# SHEEP BLOOD AGAR PLATE TMP 017

## INTENDED USE
For cultivation of fastidious organisms and studying haemolytic reactions.

## COMPOSITION

| Ingredients | Gms/Ltr |
|---|---|
| Casein enzymic hydrolysate | 14.000 |
| Agar | 12.500 |
| Sodium chloride | 5.000 |
| Sheep Blood | 5.000 |
| Peptic digest of animal tissue | 4.500 |
| Yeast extract | 4.500 |

## PRODUCT SUMMARY AND EXPLANATION
This medium is both differential and enriched medium and used for the isolation, cultivation and detection of hemolytic activity of fastidious microorganisms like *Streptococci*, and *Pneumococci*. The Blood Agar plate medium contains highly nutritious extract and the 5 % blood supplement that provides additional use in isolation, cultivation, and determination of hemolytic reactions of fastidious pathogenic microorganisms. Hemolytic patterns may vary with the source of animal blood or type of base medium used. In sheep blood, nucleotidase destroys V factors preventing the growth of *Hemophilus* species on sheep blood agar unless other microorganisms, such as *Staphylococci*, provide the V factors. Small amounts of reducing sugars inhibit the expression of β-hemolytic, and β-hemolytic *Streptococci* may develop a green zone or ring of hemolysis.

A number of streptococcal species produce substances that lyses of the red cell wall releasing of hemoglobin. Such substances are referred to as hemolysins. The activity of streptococcal hemolysins, streptolysins, can be readily observed when the organisms are growing on a blood agar plate. Different *streptococci* produce different effects on the red blood cells in blood agar.

- Those that produce incomplete hemolysis and only partial destruction of the cells around colonies are called **alpha-hemolytic** *Streptococci*. Characteristically, this type of hemolysis is seen as a distinct greening of the agar in the hemolytic zone, and thus this group of *streptococci* has also been referred to as the viridans group.

- Species whose hemolysins cause complete destruction of red cells in the agar zones surrounding their colonies are said to be **beta-hemolytic** which are small opaque or semi translucent colonies surrounded by clear zones in a red opaque medium. Two types of beta lysins are produced; Streptolysin-O, an antigenic oxygen-labile enzyme and Streptolysin-S, a non-antigenic oxygen-stable enzyme. The hemolytic reaction is

enhanced when blood agar plates are streaked and simultaneously stabbed to show subsurface hemolysis by Streptolysin-O in an environment with reduced oxygen tension. Some strains of *Staphylococci*, *Escherichia coli*, and other bacteria also may show beta-hemolysis.

- Some species of *Streptococci* do not produce hemolysins. Therefore, when their colonies grow on blood agar, no change is seen in the red blood cells around them. These species are referred to as nonhemolytic or **gamma hemolytic** *streptococci.*

## PRINCIPLE

Medium contains nutritional components like pancreatic digest of casein, neutralized peptone, and yeast extract, and the addition of sodium chloride provides an osmotically balanced medium for bacterial cells. The addition of 5% defibrinated sheep blood allows for the determination of hemolytic reactions, an important differential characteristic.

## INSTRUCTION FOR USE

Either streak, inoculate or surface spread the test inoculum aseptically on the plate.

## QUALITY CONTROL SPECIFICATIONS

**Appearance:** Cherry red coloured opaque gel forms in Petri plates.

**Quantity of Medium:** 25ml of medium in 90mm plates.

## INTERPRETATION

Cultural characteristics observed after incubation at 30-35 °C for 18-48 hours.

| Organism | ATCC | Inoculum (CFU/ml) | Growth | Recovery Rate | Colour of colony |
|---|---|---|---|---|---|
| *Streptococcus pneumoniae* | 6303 | $10^3$ | luxuriant | >=70% | alpha |
| *Streptococcus pyogenes* | 19615 | $10^3$ | luxuriant | >=70% | beta |
| *Staphylococcus aureus* | 25923 | $10^3$ | luxuriant | >=70% | beta |
| *Enterococcus faecalis* | 29212 | $10^3$ | luxuriant | >=70% | beta |
| *Escherichia coli* | 25922 | $10^3$ | luxuriant | >=70% | none |
| *Salmonella typhi* | 6539 | $10^3$ | luxuriant | >=70% | none |

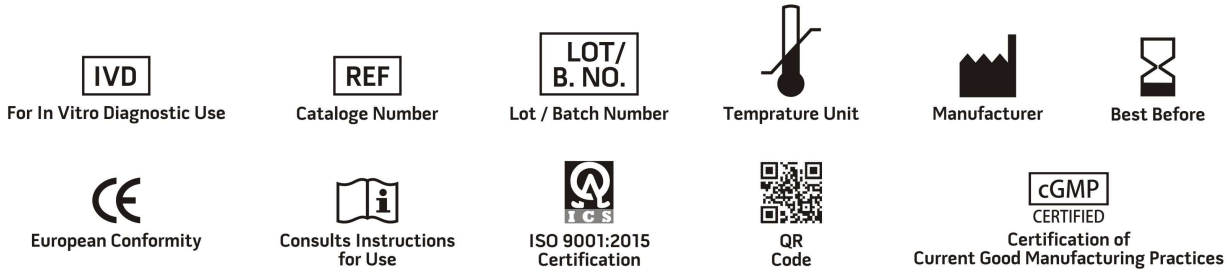
## STORAGE & STABILITY

On receipt, store the plates at 2-8 °C. Avoid freezing and overheating. Do not open until ready to use. Prepared plates stored in their original sleeve wrapping until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

## REFERENCES


1. MacFaddin JF. Media for isolation-cultivation maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
3. Isenberg HD, Ed. Clinical microbiology procedures handbook. Washington, DC: ASM, 1992.
4. NCCLS. Quality Assurance of commercially prepared microbiological culture media. 2nd ed. NCCLS document M22-A2. Wayne, PA: NCCLS, 1996. 5. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 10th ed. St. Louis: Mosby, 1998.


**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.