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# BLOOD AGAR

## (TSA w/ Rabbit Blood)

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### INTENDED USE

Remel Blood Agar (TSA w/ Rabbit Blood) is a solid medium recommended for use in qualitative procedures for the primary isolation of most aerobic organisms and for the cultivation of *Haemophilus* species.

### SUMMARY AND EXPLANATION

Members of the genus *Haemophilus* require hemin (X factor) and/or nicotinamide adenine dinucleotide (NAD or V factor). NAD-requiring *Haemophilus* spp. grow well in media containing goat, bovine, and chocolate sheep blood.<sup>1</sup> Whole sheep erythrocytes contain V factor-inactivating enzymes which make sheep blood agar unsuitable for recovery of *Haemophilus* spp. Blood from rabbits and horses does not release such enzymes.<sup>2</sup> Artman and Frankl reported that rabbit erythrocytes serve as a sole source of V factor for NAD-requiring species of *Haemophilus* due to the presence of negligible amounts of V factor-inactivating enzymes.<sup>3</sup>

### PRINCIPLE

Tryptic Soy Agar Base provides casein and soy peptones which supply nitrogen, amino acids, and peptides. Sodium chloride provides essential electrolytes to maintain osmotic equilibrium. Rabbit blood contains adequate quantities of X and V growth factor required by *Haemophilus* spp. and facilitates visualization of hemolysis.

### REAGENTS (CLASSICAL FORMULA)\*

Casein Peptone.....	15.0 g	Rabbit Blood.....	5 %
Sodium Chloride.....	5.0 g	Agar.....	15.0 g
Soy Peptone.....	5.0 g	Demineralized Water.....	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

\*Adjusted as required to meet performance standards.

### PROCEDURE

1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory. If material is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
2. Incubate plates in 5-10% CO<sub>2</sub> at 33-37°C for 24-48 hours.
3. Examine plate for typical colony morphology and hemolytic reactions.

### QUALITY CONTROL

All lot numbers of Blood Agar (TSA w/ Rabbit Blood) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

#### CONTROL

*Streptococcus pneumoniae* ATCC® 6305  
*Streptococcus pyogenes* ATCC® 19615  
*Haemophilus influenzae* ATCC® 10211  
*Neisseria sicca* ATCC® 9913  
*Staphylococcus aureus* ATCC® 25923

#### INCUBATION

CO<sub>2</sub>, 24-48 h @ 33-37°C  
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#### RESULTS

Growth w/ alpha hemolysis  
Growth w/ beta hemolysis  
Growth  
Growth  
Growth

### LIMITATIONS

1. Sheep blood agar is considered the standard medium for determination of streptococcal hemolytic reactions due to irregularities in the hemolytic activity of some streptococci (e.g., *Enterococcus* spp.) on other blood-containing media.<sup>4</sup>

### BIBLIOGRAPHY

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4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9<sup>th</sup> ed. ASM Press, Washington, D.C.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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