth factors such as nicotinamide adenine dinucleotide (factor V or NAD) and hemin (factor X), which are inside red blood cells; thus, a prerequisite to growth for these bacteria is the presence of red blood cell lysates. The heat also inactivates enzymes which could otherwise degrade NAD. The agar is named for its color and contains no chocolate products.

Preparation of Chocolate Agar:

1. Prepare the blood agar base as instructed by the manufacturer.
2. Sterilize by autoclaving at 121°C for 15 minutes.
3. Add 5-7% v/v of defibrinated blood (horse or sheep blood) and place the media in a water bath of 75 -80°C, and keep swirling gently until the color changes to dark brown.
4. Pour into sterile Petri plates under aseptic conditions after the media has cooled to 50-55°C.
5. Label the plates with the name of the media, and date of preparation, and store them invertedly at 2-8°C until use.