BD™ Trypticase™ Soy Agar II With 5% Horse Blood

INTENDED USE

BD Trypticase Soy Agar II With 5% Horse Blood is a nutritious general purpose medium for the isolation and cultivation of nonfastidious and fastidious micro-organisms from a variety of clinical specimens and for the detection of hemolytic reactions.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

The nutritional composition of **Trypticase** Soy Agar (TSA) has made it a popular medium, both unsupplemented and as a base for media containing blood. [1-3] **Trypticase** Soy Agar II (TSA II) is an improved version of the original version, providing clearer hemolytic zones than **Trypticase** Soy Agar (TSA) when used with blood. Although this formulation supplemented with sheep blood is one of the most frequently used blood media in clinical laboratories, some users prefer horse blood.

The combination of casein and soy peptones in the **Trypticase** Soy Agar II supply organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains the osmotic equilibrium. Proprietary growth factors improve the hemolytic reactions. Horse blood allows detection of hemolytic reactions and supplies both the X factor (heme) and the V factor (nicotinamide adenine dinucleotide, NAD), necessary for the growth of *Haemophilus influenzae*, which requires both the X and V factors.

REAGENTS

BD Trypticase Soy Agar II With 5% Horse Blood

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	14.5 g
Papaic Digest of Soybean Meal	5.0
Sodium Chloride	5.0
Growth Factors	1.5
Agar	14.0
Defibrinated Horse Blood	5%

pH 7.3 +/- 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

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

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate the inoculated plates at 35 ± 2°C in an

aerobic atmosphere supplemented with carbon dioxide. Examine plates after 18 to 24 h for amount of growth, colony size and hemolytic reactions.

Strains	Growth Results
Escherichia coli ATCC™ 25922	Growth good to excellent; may or may not be beta-hemolytic
Enterococcus faecalis ATCC 29212	Growth good to excellent; beta hemolysis
Staphylococcus aureus ATCC 25923	Growth good to excellent; may or may not be beta-hemolytic
Streptococcus pneumoniae ATCC 6305	Growth good to excellent green-grey colonies, alpha hemolysis
Streptococcus pyogenes ATCC 19615	Growth good to excellent; good to strong beta hemolysis
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

BD Trypticase Soy Agar II With 5% Horse Blood (90 mm **Stacker**™ plates).
Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This is a universal medium in aerobic bacteriology that can be used for primary isolation of pathogens from all types of clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.
Alternatively, if 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.
Since many pathogens require carbon dioxide on primary isolation, plates should be incubated in an atmosphere containing approximately 3 to 10% CO_2. Incubate plates at 35-37°C for 18 to 24 h or longer, if necessary.

Results

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of micro-organisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas. Further differentiation of the isolated organisms by biochemical and/or serological tests is necessary. Consult the appropriate references for the appearance and further differential tests of the organisms isolated.[2-4]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

The medium is suitable for the isolation and cultivation of many aerobically growing micro-organisms, such as *Enterobacteriaceae*, *Pseudomonas* and other non-fermenting Gram negative rods, streptococci, staphylococci, coryneforms, *Candida* species, and many others. *Neisseria gonorrhoeae* does not grow well on this medium. Instead, GC Chocolate Agar should be used for the recovery of this species.
Also, the medium is not suitable for the isolation and growth of *Mycobacterium*, *Legionella*, *Bordetella* and other organisms with highly specific nutritive requirements.

The number and types of bacterial species occurring as infectious agents is very large. Therefore, before the medium is routinely used for rarely isolated or newly described micro-organisms, its suitability must first be tested by the user by cultivating pure cultures of the organism in question.

Hemolytic properties described in diagnostic textbooks usually refer to sheep blood. On horse blood containing media, these features are different. As an example, enterococci which only very rarely hemolyse sheep blood, will produce a well visible beta hemolysis on horse blood.[1-3] *Staphylococcus aureus* which is usually beta hemolytic on sheep blood, will often be non-hemolytic on horse blood.
Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta-hemolytic streptococci. They may be differentiated by performing a Gram stain on a smear prepared from the colony.[4] The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus.*[4]
Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[1,2-4]

REFERENCES

1. Isenberg, H. D. (ed.). 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol.1, p. 1.6.1-1.6.7. American Society for Microbiology, Washington, D.C.
2. Baron, E. J, L. R. Peterson, and S. M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed., p. 415. Mosby-Year Book, Inc. St. Louis, MO.
3. Vera, H.D., and D.A. Power. 1980. Culture media, p. 969. In E.H. Lennette, A. Balows, W.J. Hausler, Jr., and J.P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
4. Vera, H.D. 1971. Quality control in diagnostic microbiology. Health Lab. Sci. 8:176-189.

PACKAGING/AVAILABILITY

BD Trypticase Soy Agar II With 5% Horse Blood
Cat. No. 212099 Ready-to-use plated media, 20 plates

FURTHER INFORMATION

For further information please contact your local BD representative.

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