

# SHEEP BLOOD AGAR - INSTRUCTIONS FOR USE

## (Ready Plated Media)

### 1. INTENDED USE:

A non-selective, highly nutritious, general-purpose medium used for the growth of fastidious organisms and to differentiate bacteria based on their hemolytic properties.

### 2. PRINCIPLE:



Blood Agar: *S. pneumoniae* colonies showing alpha hemolysis.

Blood Agar Base is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. The blood added to the base provides more nutrition to the medium by providing additional growth factors required for the growth of fastidious organisms. Hemolysins are exotoxins produced by bacteria that lyse red blood cells. The hemolytic reaction can be visualized on blood agar plates. On blood agar plates colonies of hemolytic bacteria may be surrounded by clear, colorless zone where the red blood cells have been lysed and the hemoglobin destroyed to a colorless compound. This is beta hemolysis. Other types of bacteria can reduce hemoglobin to methemoglobin which produces a greenish zone around the colonies and is called alpha hemolysis. Gamma hemolysis is no hemolysis where no change in the medium is observed. Hemolytic reactions depend on the animal blood used. Sheep blood gives the best results for Group A *Streptococci*. But sheep blood fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However, when horse blood is used *H. haemolyticus* colonies produce hemolysis and mimic *Streptococcus pyogenes*.

### 3. MATERIALS PROVIDED:

PRODUCT	TYPE	REF	PACK
AG - Sheep Blood Agar plate	Ready Plated Media	AG/SBA/22/01	10 plates in a pack

### 4. MATERIALS REQUIRED BUT NOT PROVIDED:

Sterile loops, incubator, and laboratory equipment as required.

### 5. SPECIMENS:

Clinical specimens such as faeces, rectal swab, urine, bile, non-sterile pharmaceutical products and food.

### 6. TEST PROCEDURE, READING AND INTERPRETATION:

- Allow plates to come to room temperature.
- Inoculate and streak the specimen with a loop over the four quadrants of the plate. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
- Incubate inoculated Blood Agar plates with the specimen or with a specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours.
- Observe colonies for hemolysis and other factors.

### 7. USER QUALITY CONTROL :

All manufactured lots of the product are released for sale after Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for quality control.

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CONTROL STRAINS	INCUBATION	EXPECTED RESULTS
<i>S. pyogenes</i> ATCC 19615	37°C /18-24H - 5% CO <sub>2</sub>	Good growth, colonies show beta hemolysis
<i>S. pneumoniae</i> ATCC 49619	37°C /18-24H – 5% CO <sub>2</sub>	Good growth, colonies show alpha hemolysis
<i>S. aureus</i> ATCC 25923	37°C /18-24H-Aerobic	Good growth
<i>E. coli</i> ATCC 25922	37°C /18-24H-Aerobic	Good growth

Key: ATCC is a trademark of American Type Culture Collection

### 8. LIMITATIONS OF THE METHOD:

- Addition of sheep blood is recommended to detect hemolysis. This medium does not support the growth of *H. haemolyticus*
- Addition of Horse blood or rabbit blood to base medium supports growth of *H. haemolyticus* but resemble beta hemolytic Streptococci and hence must be confirmed.
- Hemolytic pattern varies with the source of blood used.
- Other tests must be carried out in conjunction for confirmation.











### 9. PRECAUTIONS AND WARNINGS:


- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose of the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available with AstraGene and can be provided on request.

### 10. STORAGE CONDITIONS AND SHELF LIFE:

- Upon receipt, store at +2 - 8°C away from direct light in a cool, dry place. Storage below 2°C may lead to crystallization of media components.
- If properly stored, it may be used up to the expiration date. Do not use it beyond this date. The user is responsible for the storage method (temperature) of the medium.
- For optimal usage, it is advisable to utilize the entire pack of 10 plates once they have been taken out of the packaging.

### 11. SYMBOLS:

	Date of manufacture		Use-by-date		Do not use if package is damaged		Manufacturer
	Batch Code		Refer to the instructions		ISO		GMP
	In-Vitro diagnostic Medical devices		Mark of conformity				

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