

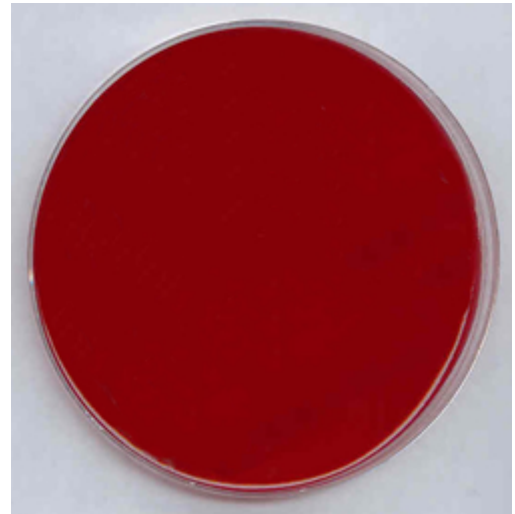
Hemolysis - Blood Agar

Intended Use:

Blood agar is used to support the growth of fastidious organisms and to determine the type of hemolysis (destruction of red blood cell walls) an organism produces.

Principle:

Blood agar is a rich medium that has been supplemented with fresh 5-10% blood. The hemolytic response can be dependent upon the type of blood. Sheep blood is commonly used, but some organisms require rabbit or bovine blood.



Test Procedure:

1. Streak a plate of blood agar for isolation.
 - Optional: Do your last streak with a needle and poke into the agar. This usually gives clear, reliable zones of beta hemolysis and is especially important to see the effects of streptolysin O which is oxygen labile. See page 84 of the Difco/BBL Manual.
2. Incubate the plates at 37°C for 24-48 hours. Strep organisms should be incubated in the CO₂ incubator.
 - The plate will be a brownish red color after 48hours.

Results:

You can differentiate four types of hemolysis by the appearance of the agar.

- **Beta hemolysis** is indicated by a clear colorless zone surrounding the colonies. There has been total lysis of the red blood cells.
- **Alpha hemolysis** is indicated by a small zone of greenish to brownish discoloration of the media. This is caused by the reduction of hemoglobin to methemoglobin and its subsequent diffusion into the surrounding medium.
- **Alpha prime hemolysis** is indicated by a zone of complete hemolysis, surrounded by a zone of partial hemolysis, a pink halo. This pattern can be easier to see if you scrape off the colony.
- **Gamma hemolysis** is indicated by no change in the media.

Limitations:

- The patterns of hemolysis can vary with the incubation atmosphere and the type of blood in the media.
- Some Staph organisms will only show hemolysis after they have been refrigerated following incubation.